A HISTORICAL PERSPECTIVE ON ZOO AND WILDLIFE HEALTH IN EUROPA

Rudolf Ippen, Prof. Dr. med. vet.
Past affiliation with Institute for Zoo Biology and Wildlife Research Berlin, POB 1103, D10252 Berlin, Germany

A retrospective view at the historic record of veterinary practice shows clearly that through extended periods of time general attention had been almost exclusively restricted to the horse. More recently, veterinary services were gradually widened to include farm animals with relevance to human nutrition. Pets, domestic and experimental animals became gradually involved, as time went by. However, zoo and wild animals, save for few exceptions, by the middle of this century continued to be totally marginalized from veterinary medicine. The annual "Report of the Society's Prosector" in the Proceedings of the Zoological Society, London, is a good example, in this context, a valuable source of information on diseases of zoo animals from the beginning of this century. A good account of diseases in huntable game is provided in "Wildkrankheiten" by OLT and STRÖSE (1914), a book with many useful suggestions.

It was not until the beginning of the second half of this century that a sub-discipline in its own right was added to veterinary medicine, thanks to growing interest in global wildlife taken by the general public in industrial nations, their accompanying desire to preserve species endangered by extinction and resulting demands for optimum veterinary attention to zoo and wildlife stocks. Larger zoological gardens were increasingly joined by full-time veterinarians. Groups which took a specific interest in diseases of zoo and wild animals were gradually set up in veterinary departments of universities and in veterinary authorities.

It was just about the same time, in 1952, when the Institute of Comparative Pathology was founded in Berlin under the supervision of Professor Dr. Johannes Dobberstein. It was the only veterinary institution of the former German Academy of Sciences and was primarily preoccupied with basic research. Involvement of the widest possible range of animal species in comparative studies was a priority that ranked high on their program. The wide variety of non-domesticated species appeared to be a natural target. The Berlin-Friedrichsfelde location of the new institute was within no distance from "Tierpark Berlin" which had been opened at almost the same time. Also, close relations existed between institute staff and "Zoologischer Garten" in downtown Berlin, then the world's largest collection of zoo animals. These two factors were to provide for ideal opportunities for studies into diseases of zoo and wild animals, an area so severely neglected in the past.

Work started by high-continuity pathological, microbiological and parasitological routine examinations of deceased mammals, birds and reptiles to establish a body of knowledge on relevant categories of diseases and incidence, which then became a basis for more specific research, for example, comprehensive studies into tuberculosis, salmonellosis and amebic dysentery in various species. The material so far obtained from postmortems is relating to far more than 42,000 cases. All of them are documented in minute detail and are now being prepared for computerized retrieval and are also available in the form of paraffin-embedded
samples, histological specimens and slides. The origin of this comprehensive material, of course, is not restricted to the two Berlin-based zoological gardens. Growing specialization of our Institute has been accompanied by growing acceptance by numerous zoological gardens across Europe, animal traders, wildlife preserves and private owners of exotic animals who submitted material of great research value. Feral animals were included at a very early point in time and provided an opportunity to work on diseases endemic in Europe and other continents, including Antarctica.

Results obtained from routine studies or special-purpose and experimental research are reflected in more than 800 scientific publications, among them several books. Lectures and seminars were staged at national and international levels. Reference should be made at this point to a book on Diseases of Huntable Game and to a Manual on Diseases of Zoo Animals (both of them published in German).

European wildlife research, of course, did not stay limited to the Berlin-based Institute of Comparative Pathology. Highly efficient working groups gradually came into being at various university centers, among them in Vienna, Austria, Utrecht, Netherlands, Zurich, Switzerland, Giessen, Germany, Uppsala, Sweden, Prague, Czechia, Budakesci, Hungary, and Sofia, Bulgaria.

Close cooperation between Institute staff and the veterinarians affiliated to the brand new, modern veterinary clinic of "Tierpark Berlin" in the late fifties led to demands for wider exchange of experience with colleagues of other zoological gardens and relevant institutions. Preparations were initiated for an International Symposium on Diseases of Zoo and Wild Animals, and in 1959 the time had come to call the First Symposium in Berlin. The invitation was jointly launched by Professor Dr. Dobberstein, Institute Director, Dr. Dathe, Director of "Tierpark Berlin", Dr. Klös, Director of "Zoologischer Garten Berlin", and Professor Dr. Schmidt-Höhnsdorf, renowned parasitologist. The scientific sessions of this first conference of its kind were staged in either part of politically divided but still pre-Wall Berlin. No one even dreamt at that time of the sustainable nature of the exercise - its uninterrupted record covering 37 years by now. I was in charge of organization from the very beginning and was later appointed chairman of the Symposium. Our initial idea to change countries every year was to work extremely well. Taking into consideration the interests and wishes of participants, we annually rotated between east and west. This, by the way, proved to be helpful in circumventing many a political problem of the time. The organizational principle provided for joint sponsorship by the Berlin Institute with a local co-organizer. Every year, a choice had to be made among several applicants for co-organization. Among them were then emergent states in Africa, such as Tunesia and Morocco, who hosted and co-organized Symposia and thus underlined their interest in optimized veterinary attention to their valuable stocks in preserves and zoological gardens. The international acceptance and appreciation of the Symposium has remained constant and unbroken up to this day, which may be seen, last but not least, from participation of some 30 countries. Communication has been greatly facilitated by regular simultaneous translation services for all lectures and discussions in English and German and, occasionally, French.
The International Symposium became a standing institution and soon turned out to be a bridge between east and west in the chilliest periods of "Cold War". Where else could one imagine a comparable chance for free discussion between colleagues from east and west? This is the appropriate place and time to extend a word of sincerest gratitude to veterinarians of the US and Canada who never ceased to come in great numbers and make invaluable contributions. They did not simply contribute their own achievements to make each of the meetings a full success but, even more, helped in the making of a community to ensure the best possible care for the treasures entrusted to us, the world's stocks of zoo and wild animals. The same spirit obviously encouraged the WDA's decision to call the Sixth International Conference on Wildlife Diseases to former East Berlin in 1990. We took greatest pleasure in playing host to numerous outstanding specialists from overseas and making them enjoy not only an excellent conference but at the same time eye-witnesses of Germany's reunification.

It is fair to say that our knowledge on diseases of zoo and wild animals would never have grown at such a tempestuous rate, had it not been for these international contacts.

Our annual Proceedings that were regularly published to coincide with the first day of the Symposium proved to be highly important elements for dissemination of results and recent findings. They carry, in full text, all papers presented at the given Symposium together with summaries in English, German and French. More than 2,000 papers have thus been published throughout the past 37 years. These scientifically founded and practice-oriented publications have increasingly become part of European standard literature on all issues relating to diseases of zoo and wild animals and also provided a sound reference basis for numerous books written and published in the meantime.

The main topics for forthcoming Symposia are democratically discussed, chosen and approved by all participants. They usually are relating to all diseases occurring in a given category of animals or to a delimited complex of diseases occurring in different species. We have always set aside plenty of time for so-called free lectures in an attempt to keep pace with latest developments. A questions and answers session is a standard component on the schedule of each Symposium and is highly appreciated by all participants, as it provides for unrestricted discussion of problems cropping up in every-day practice. The involvement, in addition to veterinarians, of zoo managers and research workers from institutes and universities proved to be highly useful in this particular context, as their contributions from different positions added much to the overall success. Many personal contacts were established over the years and were to become relations of genuine friendship.

International cooperation with scientific institutions all over the world proved to be essential to good success, not only of the Symposium. For example, studies were jointly conducted with scholars of Sukhumi, former USSR, and colleagues in Washington, D.C., into leucosis in various species. Preparatory work for the Manual on Diseases in Reptiles would not have been possible, had it not been for the close cooperation with reptile specialists in Utrecht, Netherlands, and Gainsville, Florida. Finally, many questions regarding diseases in primates were answered owing to close cooperation with our colleagues in the National Zoo Park of
Washington, D.C., and the Zoo of San Diego, California. These are just a few examples to show the great importance that must be attributed to cooperation.

The Institute of Comparative Pathology was dissolved for political reasons in the early seventies. Following tough negotiations, some of the personnel were retained in a zoological working group to set up a "Centre of Vertebrate Research". The new Centre came under the umbrella of the Academy of Sciences and was officially supervised by Prof. Dr. Dathe, Director of "Tierpark Berlin". A new building was completed for the Centre, and research underwent an enormous upswing. The area of diseases of zoo and wild animals was strongly widened, and research on behavioral and reproductive biology was added to the traditional program. A group was set up for long-term research on the animal kingdom of Antarctica. Additional material was collected in Berlin and was processed for pathological research.

The findings thus obtained with regard to health status and diseases were disseminated not only in publications but also in lectures at universities. We strongly used for that purpose an existent system of postgraduate education of veterinary practitioners and personnel affiliated to wildlife preserves. A Commission of Zoological Gardens was set up and worked well. Affiliated to that Commission were zoo directors and other experts, with myself being in charge of veterinary medicine and coordination of research projects run on the premises of zoological gardens. Similar working bodies were set up for feral animals.

We do not realize any problem with regard to the future of zoo and wild animal research, as far as a forthcoming generation of research workers is concerned. Graduate students and candidates for doctoral degrees are getting increasingly involved in research, and the interest among students is significant. They all have at their unrestricted disposal a specialized library holding more than 14,000 volumes, including most up-to-date documentation.

Germany's reunification in 1990 again gave rise to questions for continuation of this research institution. As all the other institutions of former East Germany, ours was thoroughly evaluated by the German Science Council. In view of the international importance of our excellent performance and achievement, the Council's ruling was for continuation of the line of "zoo and wild animal research". The former Centre as a whole was dissolved and was replaced by an Institute for Zoo Biology and Wildlife Research, financially sponsored by the Federal Government and the State of Berlin. Generous funding enabled smooth continuation of current activities, expansion of working areas and purchase of most modern equipment. When the two previous veterinary departments in formerly divided Berlin were to be merged, I suggested installation of a faculty position for zoo and wild animal research in its own right. The proposal was accepted, and my successor, Prof. Dr. R. R. Hofmann, the present Director of the Institute for Zoo Biology and Wildlife Research, is at the time chair holder at the Free University of Berlin. Hence, the future of education in this field is ensured.

This brief outline of 40 years of European developments in the field of diseases of zoo and wild animals has, hopefully, shown that substantive progress has been made. It is in the interest of our ethically and financially so valuable patients and to the benefit of all species
threatened with extinction, that I want to express my very best wishes for all who are committed to scientific progress and to optimized veterinary services both at this conference and elsewhere.
ECOSYSTEM HEALTH: APPLICATION OF THE CONCEPT AND WILDLIFE AS INDICATORS

N. Ole Nielsen, DVM, PhD
Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Introduction

There is general acceptance that both local and global environments are being impacted by people to an extent that exceeds nature's ability to counteract degradation and to maintain biodiversity. The concept of ecosystem health has emerged as a useful societal goal to address this situation. Furthermore it provides a paradigm or framework to guide the actions of the public, politicians, scientists and natural resource managers alike. Its application can integrate natural and social history in morally and aesthetically acceptable ways.

Ecosystem health also provides an effective context for the domain of wildlife health and disease. The health of wildlife is accepted rightfully as important because of wildlife's biological and aesthetic value. Of even greater importance, measures of wildlife health can serve as useful indicators of the health of ecosystems. Those responsible for the health of wildlife are strategically situated to identify problems and to participate in activities that seek to arrest the loss of biodiversity and to assist in remediation of ecosystems.

Ecosystem Health as a "Supergoal"

In response to the world's environmental crisis, political and scientific institutions have articulated new goals appropriate to the circumstances. These goals are embodied in terms such as sustainability, environmental quality, conservation and ecosystem health. They are "supergoals", conceptual in nature and not quantifiable by any one simple measurement. Their supremacy in a hierarchy of goals is derived from the fact that they integrate an array of lower scale, more explicit operational goals.

Sustainability, conservation and ecosystem health characteristically go beyond the biophysical world and scientific reductionism and incorporate social, economic and moral dimensions. They encompass the realm of politics and culture.

Many environmental scientists have embraced the concept of ecological integrity. I would also define this as a supergoal but one restricted to biophysical dimensions. There is no single measure of ecological integrity. Healthy ecosystems have integrity.

Ecosystem health is a particularly attractive supergoal. Both words in this phrase incorporate important ideas. The first identifies the primary unit of interest, an ecosystem. Ecosystems are self organizing, and exist in a nested hierarchy, of open but geographically consistent areas. They include all living and physical components and the interactions among them.
They are dynamic and complex and have a degree of unpredictability as they evolve through phases of birth, growth, death and renewal at different spatial and temporal scales.\textsuperscript{9,10}

As a consequence of the experience and leadership of the International Joint Commission in addressing degradation of the Great Lakes and their watershed, many agencies deal with environmental issues using an "ecosystem approach".\textsuperscript{7} This has been defined as "an integrated set of policies and managerial practices that relate people to 'ecosystems' of which they are a part rather than to external resources or environments with which they interact". The ecosystem approach is analytic and synthetic and seeks to understand the interaction among biophysical, economic and social forces. "It is a holistic perspective inter-relating systems at different levels of integration, and actions that are ecological, anticipatory, and ethical in respect to systems of Nature".\textsuperscript{7}

The second element in the phrase "ecosystem health" describes an ideal with which society has had long and positive experience, and which is understood and desired, either explicitly or intuitively, by people. Hence it has political appeal.

The concept of health is rooted in antiquity.\textsuperscript{6,13,18} The Greeks apparently distinguished health (or wellness) from illness in their mythology. Aesculapius was the god of healing and dealt with illness. Hygeia was the goddess of health. For the past one hundred years western medicine has focused on the Aesculapian approach to medicine, applying reductionistic and mechanistic methodology to understand and treat human disease. It has been said that contemporary western society has "illness" (rather than health) care systems. Some critics of ecosystem health mistakenly seem to believe that this reductionist biomedical approach, focused on individual patients, is the essence of the health paradigm. In fact, the concept of ecosystem health, where notions of wellness, balance, holism and ecological thinking hold sway, is more allied to Hygeia than Aesculapius. It is interesting to note that within human medicine there are those who advocate a health care system based on an ecological model.\textsuperscript{1}

In reference to humans, either as individuals or populations, health has been defined by the WHO not merely as the absence of disease, but as a state of complete physical, mental and social well being. This definition is so broad as to make it rather unhelpful operationally; however it is a useful ideal. It is comparable to "pristine nature", which is also an unattainable, but nonetheless useful, ideal.

Health incorporates both social and natural (or biophysical) dimensions. In veterinary medicine, health has the added dimension of productivity, including reproduction.\textsuperscript{3} Here the practice of "health management" has emerged as "a system of preventive medicine that takes into account the whole animal and the total influences, including social, with respect to relationships with others in the herd or flock, psychological and environmental factors that effect health, including nutrition, exercise, housing, freedom from crowding and boredom and from physical harassment or cruelty".\textsuperscript{3} In consequence of the above it is not difficult to see how this notion of health can be extrapolated to the wider ecosystem.
Health, whether it refers to individuals, populations or ecosystems, is a desired goal or an ideal. In none of these situations can a single measurement define health. Rather it requires a suite of measurements or indicators, in comparison to arbitrary standards, to arrive at a judgement about the degree of health. Experience in applying the concept of health to individuals and populations has made us comfortable with its utility in these circumstances despite a measure of arbitrariness or relativity in its definition. It seems reasonable to anticipate the same situation will evolve with respect to ecosystems.

The "concept definitions" (attributes) of health identified by Constanza for ecosystems also apply at the scale of individuals and populations (see Table 1). Similarly, if one defines a list of attributes or characteristics of the health paradigm, based on experience with individuals and populations, they seem to apply equally to ecosystems. Therefore the limitations and strengths of the health paradigm apply at all three levels. There seems to be no denying that the concept of health has been useful in enhancing the well-being of humans and animals individually and in populations, albeit imperfectly applied. It follows there is good reason to believe that the concept of health can be helpful in its wider application to ecosystems.

In veterinary medicine the concept of "herd health", while much talked about 30 years ago, has really only been put into wide practice more recently, once the various indicators of population health were identified, measured and incorporated into management systems. With the emergence of the concept of ecosystem health there is an element of déja vu.

Without going into detail here, it should be noted that there are critics who do not share optimism about the utility of applying notions of health to the environment. 17

Operational Goals

At levels of integration lower than health, specific goals can be classified as biophysical, economic, social, moral and aesthetic. Operational objectives can be defined for subcomponents of these categories. The first three of these categories potentially can be characterized by a specific set of measurable indicators appropriate to each scale under consideration. It is a big challenge to integrate among these five categories. Hence the emergence of concepts like sustainability, conservation and ecosystem health.

Ecosystem health holds promise of providing a framework upon which to integrate the relevant operational ecosystem goals and objectives, while making the appropriate compromises and trade-offs where necessary. Operational goals placed in such a coherent framework can serve as a map to guide society to a future with optimal well-being for people and maximal ecological integrity for ecosystems. This task remains to be carried out. It is noteworthy that human medicine also is in the early stages of a struggle to define a better framework for human health that can goes beyond illness-based medicine and incorporates determinants of health which have remained elusive.6
Indicators and Wildlife

The use of indicators in management has a rather long and extensive history in economics, social science and public health. The immediate challenge in the evolution of the ecosystem health paradigm is defining the appropriate indicators that can guide natural resource managers and decision- and policy makers in making informed choices.

Indicators are measurable attributes of ecosystems, correlated with the qualities of "health" which are used to monitor or manage the activities that lead to the attainment of operational objectives. In respect to each objective it is essential to specify indicators that apply at the appropriate scale, e.g. field, farm, community, watershed, ecoregion, etc. Indicators can relate to single objectives or they can integrate across several objectives within or among biophysical, social, moral and aesthetic dimensions. The overall assessment of the health of an ecosystem requires a judgement call based on an appropriate suite of indicators.

Indigenous flora and non-human fauna have a more intimate relationship with their immediate environment than do resident humans. They are not buffered by the effects of artificial shelter, water purification, and external food sources. It seems reasonable to posit that populations of healthy wildlife reflect healthy ecosystems and vice versa. The identification, selection and rational use of animals whose health makes them a useful indicator species would seem to be a particular challenge to this audience. There are various types of indicators involving wildlife, which may include population changes, lesions in tissues, biochemical alterations, behavioral changes, or toxicant levels.

I believe some of the best examples of the use of wildlife indicators can be found in the various programs arising from International Joint Commission initiatives directed at improving the health of the Great Lakes basin. The Great Lakes Water Quality Agreement commits the parties to an objective for the Lake Superior as follows: "...should be maintained as a balanced and stable oligotrophic ecosystem with lake trout as the top predator of a cold water community and Pontoporeia hoyi as a key organism in the food chain."

The Agreement also identifies "areas of concern" requiring action to remedy 14 use-specific impairments, that include things like "fish tumours and other deformities, degradation of fish and wildlife populations, bird or animal deformities or reproductive problems, loss of fish and wildlife habitat." Furthermore it calls for the development of early warning health indicators viz., "further development and use of reproductive, physiological and biochemical measures in wildlife, fish and humans as health effects indicators and the establishment of a data base for storage, retrieval and interpretation of data."

The occurrence of disease in fish-eating birds such as gulls and cormorants has been particularly useful in alerting the public to the degradation of the health of the Great Lakes basin. Impaired reproduction and deformities documented in these birds are indicators whose implications are easily understood by all, albeit their genesis is complex. It seems
reasonable to believe that such indicators help persuade the public to support remedial policies.

It would interesting to determine whether or not the increasing seriousness of avian cholera in waterfowl in North America is an indicator of impaired ecosystem health. The situation would seem to reflect increased population densities, at least in part caused by compression of habitat. Is this mortality a function of a salutary feed-back mechanism to control populations and insulate them from a greater catastrophe? In this case avian cholera paradoxically might reflect ecosystem health. Does this mortality portend pathological effects on other biota linked to the natural history of waterfowl species? Wetland provides other "ecological services", such as purification of water, and its loss can lead to widespread consequences. Is society trading wetland for other services or uses that have less value or unwanted dire consequences in the longer term? There needs to be some method of formally taking into account human values and conflicting goals in circumstances such as these. The application of the ecosystem health concept may lead to some practical means for society to make the necessary difficult decisions.

Some epidemiologists in the human medical community have concluded that growth in human population, coupled with the excesses of poverty and affluence, have put the carrying capacity of the earth at risk. Normal ecological mechanisms that give ecosystems resilience and the capacity for self-organization are being overwhelmed. For example, there is now strong evidence that the increase in UVB radiation brought on by thinning of the ozone layer is one of the reasons for significant declines in the populations of some species of frogs. Knowing the association of this form of radiation with skin tumours in humans, and the lag time in their clinical expression, the observation in frogs gives more immediate and graphic evidence in support of the seriousness of the situation. Recall the tragic consequences of not heeding early warning signs in animals (cats) that portended the Minamata Bay disaster in Japan associated with mercury pollution. It seems reasonable to suggest that the use of wildlife as indicators of ecosystem health may provide conclusive evidence to help motivate society to take action to correct dangerous situations before the harm is irreparable.

Conclusion

Human well-being depends on a natural world which can continue to sustain both the physical and the spiritual qualities of human existence. The task is bigger than science. Society must act to assure the long term well-being of both humans and their cohabitants on this earth by setting the necessary achievable goals or targets. Focusing on the health of ecosystems would seem to hold promise for success. There is urgency in setting societal goals for ecosystems because the options are decreasing rapidly.
LITERATURE CITED


Table 1. "Concept definitions" (attributes) of health* compared in relation to their applicability to individuals, populations and ecosystems

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Individual</th>
<th>Population</th>
<th>Ecosystem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homeostasis</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Absence of disease</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diversity or complexity</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stability or resilience</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Vigour or scope for growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Balance among system components</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Adapted from Constanza²

1995 JOINT CONFERENCE AAZV / WDA / AAWV
If we treated ecosystems as if they were patients, how would we go about diagnosing problems in the biosphere? This question was first put forward in the late 1980s by people like David Rapport and David Schaeffer. Shortly thereafter, Ole Nielsen was articulating a specific role for veterinarians within a new concept called ecosystem health. Nielsen suggested that veterinary interest in toxicology, epidemiology, and wildlife provides a logical framework for attacking problems in ecosystem health.

How might one apply such a new concept within the veterinary curriculum? The discipline, if one can call it that, is clearly in its infancy. Formulating a process for teaching such a new field presents many difficulties. Fortunately, we have received considerable help in developing such a process from the Max Bell Foundation in the form of a four-year development grant made to Bruce Hunter, a veterinary pathologist at the University of Guelph in Ontario.

Hunter gathered together interested faculty from the four Canadian veterinary colleges for a three-day curricular planning session in October of 1992. From that session a plan emerged to offer an elective two to three-week-long clinical rotation in ecosystem health within the final year of the regular veterinary curriculum. The rotation would be offered once a year and would rotate between the four Canadian colleges, with interested faculty at the "home" college taking primary responsibility for organizing the rotation. Enrollment would be open to applicants from all four colleges but limited to a maximum of 16 participants.

The first full rotation was held at the Ontario Veterinary College in Guelph, Ontario for three weeks in the fall of 1993. The rotation included, among many other events, presentations on basic ecology, a consideration of oil spills, a waste-management mini-symposium, impact assessments for a variety of environmental situations, a Great Lakes mini-symposium, a field investigation of disease outbreaks in wildlife, and field trips to the Canada Centre for Inland Waters and the Guelph landfill site.

The second rotation was held last fall for two weeks at the Western College of Veterinary Medicine in Saskatoon. This rotation consisted of introductory and summary sections sandwiched around an intensive nine day field trip through strategic sections of Saskatchewan and Alberta. The field trip took participants through feedlots; lakes; freshwater marshes; the erosional plateau and old-growth forest of the Cypress Hills; the heavy concentration of petroleum facilities, people, and cow-calf ranches in the Sundre valley; the montane forest of the Rocky Mountain foothills; and to the bison in Elk Island.
Park. This enabled the students to explore specific ecosystem problems, and to investigate the concepts of systems, sustainability, conservation, best use-of-land, animals-as-sentinels, disease investigation, biodiversity, evolution, and "ecology-as-change".

What have we learned about applying the concept of ecosystem health within the veterinary curriculum? Certainly, there is a lot more to learn during the next two years of this fascinating development project. However, I would like to put forward a few of my own observations about the introduction of such a rotation into the veterinary undergraduate curriculum, based upon our early experiences.

Making students "experience" environmental problems first-hand by getting them out of the classroom and into the field has probably contributed most significantly to the success of the rotation. We have also found it important not to offer this as some kind of general didactic course in "ecology", but to make sure that continued practical emphasis is placed upon the question "What do we, as veterinarians, have to offer to the fields of ecology and ecosystem health?"

To maintain a focus on this question we have identified environmental problems that could possibly benefit from the active presence of a veterinarian, and taken the students, whenever possible, to the area to pursue how we might get involved. These have developed into tremendous brainstorming exercises that may very well contribute to the development of the discipline. We have also identified veterinarians presently working with problems of "environmental health" and taken the students to them, so the students can visit and see "role models" who are actively involved in the field. This approach has opened the eyes of both students and faculty to some unique opportunities that either could or are being explored by veterinarians as "ecosystem health practitioners". Evaluating the usefulness of carcass recovery programs during outbreaks of avian botulism in marshes across North America, and developing the methodology of using animals as sentinels of human, "airshed", or ecosystem health are just two of the examples that have been pursued during the rotation.

I have found it useful at the beginning of the rotation to challenge the predominant worldview of what Ronald Bailey calls *apocalyptic environmentalism*.1 The students have generally been relying upon newspapers, magazines, and television news to learn about environmental problems. There is a tendency for students to accept the opinions presented in these media and to move directly to the question of how we should intervene in order to correct a certain purported environmental problem, without first applying the critical analysis that they would to more traditional veterinary subjects. Suggesting at the outset that students should demand evidence before deciding whether the new patient, the ecosystem, is unhealthy or requires assistance, has led to a more useful debate on environmental issues, and increased the chance that the students will recognize "golden" opportunities for veterinarians prepared to work in the field.

The positive feedback we have received from the students and faculty involved in the rotation suggests that there may be a place for considering the ecosystem as a "patient" within the veterinary curriculum. Growing demand for the rotation indicates that students
are prepared to devote a portion of their clinical year to exploring the opportunities that this new field presents. I am convinced that the longer-term success of the rotation will depend upon how well we can continue to focus on how veterinarians might make a living while working as environmental health consultants and practitioners.

ACKNOWLEDGEMENTS

The Ecosystem Health Elective offered among the four Canadian veterinary colleges (Ontario Veterinary College, University of Guelph; Western College of Veterinary Medicine, University of Saskatchewan; Faculté de médecine vétérinaire, Université de Montréal; Atlantic Veterinary College, University of Prince Edward Island) is supported by a generous grant from the Max Bell Foundation.

LITERATURE CITED

ENIRONMENTAL HEALTH IN AN INTERNATIONAL CONSERVATION PROGRAM

William B. Karesh, DVM,* Robert A. Cook, VMD
Wildlife Health Sciences, Wildlife Conservation Society, 185th and Southern Blvd. Bronx, New York, 10460 USA

Introduction

The Wildlife Conservation Society (WCS), founded in 1895 as the New York Zoological Society, has over 200 international conservation projects in 45 countries. The Society is also responsible for the management of the Bronx Zoo, Central Park Zoo, Flushing Meadows Zoo, Prospect Park Zoo, the New York Aquarium, and the Wildlife Survival Center on St. Catherines Island, Georgia. Over 8,000 threatened, rare or endangered wild animals are represented in the zoo and aquarium collections. Veterinary services for WCS fall under the responsibility of Wildlife Health Sciences, based at the Wildlife Health Center at the Bronx Zoo. This facility is a 20,000 square foot medical and research complex dedicated to the care of captive and free-ranging wildlife. Clinical medicine, pathology, nutrition, biotelemetry, and field veterinary services are geared to meet the needs of the WCS divisions including the various zoos, the aquarium, St. Catherines Island facilities, and the international programs. This combination of a large professional staff, experienced with a wide variety of species, coupled with ongoing overseas programs and staff allows the organization a unique opportunity to provide or supplement veterinary services and related skills where they are needed around the world.

The Field Veterinary Program (FVP) was established in 1989, to provide for and integrate veterinary services, training and research in WCS's international programs. Program goals are based on the needs identified by WCS's international efforts over the years, and include 1) providing veterinary services for conservation programs, 2) developing and applying new technologies, 3) addressing animal handling and welfare concerns, 4) crisis intervention, 5) training, 6) identifying critical health factors in wildlife populations, and 7) health monitoring of populations and environments. These last two areas of focus, are actually interrelated with the other five, and our approach to implementation is based on the recognized value of integration.

While overall organizational goals are aimed at protecting environments and habitats, research efforts have often taken an approach based on endangered or threatened species, populations, or communities. The FVP efforts follow this general rule and as such has focused on utilizing living animals in attempting to establish indicators of environmental health. While the approach described here can not provide some information as readily as harvested wildlife or direct environmental sampling such as soil or water, we feel there is a need to develop techniques to assess the health of vulnerable species or communities of species. Some of these species also migrate to poorly studied areas for parts of the year, or remain pelagic, thus limiting the possibility of monitoring their environment directly. Another theme in our efforts is that we generally work in places where little work has been conducted, and where local authorities do not have the financial or professional resources
commonly available in most developed countries. Several examples of project development serve to illustrate how we are incorporating health concerns into our international conservation programs.

**Zaire:**

Field veterinary involvement originated from Dr. Emil Dolensek’s work in Zaire in 1988, assisting Drs. John and Terese Hart with the immobilization of free-ranging okapi (*Okapia johnstoni*) for their ongoing ecological studies. The Harts were planning to begin a comprehensive, ecological study on duikers (*Cephalophus spp.*) which provided us with the opportunity to include health evaluations of the duikers as a part of the study. In this case, five species would be routinely handled for tagging and radio-collaring. Because duikers constitute 75% of the number of individual ungulates on a per area basis and approximately 67% of the ungulate biomass, they provide a good means of looking at the health of large segment of the biological community. Over a two year period, over two hundred animals were captured and released. We were able to collect blood and fecal samples and examine over 80 of these animals during three capture operations. Hematology, serum chemistries, vitamins and mineral levels, and infectious disease serology tests were performed on samples. Blood samples were also collected from domestic cattle in the area for infectious disease comparison, and serum has been banked for future studies or evaluations. This project was our first opportunity to incorporate a significant health monitoring or assessment component into a project from it’s inception.

Following our involvement in the Ituri forest, we were asked to provide veterinary services at Garamba National Park on the northeastern border of Zaire. Once again, the introduction to the area was based on clinical services, but has evolved to have a large monitoring component. Initial health surveys have started with kob (*Kobus kob alurae*), buffalo (*Syncerus caffer*), and elephants (*Loxodonta africana*). A relatively standard set of tests used in most of our projects is provided in Table 1.

**Tanzania:**

A confiscation in Europe of illegally shipped pancake tortoises (*Malacochersus tornieri*) resulted in their return to the most likely source country of Tanzania, with the hope of reintroduction. WCS staff were involved in helping with management decisions regarding the disposition of the animals. Biologists were trained for proper sample collection techniques to assess the health of the confiscated animals and also to include this type of assessment of free-ranging individuals as part of a population survey. From this work, the first baseline information on the health of this species in the wild was established. We also discovered that some of release candidates carried a *Mycoplasma spp.* which could not be found in any of their free-ranging conspecifics. The health evaluation of free-ranging animals also provided indirect evidence of dietary differences based on blood alpha- and gamma- tocopherol levels between populations which can help in guiding further ecological studies.
Peru/Argentina:

WCS has been involved with conservation projects in Peru and Argentina for many years. Long standing projects in both countries have focused on marine mammal and bird populations. Initial veterinary involvement began by responding to requests from field staff for medical supplies in 1991 and 1992. Our original field veterinary activities in Argentina were centered on assisting with the immobilization of South American sea lions (*Otaria byronia*) for the attachment of instrumentation (time-depth recorders and satellite transmitters). This provided the opportunity to meet individuals working in the region, evaluate the possibilities for expanded veterinary activities, and demonstrate some of the techniques we were interested in using in other species. During the first field season, we were able to examine and collect blood from Magellanic Penguins (*Spheniscus magellanicus*) at three geographically isolated colonies, Imperial Cormorants (*Phalacrocorax atriceps*) and Kelp Gulls (*Larus dominicanus*) at another, and from the sea lions and southern elephant seals (*Mirounga leonina*) being tagged by the field biologists. In 1994, work was expanded to include two species of penguin at 7 sites over 1000 miles of coast, more gulls and cormorants, guanaco (*Lama guanaco*) and domestic sheep (*Ovis aries*). The work from both years has been integrated into a project funded by the World Bank Global Environment Facility to develop a coastal management plan for Patagonia. This project is being coordinated and implemented by Fundacion Patagonia Natural, a conservation organization established by our key Argentinean field staff. The planning process is dependent upon obtaining and evaluating high quality biological information, and also on maintaining excellent lines of communication and collaboration between the variety of organizations and government agencies involved.

The coastal work in Peru has stemmed from providing assistance in immobilizing male South American fur seals (*Arctocephalus australis*) for ecological studies. Our involvement still includes this service, but has grown to include an annual health monitoring program for the largest colonies of fur seals and Humboldt Penguins (*Spheniscus humboldti*) in Peru. In both countries, field staff have been trained in necropsy techniques and can routinely collect samples for evaluation by WCS's Department of Pathology. Both the Argentinean and Peruvian projects provide us with an opportunity to monitor environmental changes through the evaluation of mammals and birds near the top of the food chain. Having access to high quality population biology data collected over the years at the same sites will also greatly enhance our ability to interpret findings.

Brazil/Bolivia/Ecuador

WCS has a great number of projects in the central forests of South America. Several of these projects are focused on the same or closely related species. We are currently initiating parallel health monitoring projects in this region, capitalizing on the current efforts of our field biology staff. In northern Brazil and in northwestern Bolivia, we are beginning a project to evaluate the health of white-lipped peccary (*Tayassu pecari*) populations. The disappearance of herds from established areas is not understood and we are integrating
health assessment studies into ongoing ecological research to help in elucidating possible causes. Concurrently, we have initiated health assessment studies on yacare caiman (Caiman sclerops yacare) and black caiman (Melanosucus nigra) in Bolivia and Brazil as indicator species for their aquatic environments. Both of the caiman projects have been initiated in remote, relatively pristine areas and should yield valuable baseline information. We have recently been asked to help in the development of a similar project in the Amazon basin side of Ecuador to assess the impact and/or potential impact of the petroleum industry on aquatic vertebrates. Other tropical South America health assessment or monitoring programs have included studies on free-ranging macaws (Ara spp.) in Peru and anacondas (Eunectes murinus) in Venezuela. Both of these were coordinated with WCS conservation projects.

Summary

It is still early in the development of this program and our approach to gaining additional insights into the health of ecosystems and environments. There are additional approaches that we have not yet developed such as more extensive collection of necropsy materials, or chemical analysis of soils and water. But the program has expanded in areas where we could maximize the information gained from established WCS projects and approaches. Our efforts have capitalized on current and developing conservation projects around the world to begin documenting and understanding the health of rarely studied wildlife populations and their environments. The potential for gaining access to a multitude of species and habitats is vast. A second advantage of our integration with ongoing conservation work is the inclusion of and access to biological data on the study populations or species conducted by our staff, counterparts, or collaborators. This helps to put health related findings into a more meaningful context. The involvement of individuals from non-health disciplines also distributes the “pride of ownership” of the results as opposed to creating different teams with different findings or agendas. Lastly, not even the best studies or most fascinating scientific discoveries will have any impact unless people who have the authority to make decisions are reached. WCS's long-term involvement with environmental agencies and local people around the world provides an exceptional opportunity to involve key players in our projects.

ACKNOWLEDGMENTS

We express our thanks to the many conservation professionals that have contributed to the productivity and success of this program. In particular, Dr. William Conway, General Director of the Wildlife Conservation Society for his initial vision and continued advice and support; Drs. P. Calle, E. Deirenfeld, T. McNamara, B. Raphael, M. Stetter, and the Wildlife Health Center technical and support staff for their extra efforts to make the program work; the field biologists around the world that have committed their time and efforts to advance our understanding of wildlife, and our foreign colleagues such as Drs. M. Atalia, A Hoogesteijn, M. Uhart, and M. Zalles.

LITERATURE CITED


Table 1: Standard test panels run on blood samples collected in Field Veterinary Program projects. Tests are added or eliminated to fit project specifics.

<table>
<thead>
<tr>
<th>Hematology</th>
<th>Serum chemistries</th>
<th>Vitamins</th>
</tr>
</thead>
<tbody>
<tr>
<td>hematocrit</td>
<td>inorganic phosphorus</td>
<td>alpha tocopherol</td>
</tr>
<tr>
<td>total solids</td>
<td>total protein</td>
<td>gamma tocopherol</td>
</tr>
<tr>
<td>complete blood count</td>
<td>albumin</td>
<td>retinol</td>
</tr>
<tr>
<td>WBC differential</td>
<td>globulin</td>
<td></td>
</tr>
<tr>
<td>hemoparasite exam</td>
<td>calcium</td>
<td>Minerals/metals</td>
</tr>
<tr>
<td></td>
<td>glucose</td>
<td>boron</td>
</tr>
</tbody>
</table>

**Toxicology**

<table>
<thead>
<tr>
<th></th>
<th>total CO₂</th>
<th>barium</th>
</tr>
</thead>
<tbody>
<tr>
<td>polychlorinated biphenyls</td>
<td>blood urea nitrogen</td>
<td>calcium</td>
</tr>
<tr>
<td>O, P'-DDD</td>
<td>uric acid</td>
<td>copper</td>
</tr>
<tr>
<td>O, P'-DDD</td>
<td>total bilirubin</td>
<td>cobalt</td>
</tr>
<tr>
<td>P, P'-DDE</td>
<td>alkaline phosphatase</td>
<td>iron</td>
</tr>
<tr>
<td>O, P'-DDT</td>
<td>lactate dehydrogenase</td>
<td>magnesium</td>
</tr>
<tr>
<td>P, P'-DDT</td>
<td>alanine aminotransferase</td>
<td>manganese</td>
</tr>
<tr>
<td>aldrin</td>
<td>aspartate transaminase</td>
<td>molybdenum</td>
</tr>
<tr>
<td>alpha-BHC</td>
<td>sodium</td>
<td>phosphorus</td>
</tr>
<tr>
<td>beta-BHC</td>
<td>potassium</td>
<td>zinc</td>
</tr>
<tr>
<td>dieldrin</td>
<td>chloride</td>
<td>aluminum</td>
</tr>
<tr>
<td>endrin</td>
<td>creatinine</td>
<td>sodium</td>
</tr>
<tr>
<td>heptachlor</td>
<td>cholesterol</td>
<td></td>
</tr>
<tr>
<td>heptachlor epoxide</td>
<td>amylase</td>
<td>Serology</td>
</tr>
<tr>
<td>lindane</td>
<td>iron</td>
<td>based on taxonomic group</td>
</tr>
</tbody>
</table>

1995 JOINT CONFERENCE AAZV / WDA / AAWV
WILDLIFE IN CHANGING AGROECOSYSTEM LANDSCAPES

Richard E. Warner

Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, Illinois 61801, USA

This paper describes similarities and differences in current approaches to ecosystem health and wildlife ecology, and how wildlife trends in recent decades reflect the declining health of agricultural environments of the Midwest. Further, emerging policy issues affecting agricultural settings are outlined, which provide compelling reasons why future efforts in wildlife ecology and ecosystem health should be better coordinated.

Any comprehensive measure of ecosystem health is logically, if not inextricably, tied to wild vertebrates and their habitats, and in a broader sense the ecological niches that various plants and animals occupy. Although wildlife ecology has tended to be a more narrowly focused discipline than ecosystem health, the two approaches are complimentary and, in fact, have much in common.

Ecosystem health tends be holistic, beginning with a broad assessment of the processes and patterns of environmental change and, where relevant, associating these processes with the well-being of wild vertebrates. Assessments are often made of the tolerable limits of environmental change for selected species. Thus, animal physiology and health in disturbed environments are important considerations. These approaches ideally identify testable hypotheses about factors operating at the population level. Such knowledge can directly benefit wildlife management and, for example, can be incorporated in predictive models that associate habitat changes with the demography and abundance of a species.

By contrast, wildlife ecology is a much older discipline. Knowledge in this area has typically unfolded beginning with specifics, and then moving to more general situations. For example, the study of natural history and autecology generally developed from the careful study of relatively few individuals. Founded in this manner, wildlife management has in recent decades been increasingly guided by concepts in population and community ecology based on field studies, and synthesized in highly quantitative algorithms. Habitat changes are often evaluated by population models that consider various probability functions related to birth, death, immigration, and emigration. These approaches help determine management interventions that will sustain a population in a defined area, perhaps at some target density. Unfortunately, highly quantitative and predictive population ecology tends to work best in hindsight (explaining what has happened, not what will happen), and over relatively coarse spatial scales. Many of these highly sophisticated analytical approaches are still limited by our vague knowledge of the mechanisms contributing to the likelihood that animals survive to reproduce, especially for environments where anthropomorphic disturbances abound. In short, the potentially critical physiological endpoints commonly included in ecosystem health approaches have not been studied carefully enough by wildlife managers; this is a potentially rich area of overlap for the two disciplines.
Nonetheless, wildlife populations provide a window from which to view the integrity, or health, of agricultural ecosystems. Using long-term data sets primarily from the Midwest (Illinois and surrounding states), several examples of wildlife endpoints as they reflect ecosystem health and habitat change are presented. In general, long-term wildlife studies in agricultural environments provide some important insights regarding: (1) major transitions in agricultural land use and technology, and the nature of environmental disturbances associated with farming that have profoundly affected wild vertebrates; (2) basic population- and community-level phenomena; (3) the appropriateness and/or limitations of various methods widely used to assess habitats and populations; (4) the promising potential of using physiological and other endpoints to monitor the impacts of environmental perturbations; and (5) the challenges of managing overabundant, threatened, and exploited species, often in the same region, inhabiting the same critical habitats.

There are several reasons why improving the conceptual rigor and/or overlap of systems approaches to wildlife ecology and ecosystem health are of pressing importance at present. For example, many of the critical ecological linkages between biotic and abiotic factors remain poorly understood in the constantly changing, inherently unstable agricultural environments. By most measures, the health—or ecological integrity—of agricultural environments has been declining for decades. Moreover, within our society there is increasing concern and disagreement over the magnitude of environmental problems associated with agriculture, the challenges of feeding a growing human population, and how limited federal resources can sustain agriculture and healthy ecosystems. To that end, wildlife ecology has pointed more to the problems associated with the production of food and fiber, rather than solutions. In fact, wildlife conservation alone has not been a compelling reason for society to address environmental problems associated with agriculture. Perhaps the broader perspective of ecosystem health will produce more impetus for addressing these problems.
Many salmon "stocks" (a term used in fisheries management that is roughly equivalent to "population") have declined and a significant, but unknown, number have been extirpated. Over 200 stocks are classified as "at risk." There is uncertainty over the historical number of stocks (perhaps a thousand or two), the status of individual stocks, and the causes of the decline, but the general conclusion is clear: there is a widespread salmon decline in the Pacific Northwest. California, Oregon, Washington, and Idaho represent the southern range of the species' geographic distribution and the North American location where the declines are most acute. In contrast, Alaska's salmon populations are thriving and supporting record catches. Further, the aquaculture industry can spawn and raise salmon, produce a quality product, and sell it at a moderate price. Ironically, in spite of the decline of salmon stocks in the Pacific Northwest, salmon have never been more abundant in the retail market because of supplies from aquaculture and Alaska.

The well-documented decline of the Pacific salmon has an interesting twist: no species of salmon is in danger of extinction, but many individual stocks are declining and some have become extinct. What action -- if any -- is warranted remains one of the most contentious political issues in the region. Some advocate that all salmon stocks in danger of extinction should be listed immediately as threatened or endangered, which will invoke the full force of the Endangered Species Act. An opposite view is that less disruptive approaches and decisions should be employed before the Endangered Species Act is used. Besides, the Act itself is a simplistic approach for addressing complex, ecologically-constrained public policy questions.

Economically, the consequences of listing any significant number of salmon stocks as either endangered or threatened would be massive and dwarf the impacts of listing the northern spotted owl. The geographic area would include the entire Pacific Northwest. Even though these dislocations would be great, some advocates assert that such actions are long overdue and are the only way left to save the remaining salmon stocks. After all, salmon have historically served as a cultural and natural icon for the region, as well as supporting a billion dollar industry, and ought to be preserved at any cost to society.

From a political perspective, the salmon debate has split Pacific Northwest Congressional delegations and parties, resulting in a highly polarized, partisan debate. There is agreement that restoration of salmon stocks, if undertaken in a serious way, will be expensive and socially, as well as economically disruptive. Some contend, and correctly so, that the hour is late and something drastic needs to be done now or many additional stocks will become extinct. But one opposing view holds that this position just reflects another example of environmental elitism. After all, there is little chance that any salmon species will be driven
to extinction in the foreseeable future, and hatcheries can produce salmon reliably and comparatively cheaply. The vast majority of people do not see any difference between salmon spawned in streams and those bred in hatcheries.

It is common to debate the salmon problem by focusing on public lands, especially Federally-controlled forest and range lands. What happens on forest and range lands is important, but it is the easiest part of the salmon problem to address. The more difficult -- and critical -- part of the debate deals with policies and decisions impacting agriculture, industry, electricity generation (including hydro, fossil fuel, and nuclear), national defense, urban development, transportation (including road, rail, air, and water), private property rights, community rights, the relative rights and role of local, state, and federal governments and Indian tribes, and policies on human reproduction, emigration, and immigration. Overriding all of the policy aspects of the salmon problem is the fact that over the past 100 to 150 centuries, the Pacific Northwest has changed from an uninhabited region to one supporting 13 million people, the majority of whom live in urban areas.

Viewed in broader terms, the salmon problem is a clash of fundamentally different values and priorities. One position argues that man has a moral obligation to preserve species. The reason we are in this policy conundrum with salmon is that we failed to make the right choices when they were easier to make. In short, man needs to adjust to and be part of the environment. Another view is that salmon are just one element of what man values. The future of salmon needs to be evaluated against what the alternative benefits are or might be. Balancing competing alternatives is the practical, realistic approach, not dogmatically locking into a restrictive, narrow policy position such as saving individual species at nearly any cost.

The rivers of the Pacific Northwest have been crucial to economic development. Rivers, especially the Columbia and its tributaries, were viewed as tremendous untapped resources that could be harnessed to support a strong, productive society. Electricity generation, agriculture, mining, flood control, water and land transportation, and urban development were all dependent on modifying these rivers. As one example of the importance of rivers in the Pacific Northwest, two-thirds of the electricity used in the region comes from dams in the Columbia River basin. However, one of the costs of this development was that salmon populations suffered mightily. The dams may block or delay adults on their spawning run. Young salmon migrating to the ocean may become disoriented by long reaches of slow-moving water, suffer mortality going through or over each dam, and be subject to voracious predators below some dams. However, the dams are only one factor. The Columbia River historically supported runs of 10 - 16 million salmon, but even before completion of the first mainstem dam in 1938, runs were reduced to 1.6 million, a drop of 84 - 90%.

The salmon's life history also causes serious problems for the survival of individual stocks. There are seven species of Pacific salmon and several species of sea run (anadromous) trout. Five of the seven species of salmon -- chinook, coho, chum, sockeye, and pink -- are found on both sides of the Pacific. Two, the masu and amago salmon, are only found...
on the Asian side. Of the sea run trout, steelhead is the most common and shares many of
the salmon's life history characteristics. Salmon die after spawning, while trout may not.
Salmon spawn in freshwater (rivers, streams, or lakes), spend various lengths of time in
freshwater, migrate to the ocean, and spend from one to several years at sea. Depending
on the species, salmon from the Pacific Northwest will move along the coast of North
America or make a major migration past the Aleutian Islands. Salmon return to their
stream of origin to spawn. Ocean conditions (especially El Nino events) have a major
influence on the size of a particular "year class," and can result in dramatic influences, both
positive and negative.

Salmon have always been important to people inhabiting the Pacific Northwest. The early
aboriginal immigrants developed societies dependent on the annual return of salmon. For
the past 3,000 - 5,000 years, there was a rough longterm equilibrium between salmon and
human populations. The number of salmon that could be harvested was limited by lack of
efficient fishing gear, limitations on the ability to preserve, store, and distribute the catch
on a large scale, and most important, a human population of a hundred thousand or so.
These conditions changed markedly in the nineteenth century. The early to mid part of the
century saw a major drop in human population due to exotic diseases. Starting in the mid
to late 1800s the population grew rapidly with major immigration from eastern North
America. The human population growth coincided with the advent of more powerful
harvest gear and the ability to preserve and distribute the catch in cans. The effect on
salmon stocks was massive and rapid. Within six or seven decades many stocks were
reduced below levels required to support fishing. Some were likely eliminated.

There were many other causes for the salmon decline beyond heavy fishing. Most of the
Pacific Northwest is arid and water is a valuable resource for irrigation. Water diversions
decimated many stocks. Timber resources are common, of very high commercial quality,
and extremely valuable, and the harvest of these resources had adverse effects on salmon
spawning and rearing. Floods historically have been common and devastating. Flood
control has been a societal priority for many years. Dams create fish passage problems, both
for returning spawners and migrating young fish, and has long been a challenge to fisheries
managers. Competition for salmon harvest is also severe. Recreational, commercial, and
Indian fishermen demand a share of a dwindling catch.

Some efforts to help improve salmon stocks may have actually accelerated declines. For
example, Pacific salmon can be easily spawned and raised under artificial conditions in
hatcheries. Starting in the late 1800s, when hatcheries first were used to help enhance
salmon stocks, attitudes have evolved from near universal support to widespread skepticism.
Many individuals are now openly hostile to the use of hatcheries; some contend that the
90 hatcheries releasing salmon into the Columbia River system actually worsen the condition
of naturally spawning salmon. Hatcheries may introduce diseases, compete with naturally
spawned fish, and decrease genetic diversity in the stock. Others regard this anti-hatchery
view as another example of environmental elitism; why should society pay for the costs of
maintaining wild salmon so a few, affluent individuals can fish for trophies.
Other actions have complicated the salmon situation. One especially troublesome problem is the introduction of non-native species. Non-native species, such as walleye, shad, brown trout, brook trout, smallmouth bass, and carp, have been widely distributed. As salmon stocks decline, other species move in to occupy different elements of their ecological niche. Once these exotic species are established, it is extremely difficult for salmon to reestablish viable stocks against such formidable competition and predation.

From a policy perspective, what, then, is the solution to the Pacific salmon problem? The answer to this seemingly simple question is crucial. On the simplest level, salmon stocks are declining and perhaps the elusive "public" might want to do something about this. As a public policy issue, the question is more correctly addressed as a choice among competing alternatives. But, couldn't the "problem" be equally formulated in terms of protecting agriculture? Or of maintaining the availability of inexpensive electricity? Even if we decide that the problem ought to be defined in "fish" terms, are we primarily interested in preserving all stocks, or just the most important stocks? From an evolutionary perspective, is it even possible to identify the most "important" individual stocks? Or, are we interested in maintaining relatively high stock levels so that they are fishable? Such questions are not unusual in public choice. A similar set of questions exists for policies on abortion, welfare, and disease management. Because these kinds of questions are so fundamental, yet so difficult to answer, it is often left to the province of "crats" (bureaucrats and technocrats) to implicitly answer them. When crats can't satisfactorily answer these questions, the courts will.

Nothing is free in policy analysis and the salmon issue is no exception. For every benefit, there is a cost. Costs, of course, are only partially measured in cash. Other, often more important costs, might be loss of personal freedom, civil rights, fishing rights, or property rights. Many of the options revolve around decisions about the relative importance of an individual's benefits and rights compared to societal benefits. Depending on one's values and political perspective, the good guys and the bad guys are very different people and institutions.

The political constraints for resolving the salmon issue have evolved over time. Classical natural resource management is divided into a set of decision variables (elements a manager might control such as harvest rates, habitat improvement, and supplemental stocking) and constraints (for example, species being driven to extinction without due legal process) (Lackey, 1979). What is treated as a constraint and what is treated as a decision variable can and does change over time.

*Health* is one of those amoeba-like words that changes to fit the surrounding conditions. It is also a word increasingly used to describe ecological resources or ecosystems, but in ways very different from that of an individual animal. At least in a general sense, there is societal consensus of whether an individual person, dog, or cow is healthy. However, when the concept is applied to ecological systems, there is an explicit assumption that there is some ecological condition or state that is desired or preferred. Using the human health analog, we prefer healthy individuals to sick individuals. The condition of salmon stocks is often described against the norm of a healthy stock. Further, the health of salmon stocks is often
offered as a valid surrogate for the health of ecosystems. Appealing as health might be, there are serious problems in transferring the concept of human health to ecological resources and ecosystems. The word "health" carries so much meaning in everyday life that it is difficult to use it as a descriptor of how close an ecosystem matches a desired state.

The role of science and scientists in defining ecosystem or ecological health is contentious. To categorize something as "healthy" requires an implicit determination of what the desired or preferred state is. For example, to say that a patch of land is ecologically healthy implies something good, something desired, something preferred to alternative states. However, that same patch of land might be a pristine forest, a highly productive dairy pasture, a fertile field of corn, or a bustling university campus. Which ecosystem is healthiest? In a similar light, why should we define the problem in the Northwest as a salmon problem? Does that mean that we have tacitly placed salmon ahead of the alternative policy elements? Why not define it as a problem of enhancing inexpensive urban housing, maintaining the availability of cheap food, or minimizing flood risk?

Using the health concept in ecosystem management may not help resolve the public choice; in fact, it might cloud the fundamental choices society must make. Health has moved into the political lexicon, along with words such as fairness, empowerment, reform, justice, etc., as a term with politically loaded meaning. After all, who is against any of these? It is only when these terms are defined specifically that the true policy differences are clear.

The salmon situation illustrates a class of ecological policy choices that will become increasingly common and has been described as socially violent. There are a number of general characteristics: (1) Complex -- There is an almost unlimited set of alternatives and tradeoffs to present to decision officials or the public; (2) Polarizing -- They tend to be extremely divisive because they represent a clash between competing values; (3) Winners and losers -- Some individuals and groups will enjoy positive effects, others will not, and this tradeoff is well known to the general public; (4) Delayed effects -- There is no immediate "fix" and the benefits, if any, of painful decisions are not obvious for many years; (5) Decision problems -- These are not the kind of problems that a democracy smoothly addresses because it is very easy for advocates to appeal to strongly held values; (6) The role of science is unclear -- Scientific information is important but usually not pivotal in the choice of an alternative when the choice is primarily driven by value judgements.

It is easy to sink into the mire and conclude that it is impossible to make a choice -- gridlock. The fact is, choices are being made. They may not be the best choices, best being defined as the desires of the majority and the choice being without unexpected consequences, but choices are being made. Democracy may not be efficient but history and recent world events shows the alternatives to be worse.

Informed public choice is crucial. It is not easy for the public, or anyone, to deal with the technical complexity of challenges like salmon and similar, complex environmental policy problems. One critical role of scientists and other technical people is to provide the scientific information in ways that help create an informed citizenry, but without the
advocacy of any particular political or policy choice. This is not a comfortable role for many scientists who hold strong personal views on policy choices.

Finally, those of us who are technocrats, scientists, biological resource managers, and scientific advisors should remain humble in our dealings with the public and elected officials and overcome the tendency to advocate political choices driven by strong personal interest in the resources we work with. However, it is equally important not to permit tough policy choices to masquerade in the cloak of a scientific imperative — this is a prostitution of science and scientists that provides a convenient cover for avoiding difficult social choices. The complete implications of each alternative public choice should be fully and clearly explained, including the short and long term dimensions. Great care must be exercised to not abuse our positions as advisors and councilors.

LITERATURE CITED

DEFINING AND CREATING HEALTHY WETLAND ECOSYSTEMS

Bruce Wojcik, BS
Michigan United Conservation Club, PO Box 30235, Lansing, Michigan 48909, USA

"Wetland" is a term we encounter frequently. We hear or see it in the daily news, but do we really understand the meaning of wetlands? Many people do not know what wetlands are or what they do for us.

The official definition used by governmental agencies such as the US Fish and Wildlife Service, Environmental Protection Agency, and Soil Conservation Service states wetlands are - "areas having a predominance of hydric soils that are inundated or saturated by surface or ground water for a period of time sufficient to support a community of hydrophytic plants." Regional differences in conditions result in a wide variety of wetland types.

The wetland areas in the conterminous United States has declined by over 50% since times of colonization. The strain put on the reduced wetland areas can and is resulting in a loss of wetland benefits. Increased pollution and run-off compounds contamination loads in the wetlands. This poses a serious threat to the flora and fauna of these regions.

The health and conservation of the wetlands is crucial to a balanced ecosystem. Recognizing the benefits of wetland and identifying the risks to this unique habitat is the scope of this paper.
As local, regional and global changes in the environment attributable to human impact have become increasingly significant, there has been more attention paid to that marriage of ecology and medicine: ecosystem health. In this effort, both biological and non-biological indicators are used in the effort to detect and quantify environmental change. Scientists, government agencies and the public are concerned with monitoring populations of plants and animals for several purposes: because the species are important in their own right, because these organisms can be useful measures of the state of the environment from which they come, and because they may serve as sentinels to detect threats to human health.

In our presentation we will focus on how important veterinary input can be in environmental protection efforts and the need for veterinarians to develop working relationships with other groups of environmental professionals. We will also discuss the variety of taxonomic groups that can be used to detect and monitor various environmental issues. Some of the points to be considered in choosing appropriate species include: 1. how abundant and widely distributed is the species? 2. how mobile are individuals and populations? 3. how easily can the species be identified, aged and sexed? 4. is the species of scientific, economic or esthetic interest? 5. are the organisms or the samples obtained from them of appropriate size for the projected use? 6. the nature of the environmental problem being investigated.

References: Boudou, A. and RF. Ribeyre. 1989. Aquatic ecotoxicology. (vols. 1&2) CRC Press. Furness, RW and JJD Greenwood. 1993. Birds as monitors of environmental change. Chapman & Hall. -- routes of exposure, excretion -- subclinical effects: genetic, immunol, terratogenic, carcino -- results from single measurements may reflect recent exposure or past exposure (blood & urine usually reflect recent) -- importance of choosing indicator tissues (hair, feathers, blood, urine -- alkyl Hg: lipid soluble...slower excretion, primarily a neuro tox -- Hg Cl renal tox -- enzyme bioindicators: heme enzymes for Pb, renal tubular dysfunction for Cd, neuro for Hg

An Indicator Species Should Be: 1. abundant and widely distributed 2. sedentary 3. easy to identify 4. be of scientific, economic or esthetic interest 5. convenient size for projected use

USE OF BALD EAGLES AS INDICATORS OF ECOSYSTEM HEALTH IN THE GREAT LAKES

James G. Sikarskie, DVM, MS
College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824, USA

William W. Bowerman, MS, PhD
Eagle Environmental, Inc., 6154 Columbia Street, Haslett, MI 48840, USA

Julia E. Stickle, DVM, PhD
College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824, USA

John P. Giesy, MS, PhD
Department of Fisheries and Wildlife, Pesticide Research Center, Institute for Environmental Toxicology, Michigan State University, East Lansing, MI 48824, USA

David A. Best, MS, PhD
U.S. Fish and Wildlife Service, East Lansing Field Office, 1405 S. Harrison Road, East Lansing, MI 48823, USA

The Great Lakes Basin is one of the world's largest freshwater wetland ecosystems. The economy of the area is based primarily on industry, agriculture and tourism. The somewhat closed system results in industry and agriculture contaminating the valuable natural resources that tourism depends on. The bald eagle (Haliaeetus leucocephalus) is used to assess the quality of the environment in Michigan and the Great Lakes Basin. Most eagles hatched in Michigan and adjacent areas over the last 25-30 years have been banded as nestlings as part of a population productivity study. During the 1960s, bald eagle populations reached an all time low due to the eagle's position at the top of an aquatic food chain that was contaminated with organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs). The eagle's sensitivity to these chlorinated hydrocarbons and an ongoing program of access to young birds in the nest for banding purposes made the eagle an excellent candidate for monitoring the recovery of the Great Lakes following strict protection of eagles and banning of these persistent chemicals in the early 1970s. Measurements of size in relation to age as determined by feather eruption allows researchers to determine sex of most nestlings in the field because females are consistently larger than males as in most birds of prey. All the important information needed to use an animal as an indicator of environmental quality was being gathered. Age, sex, exact location, numbers hatched and fledged and often numbers of eggs layed was information already being gathered with good data going back earlier but used for 1977-1993 for this study. Having the birds in hand for banding and measurements for the productivity research would allow easy collection of blood for genetics study and analysis for OC contaminants plus removal of a few feathers for elemental analysis. The presence and level of contaminants in these young, growing birds should reflect the level of contamination in the food chain in the area of the nest.
A cooperative study was initiated to evaluate effects of nest location and differences in source of prey as to amount of contaminants and their effects on nesting success and productivity. The study area contained ten subpopulations of bald eagles. Four subpopulations were considered to have "coastal" Great Lakes food sources with nests within 8.0 km of the shoreline or rivers to which anadromous fish from the lakes had access. Six of the subpopulations were considered to have "interior" nest sites greater than 8.0 km from the shoreline and not along anadromous fish accessible areas.

Sterile techniques were used to collect blood from the brachialus vein with heparinized glass syringes from 309 nestling bald eagles in Michigan, Minnesota, Ohio, Ontario, and Wisconsin between 1987 and 1992. Samples were kept cool in the field and spun down with plasma frozen within 48 hours of collection. PCBs and OCs were recovered from samples by lipid solvent extraction, concentration and column separation with concentrations determined by gas chromatography with confirmation of pooled samples by mass spectrometry. Individual PCB congener concentrations were determined from relative response factors calculated by comparing to an internal standard and a known calibration mixture. Total concentrations of PCBs were determined by summing individual masses of the congeners. DDT (P, p'-DDT and its metabolites p,p'-DDD and p,p'DDE), hexachlorobenzene (HCB), heptachlor epoxide, cis-nonachlor, oxychlordane, dieldrin, polybrominated biphenyl (PBB), toxaphene, mirex, alpha-chlordane and gamma-chlordane were identified by reference to the relative retention time of p,p'-DDE x 100 and quantified by comparison to authentic standards.

Concentrations of p,p'-DDE and PCBs were converted to geometric means for statistical analyses. Relationships between geometric mean concentrations of PCBs and p,p'-DDE and means of annual productivities or success rates or overall productivity or success rate for the 10 subpopulations were determined using general linear models for regression analysis. Concentrations varied among subpopulations with mean concentrations of both PCBs and p,p'-DDE being significantly greater in birds from nests in areas with access to Great Lakes coastal food sources when compared to concentrations from birds from interior nest sites. All productivity measurements including mean annual productivity, mean annual success rate, overall productivity, and overall success rate were significantly and inversely correlated with geometric mean concentrations of p,p'-DDE and PCBs. Concentrations also increased with age, and there were no significant differences due to sex of the nestlings.

Determination of values in PCBs and OCs was very expensive and time consuming, often taking months to years to get results analyzed. An attempt to evaluate hematology and biochemical constituents of blood from nesting bald eagles for biological markers that might give a quick and less expensive indication of presence and effects of contaminants was undertaken in 1993. Blood samples were collected from 55 nestling bald eagles in the lower peninsula of Michigan during May and June while they were being handled for banding and collection of toxicological samples. Nineteen of the nestlings were from coastal nest sites and 36 were from interior areas. Slides for white cell counts were made from fresh blood in the field or samples collected in EDTA tubes to prevent clotting until slides could be
made later. Most hematologic and biochemical evaluations were performed within 12 hrs of sample collection, but some biochemical evaluations were performed on frozen serum up to 21 days after collection. Syringes were flushed with heparinized saline prior to drawing blood to prevent clotting while drawing the sample, but the amount of heparin in the 10 cc syringes was small enough that samples clotted so the analyses were performed on serum rather than plasma. All analyses were done at the Clinical Pathology Laboratory at Michigan State University using standard methods and included: Calcium, Phosphorus, Glucose, Uric Acid, Cholesterol, Sodium, Potassium, Chloride, Aspartate Aminotransferase (AST), Total Bilirubin, Alkaline Phosphatase, Alanine Aminotransferase (ALT), Amylase, Creatine Kinase (CK), Magnesium, Total CO₂, Anion Gap, Sorbitol Dehydrogenase, Total Protein, Albumin, Globulin, Osmolarity, Urea Nitrogen, Serum Iron, and potential indicators of immune suppression thyroid hormones (T₃ and T₄), plus Vitamin A. The same clinical pathologist read all leucocyte differential counts to eliminate any bias from misidentification of cell type or technique. No correlation of any hematological or biochemical values was noted when geometric means of these values were compared to geometric mean concentration for PCBs and p,p'-DDE. There was a significant difference observed in leukocyte differential counts comparing counts done on slides made in the field with fresh blood to those made with blood collected in EDTA tubes. A small study was done on 4 captive juvenile bald eagles taken from nests and housed at the Michigan State University Wildlife Rehabilitation due to birth defects (cross bills and clubbed feet) suspected to be caused by exposure to PCBs. The study showed differences in leukocyte differential counts between slides made from fresh blood and slides made from blood treated with EDTA and stored chilled for 1h, 24h, 48h and 72h post-venipuncture. White counts done on the same bird consistently showed a decrease in the numbers of leukocytes on samples treated with EDTA. The leukocyte count was performed manually using the eosinophil unopette (Becton-Dickinson, Rutherford, New Jersey 07070, USA) technique.

The differentials done on slides prepared using a cover glass method showed a decrease in percentage of lymphocytes with an increase in numbers of cells that couldn't be identified. The sequential evaluations showed that the decreased white count and change in lymphocyte proportions occurred very rapidly (less than 1 hour) and wasn't affected significantly by increased storage time up to 3 days.

This study presents data that justify continued use of the American bald eagle as a monitor of ecosystem health in the Great Lakes Basin. Chlorinated hydrocarbon testing on blood samples drawn from nestling eagles while taking measurements and banding for ongoing productivity studies helps explain the cause of poor nesting success in some areas. The fate of persistent chemicals like PCBs and the metabolites of DDT can be monitored by using a sensitive indicator at the top of the food chain. Although the bald eagle population is recovering in most of its range, continued monitoring in more sensitive areas is justified. The increased numbers of eagles and expansion of nesting ranges increases their utility as an indicator of ecosystem health while showing progress from past regulations and encouraging continued surveillance.

32 1995 PROCEEDINGS JOINT CONFERENCE AAZV / WDA / AAWV
THE EVERGLADES: WHAT CAN INDICATOR SPECIES TELL US ABOUT ECOSYSTEM HEALTH?

Marilyn G. Spalding, DVM*
Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611, USA

Peter C. Frederick, PhD
Wildlife Ecology and Conservation, University of Florida, Gainesville, FL 32611, USA

The Everglades ecosystem of south Florida has been severely impacted during the past century by drainage and compartmentalization of surface waters, reduction of freshwater flows to the coastal estuary, introduction of exotic animals and vegetation, and contamination with nutrients and heavy metals. The cumulative effects of these changes has proved difficult to measure because the characteristics and functioning of the pre-drainage ecosystem was poorly described, because change has been gradual, and because the physical characteristics of the ecosystem are extremely variable from year to year. Populations of breeding wading birds in the Everglades (order Ciconiiformes, herons, egrets, ibises, storks and spoonbills) have been a focus of monitoring interest for many years, and some reports go back to the 1880's. Following a rapid rebound from plume hunting, breeding populations of many species have declined by over 90% since 1930.

This unusually rich mosaic of historical information on bird populations, coupled with a modern understanding of wading bird responses to ecological variability, has led directly to a detailed reconstruction of how the ecosystem once functioned. For instance, a major shift of colony locations away from the coastal region between the 1950's and the 1980's suggested that the estuarine area had become degraded in some fashion. This also implied that secondary productivity had declined in the estuary, a conclusion confirmed much later in commercial fishery declines. Research also has shown that salt tolerance is quite low in nestling birds, suggesting that the birds also abandoned the estuary because it had become saltier. The increase in salinity was then confirmed independently, both through banding patterns of long-lived corals, and by simulation modeling of changes in water behavior in response to diking and canalization. In each case, the breeding responses of birds were the first suggestion of problems in ecosystem function, all of which were difficult to otherwise discern in the noise of a hypervariable physical environment.

Historical records and databases have been essential to the use of birds as bioindicators in the Everglades, and modern data alone would present an extremely misleading picture of ecosystem function. Successful breeding years now occur only during conditions that lead to rapid drying of the marsh and subsequent entrapment of prey animals. Yet careful analysis of historical accounts suggests that in the pre-drainage Everglades, successful breeding occurred in both wet and dry conditions. Concurrently, fisheries biologists have shown that populations of prey fishes and invertebrates are greatly reduced wherever the marsh has been over-drained. Over time, over-drainage may have caused a general reduction in prey populations, making wading bird breeding dependent upon mechanisms such as drying in order to sufficiently concentrate the few remaining prey. The apparent

1995 JOINT CONFERENCE AAZV / WDA / AAWV
needs of birds breeding in the currently degraded marsh would suggest annual drying as a priority management strategy. Yet the full picture in the context of historical information indicates exactly the opposite as a restoration strategy.

Detailed monitoring studies of avian health in the Everglades have also turned up several unexpected problems that have effects felt at the population and ecosystem level. Infection of breeding wading birds with the nematode parasite *Eustrongylides ignotus* has proved to be a major source of breeding failure in recent years, and infected fish have been shown to be found in nutrient-polluted water. Historical comparisons show the parasite has become much more common than in the past, perhaps due to the use of large quantities of agricultural phosphorus and sewage wastewater. Wading birds have also shown a sharp increase in mercury contamination since the 1970’s, and in combination with surveys of other predators, have helped established the major geographic foci of contamination.

The wading bird guild has also provided evidence that choice of species is important in the use of indicator species. Great Egrets, for instance, breed successfully in nearly all years in the Everglades and have not proved to be successful weathervanes of hydrological change. In contrast, White Ibises (*Eudocimus albus*) are extremely sensitive both to water depth between years, and to sudden interruptions in drying within breeding seasons. In contrast, the Great Egret is the obvious choice of indicators of mercury contamination because of its elevated position in the food chain compared to White Ibis. Choice of contaminant accumulators may not be easy - although alligators and panthers also represent top carnivores in the Everglades wetlands, it has taken a detailed simulation model to show that their accumulation potential is lower than wading birds. This is because fish comprise a larger proportion of the diet in wading birds, and wading birds consume fish at a greater rate due to a higher metabolic rate.

Choice of indicator species is often confounded by protected status. Many of the first-pick indicator species in any ecosystem are highly sensitive to environmental change, often so much so that they are rare or endangered. The protected status usually requires that closely related species be studied, or that indirect approaches are taken to answer pertinent questions. This increasingly common problem can result in a difficult choice for researchers, as misleading conclusions result from study of a species that is clearly not responding to change by becoming rare.

Interdisciplinary collaborative work is absolutely essential in the use of any species for ecosystem health studies. In the case of eustrongylidosis in wading birds, the importance of the disease went unnoticed by field ornithologists who could not recognize the parasite as a cause of death. The unraveling of the entire Eustrongylides story will require input from fisheries biologists, soil and water chemists, invertebrate zoologists, pathologists, epidemiologists, and ornithologists. Similarly, piecing together the story of coastal degradation has required the input of hydrologists, ecologists, historians, computer modelers, and marine biologists.
The Everglades experience has demonstrated the profound impact of historical data on the interpretation of current problems and patterns. However, it is rare that quality historical information is available. In most ecosystems, "baseline data" is represented by only a handful of years effort, often recently collected. More often than not, studies tend to be short term, directed toward specific questions, and allow little opportunity for comparisons between years and between locations. In general, it is useful to ask whether such data may or should be used as baseline information. For example, wading bird reproductive parameters such as clutch and brood size were until recently assumed to be indicative of wading bird feeding opportunities in the Everglades. Yet a five-year summary of this information reveals that these parameters change little in response to even drastic environmental change. Opportunities for the collection of historical data exist and should always be taken. Examples in the Everglades include mercury analysis of bird feathers in museums, parasite prevalences in spirit collections, and peat core sampling for detecting mercury and phosphorus histories.

In conclusion, indicator species such as wading birds have revealed a great deal about ecosystem processes and restoration in the Everglades that was not obtainable in any other way. However, it should be remembered that the successful use of indicator species in this case is almost completely attributable to the availability of historical information, the monitoring of a large number of species for different purposes, careful choice of indicator, and the collaboration of disciplines.
HEALTH PROBLEMS IN WETLANDS IN EUROPE

Torsten Mörner*, Dolores Gavier-Widen and Thomas Jagas
Division of Wildlife, National Veterinary Institute, PO Box 7073, S-750 07 Uppsala, Sweden

The major breeding grounds for waterfowl in Europe are found in the arctic and subarctic in Iceland, Scandinavia, Finland and Russia. Two main migrating routes exists. The first one follows along the Atlantic coast and the other passes through eastern Europe. Major winter grounds are found in southern scandinavia, along the British Isles, along the Atlantic coast in the Mediterranean Sea and in fresh water wetlands in eastern and southern Europe. Most important wetlands in Europe are listed on the Convention on Wetlands (CW-list), also called the Ramsar Convention. In 1989 twenty-two European countries were contracted to the convention, covering around 26,00 hectares. Two other conventions - the Bern Convention, concerning protection of animal and plat species and hunting methods in Europe, and the Bonn Convention, concerning migrating birds and mammals, also have an impact on the status on wetlands and waterfowl.

The status of the winter habitat are for many waterfowl species, very important and changes in some habitats in south-eastern Europe and western Asia have had a negative impact on many species and especially on the populations of lesser white-fronted goose (Anser erythropus). Because of this, migrating routes and wintering grounds for this goose species has been changed by raising Lesser white-fronted goosling by foster parents of Barnacle goose (Branta bernicla) and by that chaning the winter area from the Black Sea to the Atlantic cost of Europe.

Massive die-offs of waterfowl, as reported from North America seldomly occurs in Europe. Viruses causing Duck Plague, Avain Influenza and Newcastle Disease are occasionally isolated in Europe, but does not seem to be a great factor causing massive disease among waterfowl. Outbreaks of Avain cholera are not reported from Europe along Pasteurella Multocida occasionally is isolated from waterfowl. Salmonellosis causes high mortality in areas with high density of waterfowl in some parts of Europe.

The major disease problems in wetlands in Europe includes lead poisoning and botulism. Lead shot densities are rather high in many parts of Europe and lead poisoning among waterfowl is historically outbreaks with high mortality in waterfowl have occurred. Mortality in waterfowl due to botulism is found regularly in most countries and wetlands in Europe, but does not seem to be such a big problem in North America.
BOTULISM IN FREE-RANGING AND CAPTIVE WILDLIFE: OCCURRENCE, DIAGNOSIS AND CONTROL

Tonie E. Rocke, PhD*
National Biologic Service, National Wildlife Health Center, 6006 Schroeder Rd. Madison, WI 53711, USA

Since the early 1900's, avian botulism has been our most severe disease problem in wild birds in the U.S., killing tens to hundreds of thousands of waterbirds every year. Although the disease was originally considered a "western duck sickness" because most outbreaks were reported from marshes in western North America, both the distribution and frequency of botulism outbreaks have increased. Today, botulism outbreaks are reported in waterbirds throughout the U.S., including Hawaii, and with increasing frequency in zoos, urban settings and other wildfowl collections. The disease has been diagnosed in wild birds on every continent with the exception of Antarctica, and in at least 17 countries.

Botulism is caused by exposure to neurotoxins produced by a heterogenous group of anaerobic, spore-forming bacteria classified as Clostridium botulinum. At least 7 different neurotoxins are produced by strains of C. botulinum, denoted types A - G. Most outbreaks of botulism in wild birds are caused by strains which produce type C toxin. Production of type C neurotoxin depends on infection of C. botulinum with specific bacteriophages (TOX+). DNA hybridization analysis has shown that the structural gene for type C neurotoxin is located on the genome of TOX+ bacteriophages.

In-vivo, neurotoxin exerts its paralytic effects by blocking release of the neurotransmitter acetylcholine in a 3-step process. First the toxin binds rapidly and irreversibly to the presynaptic cell membrane, then the bound toxin penetrates the cell membrane and enters the cell. Finally the intracellular toxin disables the mechanism for acetylcholine release. Recent work has shown that once inside the cell, the toxin acts enzymatically, cleaving synaptobrevin, a protein located in the membrane of synaptic vesicles which is apparently critical for the release of acetylcholine.

Birds contract botulism by ingesting toxin-laden food items (food poisoning) or through "gut toxigenesis" when spores of C. botulinum carried in the gut or other tissues germinate and produce toxin. Food poisoning is the most likely route of exposure in birds, and there may be several sources of toxic food items, including decaying organic matter, free-living invertebrates that have ingested toxin, decaying invertebrate carcasses, and scavenging invertebrates (fly larvae) which ingested toxin produced in decaying vertebrate carcasses. The diagnosis of botulism in sick birds is based on clinical signs and the demonstration of botulism toxin in the bloodstream. Determination of botulism as the cause of death in carcasses also depends on demonstration of toxin usually in heart blood or tissues, but can be confounded by postmortem toxin formation. The in-vivo mouse neutralization test is most commonly used for the detection of botulism toxins. However, recently, a simple, in-vitro ELISA was developed for type C botulism toxin which can replace the mouse test for some applications.
Attempts have been made to vaccinate birds at several zoos in order to prevent botulism outbreaks in valuable collections, however, the vaccines commercially available for type C toxin have not been tested in birds and their efficacy is questionable. Other practices which could reduce the risk of botulism outbreaks in captive wildlife include improvements in the construction of impoundments to increase water flow and facilitate cleaning, alteration of feeding practices to reduce protein loads and organic buildup in impoundments, and careful carcass surveillance.

Prevention of botulism in free-flying waterfowl is more problematic. Mass immunization of wild waterfowl is not feasible at this time and wetland characteristics which precipitate botulism outbreaks have been difficult to define. However, ongoing studies designed to identify environmental conditions which increase and decrease the risk of botulism outbreaks in wetlands may offer management options in the future. Once an outbreak begins in either free-flying or captive birds, carcass pickup is a critical factor in controlling the magnitude of losses. At locations where water control is feasible, attempts have been made to control botulism outbreaks by draining affected wetlands, thereby discouraging bird use, or flushing them with freshwater to dilute the source of toxin. Unfortunately, no systematic attempts have been made to evaluate the effective impact of these strategies.
EFFECTS OF AGRICULTURAL, INDUSTRIAL, AND MUNICIPAL POLLUTANTS ON WETLANDS AND WILDLIFE HEALTH

Kathryn A. Converse, PhD
National Biological Service, National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711, USA

Wetlands accumulate pollutants from adjacent areas through intentional discharge of sewage or industrial wastes, runoff of agricultural fertilizers and pesticides, and discharge from municipal storm drains. Coastal wetlands receive more pollutants indirectly as the endpoint for upland drainage systems and directly through petroleum spills and insect abatement. Wetlands that serve as evaporation basins during seasonally high water, especially in more arid climates, concentrate natural compounds and as well as pollutants. The ability of wetlands to be effective filtration systems for wastewater nutrients through microbial transformations, uptake by plants, and deposition of particulate matter, and the shortage of water in arid climates has resulted in revision of wetland regulations. Wetlands can now be developed for wastewater treatment and natural wetlands can be restored or converted to wastewater treatment systems. The effect of these accumulating pollutants on wetland ecology and wildlife health needs to be recognized.

Inadequate understanding of wetland processes can lead to alteration of the hydroperiod and hydrology by conversion to constant rather than naturally fluctuating water levels. The changes in wetland hydrology along with the addition of pollutants can alter aerobic and anaerobic processes in the sediment, convert plant growth, and change food web structure. Modifications in the vegetation community may affect the attractiveness of the wetland to wildlife. Incoming and accumulated pollutants provide a source of pathogens and toxic chemicals that pose a threat to wildlife health.

Inadequate understanding of the risks to wildlife using polluted wetlands can result in their exposure to bacterial, viral and parasitic pathogens, organic chemical compounds, heavy metals and pesticides. Bacteria, viruses and parasites, primarily from animal feces, can cause disease directly. Because microbial transformations are unpredictable, pollutants may be transformed to more toxic forms, the breakdown of natural compounds may be inhibited and compounds may accumulate in detritus. Wildlife ingest detritus feeding organisms that accumulate contaminants at levels higher than the level in the water. In addition to direct toxic effects, concern exists that contaminants may affect immune function, increasing susceptibility of wildlife to infectious disease.

Greater understanding of the dynamics of wetland ecology and risks to wildlife health is needed to promote wise and effective use of wetlands as natural treatment systems. Recognition of the pollutants present in wetlands needs to be supplemented by research to determine their impact on wildlife.
OIL POLLUTION IN THE AQUATIC ENVIRONMENT: EFFECTS ON BIRDS AND MAMMALS

Frederick A. Leighton DVM, PhD
Canadian Cooperative Wildlife Health Centre, Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, SK S7N 5B4 Canada

While there are many oils derived from petroleum, those most often spilled in large quantity into the aquatic environment are crude petroleum and the major distillates used as fuels: fuel oils of various grades, gasoline, diesel fuel, jet fuel and kerosine. The chemical composition of crude oils and the distillates derived from them varies over a wide range. This greatly complicates assessment of petroleum oils as toxic substances. Petroleum oils may enter the aquatic environment as point-source spills and as constant discharges of lower concentration in terrestrial (urban, industrial) run-off. Very little is known about the effects of this latter form of oil pollution. Since most oil is transported in large quantity by ship and petrochemical complexes also usually are located on shorelines, coastlines of navigable waters are most at risk of oil spills. Once spilled into the environment, oil undergoes changes in chemical composition and physical characteristics collectively referred to as weathering. Average residence time (the duration of the weathering process) for oil in the aquatic environment is approximately one year but this is highly variable. The biological impact of spilled oil depends as much on location, date and local conditions as on the absolute amount of oil spilled; some rather small spills have had large impacts and vice versa. There are three different direct, negative effects that oil can have on birds. External contamination disrupts the normal physical properties of feathers, reducing buoyancy, waterproofing, insulation and flight. Death from hypothermia and starvation are a common result. Contamination of the shell of eggs during incubation can lead to high rates of mortality in embryos. Ingestion of oil by birds attempting to clean their feathers by preening also can result in systemic poisoning; ingestion of oil has been shown to invoke stress, to reduce reproduction and to damage red blood cells, and experimental evidence suggests additional toxic effects for some oils. Aquatic mammals that rely on pelage for insulation and waterproofing are the most vulnerable to external contamination with oil; these include otters, mink, fur seals, polar bears, muskrats, and beavers. As with birds, combinations of hypothermia and starvation can cause death in these species. Mortality associated with oil exposure has been observed in harbor seals but, in general, the impact of oil pollution on pinnipeds and cetaceans that use blubber for insulation appears to be much less than on fur-dependent species. Observations made subsequent to the Exxon Valdez oil spill indicate that systemic toxicity also may occur in mammals exposed to oil. Oil can also affect birds and mammals indirectly through habitat alteration and changes in food supply. Few data are available to directly assess such impact; but there are abundant data on alterations in marine habitat such that reasonable inferences may be drawn. Equally, data on long-term effects of oil pollution on populations of mammals and birds are not available; it will remain very difficult to separate the effects of oil at the population level from those of other variables such as food supply, weather and other pollutants. The estimated mortalities of common murres, harbor seals and sea otters associated with the Exxon Valdez spill suggest significant effects on local and regional populations of these species.
REPRODUCTIVE EFFECTS OF PETROLEUM PRODUCT EXPOSURE ON AMERICAN MINK (*Mustela vison*) AS A LABORATORY MODEL FOR SEA OTTERS (*Enhydra lutris*)

Jonna AK Mazet, DVM, MPVM*, David Jessup, DVM, MPVM, ACZM
California Department of Fish and Game, office of Oil Spill Prevention and Response, PO Box 944209, Sacramento, California 94244, USA

Ian A. Gardner, BVSc, PhD
Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California 95616, USA

Linda J. Lowenstine, DVM, PhD
Department of pathology, San Diego Zoo, PO Box 551, San Diego, California 92112, USA

The acute effects of crude oil on the sea otter (*Enhydra lutris*) are well documented as a result of the *Exxon Valdez* oil spill (March 24, 1989) in Prince William Sound, Alaska. However due to the limitations on reproductive data collection in the field, documentation of the effects of sublethal petroleum product exposure on sea otter populations is very difficult. In order to investigate the potential consequences of petroleum product exposure, American mink (*Mustela vison*) were used as a laboratory animal model for sea otters in an experimental trial. Characteristics of the mink which are indicative of its usefulness as an experimental model for the sea otter include: mustelid family member; high metabolic rate; intense grooming behavior; semi-aquatic nature; utilization of a diverse group of prey items; and exquisite susceptibility to a variety of environmental contaminants.

Mink were exposed either externally on the one occasion sixty days prior to breeding or via low level contamination of their diet daily from sixty days prior to breeding until weaning. The externally exposed mink were placed in either a slick of bunker C fuel oil on sea water, a slick of Prudhoe Bay crude oil on sea water, or sea water alone for one minute (External Trial). The dietary exposed mink were fed diets containing 500 ppm of bunker C fuel oil or 500 ppm of Prudhoe Bay crude oil (Internal Trial). The results of this reproductive trail in mink are summarized in Table 1. While number of liveborn kits per female bred did not vary significantly between the mink exposed acutely (External) and those not exposed to petroleum products (Control, 5.32 kits/female bred), only 2.43 and 0.70 kits were produced per female bred for those exposed dietarily to crude oil and bunker C fuel oil, respectively (Internal). These females with reduced reproductive success did not show any clinical signs of toxicosis and showed no behavioral abnormalities related to eating, grooming, and breeding. In addition, the kits of those females exposed to petroleum products in their diets had reduced survivability. Therefore, it is possible that sea otter populations utilizing potentially contaminated food sources when colonizing previously oiled habitats well have reduces reproductive success.
Table 1. The effects of petroleum product exposure on the reproductive system of American mink (*Mustela vison*).

<table>
<thead>
<tr>
<th>Exposure Group</th>
<th>Number of Females Bred</th>
<th>Percent of Females Whelping</th>
<th>Number of Liveborn Kits per Female Bred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>88.0</td>
<td>5.3</td>
</tr>
<tr>
<td>External: Prudhoe Bay crude</td>
<td>8</td>
<td>87.5</td>
<td>5.0</td>
</tr>
<tr>
<td>External: Bunker C fuel oil</td>
<td>15</td>
<td>100</td>
<td>6.5</td>
</tr>
<tr>
<td>Internal: Prudhoe Bay crude</td>
<td>21</td>
<td>76.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Internal: Bunker C fuel oil</td>
<td>20</td>
<td>25.0</td>
<td>0.7</td>
</tr>
</tbody>
</table>
THE USE OF VIRAL VECTORED IMMUNOCONTRACEPTION FOR FERAL PEST CONTROL IN AUSTRALIA

Michael K. Holland, PhD* and A. J. Robinson, BVSc, PhD
Cooperative Research Center for Biological Control of Vertebrate Pest Populations and CSIRO Division of Wildlife & Ecology, PO Box 84, Lyneham, ACT 2615, Australia

Introduction

The European wild rabbit (Oryctolagus cuniculus) and red fox (Vulpes vulpes) are not native animals to the Australian ecosystem. Both were introduced approximately 130 years ago by European settlers. In that time they have expanded to cover the major portion of the Australian continent. Their impact on Australia's fauna has been profound. Eighteen species of marsupial, half the World total, have become extinct over this period and the survival of others remains jeopardized. Foxes and rabbits were not solely responsible for these losses. Other introduced predators played their part. However, arguably most important was habitat modification through the clearing of large tracts of land for agricultural purposes and competition with introduced herbivores, which meant that suitable habitat often remained only in small, restricted areas of low agricultural value where native animals are most susceptible to competition and predation. In specific cases other causes such as hunting also contributed.

Rabbits compete for food and shelter with the native fauna and by ringbarking, grazing and selectively browsing they significantly affect the distribution if the native flora. Collectively, these affects have impact on the land use and degradation particularly in the very large areas of Australia where rainfall is marginal. In these arid zones rabbit densities as low as 1 rabbit/hectare prevent regeneration of the seedlings needed to provide the trees, shrubs and grasses which stabilize the soil. Given the long life span of many of these trees (eg mulga trees commonly survive 250 years) destruction of the seed bank and limited regeneration of seedlings has implications for vegetation cover into the long term future.

Foxes prey on a range of native species as well as rabbits. Detailed studies on prey preference have not been done but the impact on native animals is clear from studies in which the distribution and abundance of selected native species have been monitored both before and after foxes have been controlled over large areas. In such cases the resurgence in numbers of selected indicator species is documented.

The deleterious effects of rabbits on agricultural and grazing land were realized within 30 years of their introduction and a number of methods intended to control rabbit numbers were quickly employed. These ranged from the construction of thousands of miles of fencing in an attempt to prevent the advance of rabbits into new areas, to attempt to eradicate animals locally by shooting, poisoning, or ripping or fumigation of rabbit warrens. While all of these methods, at various times, achieved some success at a local level the Australian rabbit population in 1950 was estimated to number 600 million or approximately 60 rabbits for every man, woman, and child! By far the greatest long term
impact on rabbit numbers came from the introduction of the myxoma virus in 1950/51.\textsuperscript{8} This virus is endemic in the Americas where it causes a relatively benign disease of cotton tail rabbits \textit{(Sylvilagus spp.)}. However in European rabbits the virus induces a virulent, generalized infection which frequently proves fatal. The exact cause of death remains unclear but secondary bacterial infections particularly of the upper respiratory tract certainly result in the death of many animals.\textsuperscript{9} Initial case mortality was 99.9\%, but, within three years of release, less virulent strains of virus were being isolated from the field.\textsuperscript{8} Both the host and virus were rapidly evolving toward the state seen with the virus and cottontail rabbits in America. Nonetheless, forty years on the myxoma virus remains our most effective means of controlling wild rabbits. The rabbit population has never recovered to the levels achieved prior to introduction of the virus.\textsuperscript{8,10}

Despite this there is a perception amongst some in the agricultural community of a slow increase in rabbit numbers over recent years. There are a number of factors contributing to this perception not the least being the anecdotal observation that more rabbits survive the annual outbreaks of myxomatosis leading inescapably to the conclusion that myxomatosis is declining in efficacy as a control procedure. More rabbits, of course, means in some situations more foxes. These concerns of the agricultural community are exacerbated by an emerging reservation in the wider community about poisoning, because of effect on nontarget species, and trapping and shooting which many no longer considered humane. Several of these concerns combine with the financial pressures on many farmers from decreasing remuneration for their product, interest in development of new humane, cheap and effective procedure for control of rabbits and foxes is high. This is despite the fact that in the case of both species considerable control can still be achieved by correctly applying conventional procedures and by seeking new ways to integrate and apply existing approaches.\textsuperscript{10} Nonetheless, there is significant interest in developing novel control strategies which would be:

- species specific
- humane
- easy to apply
- inexpensive
- applicable over large geographical regions with differing ecosystems

One such approach is to substitute control of the birth rate for control of the death rate.

\textbf{Fertility Control}

The control of fertility can be aimed at either or both sexes. The ideal method of fertility control would target both sexes to maximize efficacy but in many situation it is the female sex which is targeted as she is the breeding unit of the population. However, in species where monogamous relationships of significant duration exist the male can be an equally appropriate target. This condition remains relatively rare and definitely does not apply to rabbits.\textsuperscript{11,12} The situation in foxes is less clear.\textsuperscript{13}
A number of aspects of the reproductive process are susceptible to fertility control. Broadly these can be divided into effects on:

- gametogenesis
- the gametes
- the pre-implantation embryo
- the implantation process
- the post-implantation embryo

Naturally, an agent which affects more than one of these would be both highly effective and also make it difficult for resistance to emerge. Reproductive processes are highly conserved and individuals with altered reproductive function are generally infertile. Which steps are most readily affected depends on: the reproductive biology of the target species (e.g., multiple or single mating, single offspring or litters, duration of pregnancy, seasonal or nonseasonal breeding, etc), the means of induction of infertility (e.g., chemical, endocrine or immunological, etc), whether contraception or castration is desired and how the agent is to be delivered (e.g., bait, dart, virus, etc). In addition, factors other than scientific ones may affect the choice of fertility control agent, e.g., an agent which caused abortion in midpregnancy may be acceptable to the community when the target species is the mouse but would likely be considered inhumane in deer.

**Viral vectored immunocontraception**

The concept if virally vectored immunocontraception seeks to combine the well studied concept of immunocontraception with one of the latest concepts from vaccine technology, the use of live viruses to carry antigens. There is a large literature on the use of immunocontraception to control fertility, although its application to control of wild animal populations has only recently been pioneered by Jay Kirkpatrick and his colleagues. Similarly, the concept of utilizing live viruses to deliver antigen is a recent development which has had success when used to immunize wild animals against rabies. Viral vectored immunization unifies these concepts. How it is proposed to achieve the immunocontraceptive response is broadly similar for both the rabbit and fox although the mechanism of delivery of the immunocontraceptive differs. Briefly stated (Fig. 1), in the case of the rabbit components of the gametes which are both immunogenic and fulfill an essential role in fertilization will be identified and the genes encoding these components cloned into intergenic sites in the genome of myxoma virus. The resultant recombinant virus, when it infects a rabbit, will replicate and in so doing produce the antigenic gamete protein. The rabbit will mount an antibody response not only to the virus but also to the gamete protein. These antibodies will bind to the gamete protein wherever it is present, including on the gamete itself. This will compromise the gamete function and result in infertility. The details of the antigens being considered for inclusion into the myxoma virus and the mechanisms for construction of recombinant virus have been previously reviewed.
A similar strategy will be employed in the fox, although there are differences in how this concept would be applied. In the fox no species-specific disseminating virus has yet been identified which would be amenable to genetic manipulation. Hence, while the search for a virus continues, a bait-driven intermediate strategy is proposed. Several different delivery systems are being evaluated for incorporation of the immunocontraceptive into a bait ranging from recombinant, non-disseminating vaccinia virus, attenuated AroA strains of *Salmonella typhimurium*, to synthetic systems such as microspheres, immunostimulating complexes or protein cochelates. These systems as yet remain in the early stages of development. It is important that a number of different approaches to delivery of antigen be considered if we are to view immunocontraception as a generic concept for control of animal populations. Flexibility in antigen presentation is as crucial as flexibility in antigen selection given the differences in reproductive behavior and biology between species. This becomes clearer if we consider what are the features required in an immunocontraceptive vaccine (Table 1).

**Table 1**

**Requirements of an Immunocontraceptive Vaccine**

- An antigen essential to fertilization
- Means to ensure the immunocontraceptive effect is target specific
- Genetically stable delivery system
- Provocation and targeting of the immunocontraceptive response
- Easy to apply and long-lasting effect
- No interference with social hierarchy
- Cheap and easy to produce

Consider each of these properties in turn.

**Antigen selection**

The most important characteristics of an immunocontraceptive vaccine are species-specific antigens to provoke immunocontraception and a delivery system which only targets the intended species. In the case of both foxes and rabbits substantial effort is being directed to the first of these aims. Regrettably, our knowledge of the molecular events underlying the well-described cellular events which occur during fertilization is far from complete. It is not even yet clear how many molecules participate in the various steps of fertilization nor whether molecules involved in the recognition of gametes are species-specific or indeed whether the same molecules with slight differences in structure, such as the degree and nature of glycosylation, occur in all mammalian species. Such information has profound implications for antigen selection. If gamete recognition molecules are species-specific, the fear of cross-reactivity between other than closely related species is minimal. However, if gamete recognition molecules are highly evolutionarily conserved, as are many of the cell biology aspects of fertilization, then the gamete recognition molecules will differ only in small molecular details. Such an eventuality implies that to maximize the possibility of
producing a species specific immune response the construct which is inserted into the virus may not be the entire gene encoding a gamete recognition molecule but rather an epitope or series of peptide epitopes linked together and regulated by a synthetic promoter. A construct of this type will be more difficult to produce and it is difficult to predict what the nature and magnitude of the immune responses such a construct would generate. Nevertheless, significant advances are being made in identifying and characterizing gamete recognition molecules and we are developing procedures for producing species-restricted, antigenic constructs appropriate for use in immunocontraception.

Effects on nontarget species

Species specific antigens provide an immunocontraceptive effect which can only compromise fertility in the targeted species. If a totally species specific antigen cannot be identified and only species restricted antigens have to be employed to provoke immunocontraception the species most susceptible are those most related to the target species. In the case of rabbits this is other lagomorphs. In Australia this mean only the European hare which was also introduced to our country last century. From the environmental perspective it would not be a disaster if this species were affected by the viral vectored immunocontraceptive. Whilst hares remain a pest species they have never become the problem that rabbits have. Nevertheless, we have commenced an investigation into the molecular relatedness between gamete recognition molecules in hares and European rabbits in an attempt to assess whether it is likely that the antigens selected for use in rabbits or ones closely related to them, are present in hares. Should related molecules be identified, a study of the immune response of hares to injection with rabbit gamete antigens and whether there is any effect on fertility will be undertaken.

Of course, outside Australia, there are a number of lagomorph species which are either known to be or are potentially susceptible to myxoma virus and could thus be affected if gamete recognition molecules are sufficiently conserved. Most of these belong to the genus Sylvilagus, a few of which are rare. We are therefore in the process of trying to establish collaborations with investigators in North America which will allow us to determine how related are gamete recognition molecules in Oryctolagus and in Sylvilagus spp. Since Sylvilagus rabbits were the source of the original viral isolation we already know that myxoma virus is not species specific. Thus, in closely related lagomorph species, to prevent an unwanted immunocontraceptive effect specificity must be maintained at the level of the antigen. As we move to more distant species which are not susceptible to infection by the myxoma virus the viral delivery system confers a second, quite considerable, level of specificity.

In the case of the fox, species specificity of antigen could be crucial as baits can be taken by a number of other species of carnivore. Those most closely related to foxes would be domestic dogs and the Australian native canid, the dingo. No reports are available on the gamete recognition molecules in these species although crosses between dingos and domestic dogs are known and anecdotal reports of fox-domestic dog hybrids exist. Given the geographical and ecological distribution of the dingo and fox, which shows limited overlap,
the risk to dingos may not be large if baits remain the delivery vehicle. The same comment cannot be made about domestic animals.

**Delivery systems**

Poxviruses in general have quite specific and restricted host ranges. Forty years of experience with myxoma virus since its introduction into the Australian environment coupled with the longer experience in the Americas suggest myxoma follows this pattern. Host range is confined to at least lagomorphs and apparently only to *Sylvilagus* spp. in which resistance to disease is probably high, possibly *Lepus* spp. and our target species *Oryctolagus*. The issue of whether abortive infections exist in other species has not been addressed directly. Certainly, disease occurs only in lagomorphs. It is not likely an abortive infection would provide sufficient antigen to trigger an immune response. Evidence from other pox viruses suggests that it requires very large quantities of virus to do so, much greater, by 5-7 orders of magnitude, than either an arthropod vector or close contact could deliver. In any case, this would only become an area of concern if the targeted gamete recognition molecules proved to be conserved across species.

In bait delivered systems limited species specificity can be achieved by the system itself. By selecting areas in which non target canid populations are low and by presenting baits in various ways it is possible to minimize contact with non target species. In the future whether a fox specific virus, which leave other canids unaffected, can be isolated remains to be seen. Even if this can be achieved the virus must be amenable to genetic manipulation, a feat which can only be accomplished with a few classes of virus.

Other aspects such as genetic stability of the virus are important for the success of the recombinant virus. Should the insert be so unstable as to be readily lost the immunocontraceptive effect will also be lost and the virus will revert to wild type phenotype. This issue of exchange of the insert through recombination with co-infecting wild type virus must also be considered. Co-infection of rabbits with two different, genetically distinct strains of myxoma virus can be achieved in the laboratory but there is no evidence from a number of viral isolates that it occurs frequently in the wild (P. Kerr, pers comm). Should viral particles of both strains be replicating in the same cell at the same time exchange of the immunocontraceptive insert is possible with production of a new strain of recombinant virus. Experiments are planned to assess directly the ease and frequency with which this can occur. However, such an event may prove beneficial as the new recombinant strain may have some advantage and thus help spread the immunocontraceptive effect. If the co-infecting virus were another pox virus with sufficient homology to myxoma virus to permit homologous recombination then the insert could theoretically be transferred to this virus. Experiments to investigate this are planned.

**Immune responses**

Another important property of an immunocontraceptive antigen is that the immunocontraceptive effect be long lasting. This will be part determined by the nature of
the construct and the presentation of the antigen. High antibody titers to the antigen will likely be required while, at the same time, minimizing the cytotoxic T cell response which could result in an autoimmune disease that destroyed the gonads.\textsuperscript{26} The relative importance of mucosal versus systemic antibody response is the subject of active experimentation. In the case of the fox, where antigen is to be delivered orally, clearly a mucosal response is likely to be favored. Contraception would then depend on IgA responses being generated within the female genital tract. Preliminary studies (M. Bradley, pers comm) in which antigen has been administered directly into the Peyer's patches in the gut has resulted in vaginal IgA and IgG responses which, in some cases, have affected fertility. Experiments in which rabbits were infected with a recombinant myxoma virus containing the influenza HA gene resulted in IgG and IgA antibodies to HA being detected in the reproductive tract and IgG responses in the serum.\textsuperscript{27} Whether high antibody titers within the region of the reproductive tract where fertilization occurs can be achieved solely through selection of a highly immunogenic antigen and presentation of that antigen in a manner that is immunostimulatory remains to be determined. There is the option of genetically engineering rabbit immunostimulatory molecules to become part of the construct to achieve this purpose. Current experiments are exploring this option. However, this may prove unnecessary as it has been shown\textsuperscript{27} that antibody titers to the virus rise to very high levels in animals which have recovered from natural infection and that there is little change in these titers over at least a two year period. If a similar response to the inserted antigen could also be generated infertility would seem to be assured. Should titers drop there is the possibility of reinfection with consequent boosting of titers. The theoretical possibility that mating will provide a natural boost in titers to a sperm antigen seems unlikely. Women who are infertile due to the presence of antisperm antibodies do not show changes in antibody titer as a consequence of sexual activity,\textsuperscript{28} but it is worth experimental consideration. In conclusion, given that the average lifespan of breeding female rabbits in many regions of Australia is in the range 2-3 years\textsuperscript{8} then it seems likely that immunity which covers this interval can be induced.

Dissemination

The arthropod vectors responsible for the spread of myxomatosis deliver relatively few virions each time a single flea or mosquito bites a rabbit\textsuperscript{8} but, as multiple exposure is the obvious norm, transmission rates are high given an infected pool of rabbits of reasonable size.\textsuperscript{5} The key to successful dissemination of a recombinant virus would thus seem to be the existence of sufficient susceptible rabbits and the establishment of an appropriate sized infective pool of virions. For the recombinant virus to achieve this and to compete successfully for susceptible animals with a number of other endemic strains of virus it is possible that the recombinant virus will need an advantage. This advantage could take many forms. Perhaps the simplest is that, initially, the recombinant virus will need to be introduced at a time before other strains are established. These issues are currently the subject of modelling experiments which will develop predictions which will be tested in the field.
These issues are important also in bait delivered systems where cost pressures require that a single application be used. Single application requires very high efficacy. This means high rates of bait uptake as well as high percentages of animals taking baits becoming infertile. This adds further pressure on the selection of a highly immunogenic and contraceptive antigen.

The importance of mating behavior

An important issue is whether the effect on fertility should also affect aspects of behavior. In some species immunocastration may be appropriate. In such a response both the gametogenic as well as the endocrine functions of the gonads are destroyed. Naturally such a response affects the sexual behavior of the animal which includes its ability to maintain its position in the breeding hierarchy. In species where only dominant animals breed successfully this could result in subordinate animals being released to breed making the task of decreasing the breeding population more difficult. In certain ecological situations foxes fall into this category. In an immunocontraceptive response the endocrine functions of the gonads remain unaffected while the gametogenic function is impaired. Such responses leave reproductive behavior uncompromised and position in the social hierarchy may be retained. In the case of rabbits the issue of the importance of social dominance is unclear. Most females breed but the subordinates are relegated to the least favorable burrows and hence survival of their offspring is less assured. Also unclear is the role of dominant males in siring offspring. We are currently using DNA fingerprinting to determine the parentage of kittens produced from all females within a confined population and relating this to position of the parents in the social hierarchy. In conclusion, our current approach in both the fox and rabbit is to achieve immunocontraception rather than immunocastration. Whether this strategy is continued as we extend the technology into other species remains to be seen.

Vaccination production

A successful vaccine will need to be produced in very large quantities, at low cost per dose and also be easy and inexpensive to distribute. In the case of bait driven strategies these may be difficult aims to achieve given the vast geographical areas inhabited by foxes. The use of baits may need to be restricted and targeted to areas of highest damage, which frequently is where animal numbers are highest.

In the case of rabbits, where we are using a disseminating virus, production costs should be low, although as we scale up production to the quantities likely to be needed unforeseen difficulties may aries. Introduction of the virus to the rabbit population is more problematic. Ideally, the recombinant virus will only need to be introduced in a limited number of locations before it becomes established and becomes endemic just as the present field strains of virus have done. Should the virus fail to establish and repeated introductions prove necessary cost-benefit analysis will be required to determine the optimal course.
Ecological Issues

In the 130 years or so since their introduction foxes and rabbits have become part of the Australian ecosystem. Hence their eradication or substantial reduction in their numbers will impact in diverse, often unpredictable ways. This is further complicated by the fact the two species are inter-related with rabbits being the major prey species for foxes in many situations. It is for these reasons that the parallel programs in fox and rabbit control exist.

A number of scenarios are imaginable given different outcomes in our attempt to control both species. Limited experimental data suggest that when rabbit populations decline rapidly from very high numbers, for example during a drought then fox numbers also decline but with a lag phase of 8-12 months. However, such a situation may be atypical in that the fox population may have expanded as a result of the large numbers of rabbits and what is observed as a decline in fox numbers is an adjustment down to the fox population size sustainable by smaller rabbit populations. Nevertheless this does constitute a clear demonstration of a relationship in the population size of the two species.

A concern often expressed is that as rabbit numbers declined foxes would switch prey and perhaps put further pressure on populations of native species. This is a subject of current experimental investigation. What seems clear is that when fox numbers are reduced to very low levels by large scale poisoning operations the populations of several native species such as rock wallabies and numbats increase. This is not always an immediate response and may take a number of years to be manifested but it does show a relationship between fox numbers and the numbers of several native species suggesting that were our program to succeed in reducing fox numbers the desirable consequence of a resurgence in some native species may well result. The outcome will depend on whether the native species are primary or secondary prey for foxes.

Some consequences are clearer. Control of rabbit populations definitely results in increases in the species richness of native flora, particularly the native grasses and the trees which are so important in soil erosion in the arid regions. In addition undesirable species such as the woody weeds proliferate where rabbit numbers are high. The effect of rabbits on native fauna is not well documented. Rabbits will use the burrows of other animals as their own, in the process evicting the native species. Declines in the population of the rufous hare-wallaby correlate temporally with the invasion of the habitat by rabbits although no clear cause and effect relationship has been established. Similarly, the burrowing bilby (Macrotis lagotis) now is found only in regions where rabbits and foxes are rare or north of the limit of the rabbit distribution. How much of these effects is directly attributable to competition from rabbits or due to predators such as foxes which follow the rabbit distribution is not totally clear.

Rabbits also compete with native species for food. In good environments this may have little impact but the small to medium size herbivores within the arid zone are vulnerable, particularly so in drought. Under these conditions even species as large as the red kangaroo and the common wombat are vulnerable to competition as they congregate in

1995 JOINT CONFERENCE AAZV / WDA / AAWV

51
isolated, watered patches. Island studies often provide the best examples of some of these effects. Stevens and Weisbrod \textsuperscript{40} showed that on a large island off the Washington coast, where European rabbits were endemic, in areas where rabbit populations were highest deer populations were 10\% of that in low rabbit areas. Rodents were similarly rare in high rabbit regions but on the other hand raptors were particularly abundant. On several islands rabbits have been shown to have a major impact on vegetation and thus on bird populations with ground nesting birds being especially susceptible. In one example in the Frioul Archipelago off Marsailles, the numbers of Cory’s Shearwater were very low and breeding success was poor. \textsuperscript{41} An aggressive rabbit control campaign has seen bird numbers increase and breeding markedly accelerate. Of course, not all situations are as clear cut. On some islands control of rabbits has allowed proliferation of weeds which has made conditions less suitable for seabirds and nesting penguins. \textsuperscript{42} Whether this is a transient effect remains to be determined. In other cases the sequelae of rabbit reduction are still less clear. Habitat modification by rabbits at Macquarie Island appears to have benefitted Antarctic prions but disadvantaged white-headed petrels and sooty shearwaters. \textsuperscript{43}

In conclusion, it would seem that consequences of rabbit and fox control are going to prove complex. There will be short term and long term consequences which differ in different environments. There is, however, a strong perception of overall benefit to the environment as well as to other areas of the community such as agriculture.

Future directions

The research being undertaken in the Center for Biological Control of Vertebrate Pest Populations will provide an integrated examination of the potential of fertility control as a tool for feral pest management. All aspects, from the identification, isolation and utilization of immunocontraceptive antigens, through the development of systems for delivering them to the ecological aspects relating to both how to achieve the degree of control required to the consequences of that control on the environment are being investigated in an integrated manner in a small number of species, currently the rabbit, fox and more recently the mouse. Much of what we learn about areas as diverse as antigen selection, viral engineering and impact assessment will prove generic, so that as interest in applying fertility control to other species develops, the task may be easier. Each species will present its own challenges in terms of its reproductive biology, methods for delivering contraception and the environmental significance and consequences of successful control. Each case must thus be considered on its own merits but the potential of immunocontraception as a control procedure for wild animal population remains high given our current state of knowledge.

ACKNOWLEDGEMENTS

We wish to thank many colleagues in the Center for Biological Control of Vertebrate Pest Populations for their generosity in discussing many of the issues dealt with herein and directing me to relevant literature. I am particularly grateful to Peter Kerr, Ian Parer and Kent Williams for clarifying some of the issues in ecology and virology pertaining to the rabbit project and to Mark Bradley, Alan Newsome and Roger Pech for introducing me to foxes.

Particular thanks are due Hugh Tyndale-Biscoe whose drive and initiative resulted in creation of the Center and who was most helpful in critiquing this manuscript.
LITERATURE CITED


Figure 1. The concept of viral vectored immunocontraception. The genes encoding proteins involved in gamete recognition which are present in sperm membranes or the zona pellucida of the oocyte are cloned into the virus. When an animal is infected with this recombinant virus the immune system is exposed to both viral proteins and the protein encoded by the inserted gene. The result is that antibodies are produced. These antibodies bind to the gamete protein and in so doing prevent fertilization.
BIGHORN SHEEP HEALTH MANAGEMENT IN CALIFORNIA: A FIFTEEN YEAR RETROSPECTIVE

David A. Jessup, DVM MPVM Dipl ACZM
California Department of Fish and Game, 1701 Nimbus Rd. "D", Rancho Cordova, CA 95670, USA

Walter M. Boyce, DVM PhD
Department of Pathology, Microbiology, Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

Steven G. Torres, MS
California Department of Fish and Game, 1416 9th Street, Suite 1270, Sacramento, CA 95814, USA

In 1979, following one hundred years of full protection and passive management, the California Department of Fish and Game (CDFG) began an aggressive management program for bighorn sheep. This included capture, sampling, and marking of animals coupled with selected translocations and various types of research. This work was greatly aided by a legislative mandate and special appropriations. Capture methods were compared and improved with a reduction in capture associated mortality to <1%. Demographic studies documented movements between ranges and the existence of a number of metapopulations, and genetic and morphometric studies showed that accepted taxonomic classifications were questionable. Serologic and pathogen isolation surveys revealed exposure to a number of potentially pathogenic parasites, bacteria, and viruses. Several of these disease organisms appear to be able to influence the health of bighorn sheep populations, while the role of others is less clear. Several successful translocations resulted in range extensions, but other translocations failed. In retrospect, 15 years of active management of desert bighorn sheep resulted in an explosion of biological and biomedical information, much of which should improve future management programs.

History: Mountain sheep in California have always attracted attention as an important symbol of the state's wildlife heritage. John Muir was one of the early naturalists who reported the presence and natural history of these animals in California. Unfortunately, bighorn sheep in California were historically more numerous and widespread (Fig. 1). Indeed, bighorn sheep no longer occur in northeastern California and north of Mono County in the Sierra Nevada.

Currently, mountain sheep occur in a diversity of habitats. Common features influencing their distribution include steep rocky terrain with little visual obstruction, summer water resources, and little disturbance from human activity. As such, bighorn sheep represent an ecological indicator of desert and mountain ecosystem quality, and the conservation effort dedicated toward bighorn sheep has also played a role in protecting ecosystem structure and biodiversity.

In 1878 the California Legislature chose to end unregulated hunting of bighorn sheep and give them full legal protection. This action followed about 40 years of market hunting by miners and settlers, significant habitat loss, major die-offs thought to be related to contact
with domestic livestock, and extinction of many populations.\textsuperscript{38} This full protection status continued through the early 20th century while habitat loss and degradation accelerated. Only occasional and sporadic biological monitoring of remaining bighorn populations was conducted during this time.

Restocking of vacant bighorn sheep ranges in California was first attempted in 1971 when 10 captive-reared bighorns from British Columbia were transplanted to the Lava Beds National Monument. In 1979 CDFG conducted the first capture and relocation of free-ranging bighorn sheep and began a program of routine collection of disease and physiologic data. Clark et al.\textsuperscript{9} and Bleich et al.\textsuperscript{2} provided histories of relocations of bighorn sheep within California and some of the attendant problems and results.

\textbf{Economics of a Conservation Effort:} The conservation and management of California’s bighorn sheep resource must rely on adequate and stable funding commitments. This revenue has been required to offset costs associated with insuring the long-term survival and persistence of mountain sheep. The wildlife values associated with the persistence of these populations, such as viewing and knowledge of existence, are greatly appreciated, but not easily quantified. Although these “uses” do not directly translate into funds for bighorn sheep management, the Department recognizes their importance toward successful bighorn sheep management. Public knowledge of the status of bighorn sheep populations can help support important bighorn sheep management efforts, such as local and regional land-use planning.

In 1986 the California legislature decisively ended an era of benign neglect by passing Assembly Bill 3117. This bill mandated statewide bighorn sheep inventory, demographic, disease and genetic studies, and selected relocations. It also allocated sufficient funds to support this work. The bill allowed the California Department of Fish and Game to sell from 8 to 16 hunting permits per year for mature males in large populations which could sustain this rate of harvest. In the late 1980’s and early 1990’s the additional funds from this hunting program were added to the legislative appropriation and supported research and relocation efforts.

Although revenues from hunting programs have continued, special non-dedicated funding for bighorn sheep research and management declined sharply beginning in 1994. Currently, the Department’s bighorn sheep management program receives 80\% of its funding from its bighorn sheep hunting program, and the remaining funding comes from non-dedicated revenue (California Environmental License Plate Fund, Federal Pittman-Robertson Funds). Additional funds, equipment, and volunteer effort are contributed by county Fish and Game Commissions, sportsmen associations, and conservation groups.

\textbf{Capture:} An attempt to capture bighorn sheep from a large enclosure in California in 1980 yielded rather poor success and excessive mortality.\textsuperscript{9} This brought severe criticism, but also a donation of $55,000 from a hunter/conservation organization to support research and improvement of capture technology.\textsuperscript{19} This seed money supported the first systematic collection of biomedical and genetic data from bighorn sheep. When legislative funding
became available, this project was eventually expanded and included over 1000 bighorn sheep from eight western states.

The five most common methods of capture were evaluated and compared with regard to effects on physiological responses, morbidity and mortality, and cost effectiveness and safety. Application of these findings allowed us to reduce capture associated mortality from 5-10% in 1979 to 2-4% in 1985, and subsequently to 0.4-1% today. Although several of the methods of capture we studied are still in use, net-gun capture proved to be clearly the least stressful, least often fatal, most flexible, most successful, and most cost effective method for capturing relatively large numbers of bighorn sheep in California's desert and mountain conditions.

Population status and demographics: Only through understanding the factors influencing the historic and current distribution of mountain sheep can resource managers determine the appropriate strategies to maintain and enhance the resource. Therefore, survey, assessment and inventory of mountain sheep populations is an essential part of long-term management. The current and historical distribution of bighorn sheep in California is detailed on Figure 1. A detailed inventory of these populations has been published by Torres et al.

Populations of mountain sheep in California have been organized into metapopulations, or 'systems' of populations, that best represent logical regions for managing for the long-term viability of this species (Fig. 2). This regional approach recognizes the importance of inter-mountain areas that allow movement and exchange of individuals between populations, the recolonization of vacant habitats, and the interagency coordination of land management. This definition of regional populations considers not only vegetative and geographic boundaries, but also man-made barriers that define distributions and which have resulted in the fragmentation of habitat.

Several investigations have alluded to the importance of population size and genetic diversity in the long-term viability of bighorn sheep populations. Given the need to understand the status and dynamics of these regional populations of bighorn sheep, Torres et al categorized the California populations by size class. Historical and current data from ground, water-hole, and aerial surveys were used to classify these populations. Although the population estimates are of varying precision, the size classes are large enough to provide an accurate yet conservative assessment. The defined metapopulations are summarized by size classes in Table 1, and population estimates are subsequently computed by totaling the high, low, and median interval estimates (Table 2). This inventory should provide an index for documenting regional population changes over time, and help optimize future reintroduction and management efforts to ensure population viability.

Although the traditionally defined subspecies are questionable, CDFG in the past has reported the population estimates for Nelson (Ovis canadensis nelsoni), Peninsular (O. c. cremnobates), and California (O. c. califomiana) bighorn sheep, because the latter 2 of these remain State listed as Threatened. The population estimate for Peninsular bighorn simply
corresponds with the Peninsular Ranges metapopulation total, while that for California bighorn is for the Sierra Nevada (Fig. 2, Table 2). The total statewide estimate of bighorn sheep is 4,653 animals, consisting of 3,875 Nelson, 426 Peninsular, and 352 California bighorn sheep. All populations of Nelson bighorn, with the exception of those found in 4 areas open to hunting, are fully protected by state law.

Genetics and Morphometrics: Bighorn sheep in California have traditionally been considered and managed as three subspecies based on early morphometric studies. A recent reexamination of this original morphometric data revealed several problems with this classification. These morphometric studies, molecular analyses, and alloenzyme studies (Jessup and Ramey, unpublished) all strongly suggest that there may only be two distinct subspecies of bighorn sheep in California: *O. c. nelsoni* in the deserts, and *O. c. californiana* in the Sierra Nevada Mountains. The issue of taxonomic classification is not academic since several populations in California have been proposed for listing as endangered under the Endangered Species Act. Additional genetic studies are underway that are attempting to provide further insights into these questions.

Translocations: Between 1971 and 1988, 340 bighorn sheep were relocated to 13 release sites in 11 mountain ranges in California. In 1989, 45 desert bighorn were relocated to the Chuckwalla Mountains. During October 1992, 5 rams (1-3 years old) were relocated from the Kelso Peak/Old Dad Mountain Management Unit to the Avawatz Mountains. During November 1992, 30 ewes and 14 rams were translocated from Kelso Peak/Old Dad Mountain to the Bristol Mountains (15 females, 6 males), the Bullion Mountains (15 females, 4 males), and Sheephole Mountains (4 males). The translocations to the Bristol and Bullion Mountains established new populations, and the relocations to the Sheephole and Avawatz Mountains augmented existing small populations.

Of the fourteen historic mountain ranges in California to which bighorn sheep have been relocated between 1979 and 1995, it appears that viable populations may exist in 10. Because of access restrictions to the area of the China Lake Naval Weapons Station there was no way to follow the fate of bighorn released in the Argus Mountains, but this relocation appears to have failed. The failure of translocated bighorn to survive in two locations (San Rafael Peak and Northern San Gabriel Mountains) may be related to the brushy sub-optimal habitat and the high density of mountain lions in the release areas. The results of both of these translocations efforts are insufficiently documented by field studies to draw definitive conclusions, however. As reported by Clark et al. and Jessup and Boyce the entire herd of bighorn sheep in the Warner Mountains of Northern California, which had increased from 12 to over 60, died from *Pasteurella* pneumonia, probably contracted from domestic sheep.

Health Studies: An initial serologic study of patterns of exposure to infectious diseases in bighorn sheep in California was completed in 1985. Protostrongylus lungworms were found in low numbers in only 2 of 17 mountain ranges and *Pasteurella* spp. were seldom found. Serologic evidence of exposure to a number of potentially pathogenic viruses was
documented and PI-3 virus was isolated from 6 bighorn in 3 herds, and 4 serotypes of bluetongue virus were isolated from 3 bighorn herds. This work indicated that treating bighorn sheep for health problems was impractical, and that optimal management strategy appeared to be the maintenance of populations within carrying capacity and separated from livestock.

Pneumonia caused by Pasteurella hemolytica are rapidly fatal to bighorn sheep. Two outbreaks in California, both following contact with domestic sheep, resulted in the extinction of entire herds of "threatened" California bighorn sheep from Northern California. Although non-hemolytic P. hemolytica can be isolated from the nasal passages and tonsils of desert bighorn sheep, this is not the biotype commonly associated with bighorn pneumonia outbreaks. Separation of bighorn sheep from domestic sheep remains the primary management strategy to avoid bacterial pneumonia epidemics in California.

Concern over the potential for respiratory viruses to predispose bighorn sheep to pneumonia lead to additional serosurveys and attempts to isolate viral pathogens. In some cases pneumonia in bighorn lambs appeared to be initiated by parainfluenza-III (PI-3) and/or bluetongue virus (BT). A vaccination trial in two populations of free-ranging bighorn sheep using a freeze-dried PI-3 vaccine in biobullets did not result in significantly improved lambs survival when compared to an adjacent unvaccinated herd. Evidence of infection with PI-3, BRSV, IBR, EHD and BT in healthy adult bighorn was found, and IBR, BT, and PI-3 viruses were isolated, most often from clinically normal animals. However, no real pattern or association of clinical disease with exposure to these viruses was apparent.

An outbreak of psoroptic scabies in bighorn sheep in New Mexico in the late 1970's resulted in an approximately 90% population decline. Despite various attempts to manage this disease and/or to allow the population to respond by itself, this population has not recovered significantly in 15 years. Morphometric studies of Psoroptes has shown them to be distinct from those infesting other species with the exception of mites found on mule deer in mountain ranges adjacent to infested bighorn in New Mexico. Bighorn sheep produce specific antibodies to Psoroptes and this information was used to develop a kinetic ELISA and to determine the prevalence of infestation. Many bighorn sheep herds in California have evidence of infestation with Psoroptic scabies, but the vast majority of infestations are inapparent or of no major clinical importance. However, observations in New Mexico and experimental evidence from Arizona suggest that, even when confined to the ear canal, scabies can predispose bighorn sheep to predation and/or fatal falls.

Brucellosis does not appear to be present in bighorn sheep in California. Several diseases associated with overpopulation or stress have been seen. Contagious ecthyma was diagnosed in lambs with pneumonia and in one large herd of bighorn. In the later case, hay from fields grazed by domestic sheep appeared to be the source of the virus. Lesions compatible with CE have been seen at other locations, but these usually appear to resolve spontaneously. Coccidioidomycoses was also diagnosed in one bighorn sheep that had been malnourished and held in captivity.
In March of 1990 a *Babesia* spp. was isolated from whole blood from bighorn sheep and inoculated into a variety of test animals (black-tailed deer, white-tailed deer, bighorn sheep, splenectomized calf, and domestic sheep). Although similar to *Babesia capreoli*, this *Babesia* spp. appears to be a previously undescribed organism which was quite pathogenic when inoculated into naive bighorn sheep, causing severe anemia 8-12 days post inoculation. Subsequent research has shown this *Babesia* is also present in mule deer in the same portion of the San Bernardino Mountains as the infected bighorns. *Ixodes pacificus* ticks, which feed on both deer and bighorn, are the likely vector. An indirect immunofluorescent assay was modified to assist in diagnosis and methods for in vitro cultivation were developed.

In 1991 *Anaplasma ovis* was isolated for the first time from a bighorn sheep whole blood sample. The source herd at Old Dad Peak/Kelso Mts. is one of the largest and most productive in California. Previous inoculation trials had shown that captive reared Rocky Mt. bighorn sheep were quite susceptible to challenge suffering severe, potentially fatal, anemia. A retrospective serosurvey revealed that the prevalence of anaplasmosis varied widely, and herds with seropositive animals were from areas where *Dermacentor hunteri* commonly parasitizes bighorn. These findings, and those cited above for *Babesia*, suggest caution in the relocation even of apparently healthy bighorn sheep to locations where they may contact naive bighorn in the presence of appropriate tick vectors.

Epidemiologic studies of population clusters of bighorn, roughly similar to the metapopulations discussed above, revealed distinct differences in patterns of exposure to potential pathogens. A distinct set of bighorn populations in the Peninsular Ranges had higher exposure rates to several pathogens than other populations in the state. Further work on causes of morbidity and mortality in these populations continues. It is clear that these populations are currently being impacted by high levels of mountain lion predation.

Bighorn sheep, deer and cattle that share rangeland in the San Bernardino Mts. have been sampled over a three year period. Bighorn in this area are infected with psoroptic scabies and *Babesia* spp. and cattle evidence exposure to bluetongue and several other pathogens. However, these organisms do not appear to be transmitted between these two species. In contrast, diseases and parasites of domestic sheep may cause serious mortality in bighorn populations. Disease considerations should be seriously considered when relocation of bighorn and/or when changes in grazing practices are considered.

Summary: The amount and quality of information available to the California Department of Fish and Game and other land management agencies on bighorn sheep population status, biology, health and genetics has increased dramatically in the last 15 years. Some of the lessons gained from this are enumerated below.

1). The quality of management and conservation programs for sensitive, widely scattered wild ungulates like bighorn sheep are directly proportional to the quality and quantity of demographic, genetic, and biomedical data available for them. However, quality research and management programs rely on adequate funding.
2). Combined capture, marking, telemetry and biomedical sampling programs can result in tremendous amounts of data being generated over a relatively short period of time.

3). Capture methods for free-ranging wild ungulates can and should be improved to the point that immediate mortality is less than 1% and total combined morbidity (capture stress/myopathy) and mortality (any deaths within 7-10 days of capture) do not exceed 2-5%.

4). Thorough sampling at the time of capture and expeditious processing of samples can allow simultaneous investigation of infectious diseases, genetics, reproduction, physiology and nutrition. Each opportunity to capture and sample free-ranging wild animals is unique and valuable.

5). Current taxonomic designations (subspecific) may not be supported by mitochondrial and/or nuclear DNA and protein electrophoretic studies. You cannot accomplish genetic conservation if you do not know the genetics of your populations.

6). Population management strategies must be based on factual information regarding the genetics and demographics of each population. Geographically scattered populations may not be genetically isolated, and their management requires a sound understanding of the degree and importance of gene flow.

7). Diseases may limit or eliminate some bighorn populations. Pasteurella spp. from domestic sheep are particularly deadly. This same negative relationship may not hold for cattle and bighorn sheep. Diseases may have long term consequences for populations, and in some cases the consequences may not be what we would expect. For example, psoroptic scabies is common and unimportant in California, yet this parasite has devastated herds in other states.

8). Wild animals are biological packages. You potentially relocate viruses, bacteria, protozoa and parasites along with them.

9). The success or failure of wildlife relocation efforts should be investigated, documented, and publicized. We learn from success as well as failure.

ACKNOWLEDGMENTS

The majority of funds supporting the studies summarized above came from the CDFG Bighorn Sheep Management Program. The entire staff of the CDFG Wildlife Investigations Laboratory helped with capture and sampling. Dick Weaver kept the dream of "active" bighorn sheep management alive when the powers that be were "passive", and he infected several young veterinarians and biologists with his love of bighorn sheep. Drs. Vern Bleich and John Wehausen initiated and emphasized the importance of regional management of mountain sheep. The late Dr. Wilmir Hansen of Shikar Safari Conservation International had the vision and tenacity to insist on studies to improve capture methods when nobody else would fund this work. Mazuri Wildlife Foundation, Bighorn Institute, Sacramento Safari Club, and Foundation for North American Wild Sheep helped fund various research efforts described. Steve DeJesus, the late Don Landells, and all the crew at Landell's Aviation facilitated bighorn sheep captures in innumerable ways. Rick Clark, Mike Kock and Nancy Kock of IWVS contributed their professional time and enthusiasm. Grace Lee-Yamagata, Dr. Joanna Mazet, and many other students from the University of California, Davis, helped with field and laboratory aspects of this work.
LITERATURE CITED


Table 1. Bighorn sheep population size class profile and summary by metapopulation (1995).

<table>
<thead>
<tr>
<th>Metapopulation</th>
<th>0</th>
<th>&lt;25</th>
<th>25-50</th>
<th>51-100</th>
<th>101-150</th>
<th>151-200</th>
<th>201-300</th>
<th>&gt;300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peninsular Ranges</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>San Gabriel</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Western Transverse Range</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sonoran</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>South Mojave</td>
<td>7</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Central Mojave</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Central North Mojave</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>North Mojave</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Very Southern Sierra</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Southern Sierra Nevada</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Central Sierra Nevada</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Northeastern California</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>14</td>
<td>17</td>
<td>14</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Bighorn sheep population estimates by metapopulation (1995).

<table>
<thead>
<tr>
<th>Metapopulation</th>
<th>Low</th>
<th>Median</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peninsular Ranges</td>
<td>328</td>
<td>426</td>
<td>524</td>
</tr>
<tr>
<td>San Gabriel</td>
<td>401</td>
<td>500</td>
<td>600</td>
</tr>
<tr>
<td>Western Transverse Range</td>
<td>1</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td>Sonoran</td>
<td>428</td>
<td>514</td>
<td>600</td>
</tr>
<tr>
<td>South Mojave</td>
<td>690</td>
<td>1004</td>
<td>1318</td>
</tr>
<tr>
<td>Central Mojave</td>
<td>355</td>
<td>514</td>
<td>672</td>
</tr>
<tr>
<td>Central North Mojave</td>
<td>278</td>
<td>364</td>
<td>450</td>
</tr>
<tr>
<td>North Mojave</td>
<td>685</td>
<td>967</td>
<td>1248</td>
</tr>
<tr>
<td>Southern Sierra Nevada</td>
<td>202</td>
<td>276</td>
<td>350</td>
</tr>
<tr>
<td>Central Sierra Nevada</td>
<td>51</td>
<td>76</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>3419</td>
<td>4654</td>
<td>5886</td>
</tr>
</tbody>
</table>
Status

- Native
- Reintroduced
- Extirpated

Figure 1. Current and historical distribution of bighorn sheep populations in California.
Figure 2. Current and historical distribution of bighorn sheep metapopulations in California.
HEALTH PROBLEMS OF WILD POPULATIONS OF DESERT TORTOISES, *Gopherus agassizii*, IN THE SOUTHWESTERN UNITED STATES

Elliott R. Jacobson, DVM, PhD and Bruce L. Homer, DVM, PhD
College of Veterinary Medicine, University of Florida, Gainesville, Fl 32610, USA

In 1988, desert tortoises (*Gopherus agassizii*) with upper respiratory tract disease (URTD) were seen in the Desert Tortoise Natural Area (DTNA), Kern County, California. In 1989, a detailed survey of the DTNA and nearby areas in the Rand Mountains and Freemont Valley indicated that 43% of 468 live desert tortoises encountered on the sections surveyed showed signs of this disease. Additionally, carcasses of 627 tortoises were recovered from the sampled areas. Since first being seen in desert tortoises in the DTNA, desert tortoises with URTD have been seen in multiple locations throughout the Mojave Desert of southern California. Desert tortoises with URTD have also been seen in the Las Vegas Valley, Nevada, the Beaver Dam Slope, Utah/Arizona, and the Sonoran Desert, Arizona.

Pathologic studies of 17 ill desert tortoises from the DTNA and one ill desert tortoise from Utah indicated that major microscopic lesions were confined to the upper respiratory tract (URT) of ill tortoises. Electron microscopic studies revealed small (350 to 900 nm), pleomorphic organisms resembling *Mycoplasmia* in close association with the surface epithelium of the URT of ill tortoises. A *Mycoplasma*-like organism was cultured from the nasal passageways of four ill tortoises and was ultrastructurally similar to the pleomorphic organism present on the mucosa in tissue sections. The species name proposed was *Mycoplasma agassizii*; strain PS6 is the type strain. In a recent transmission study, this organism was demonstrated as the cause of URTD in the desert tortoise.

High mortality rates and a shell disease originally described as shell necrosis were observed in the population of desert tortoises in the Colorado Desert, on the Chuckwalla Bench Area of Critical Environmental Concern, Riverside County, California, USA. In a retrospective review of photographic slides of desert tortoises from the Chuckwalla Bench, the disease was evident in 1979 when tortoises on a permanent study site were first photographed. In those tortoises where sequential photographs were taken, the most severe lesions were seen in 1988. While the disease was present on the carapace, plastron and thickened forelimb scutes, the plastron was more severely affected than other areas of the integument. The lesion commenced at seams between scutes and spread toward the middle of each scute in an irregular pattern. Shell biopsies of nine affected tortoises were evaluated by light microscopy. No inflammatory infiltrates were in the lesions and while bacterial organisms were identified in tissue sections, they were superficially located and were considered to be secondary invaders. For the most part, the epithelial cells which formed a pseudostratified layer under affected portions of each scute remained intact. While the location and histological appearance of the lesion was compatible with a dyskeratosis and was suggestive of either a deficiency disease or toxicosis, the exact cause of the disease could not be determined.
Pathological evaluations of 22 desert tortoises, received over a 31-month period from the Mojave and Colorado deserts of California and the Sonoran Desert of Arizona indicated a variety of problems. Seven of these tortoises had shell lesions typical of cutaneous dyskeratosis. Three tortoises had respiratory tract disease, one with fungal pneumonia and the other two with lesions typical of mycoplasmosis. Septicemia was seen in two tortoises, of which one had a cutaneous fungal infection secondary to being entombed within its burrow. Another tortoise had lesions associated with a burn injury. Osteopenia of the shell was seen in two cases.

LITERATURE CITED

REARING AND DISEASES OF CAPTIVE GREATER AND ATTWATER'S PRAIRIE CHICKENS

Mark L. Drew, MSc, DVM*
Department of Large Animal Medicine, College of Veterinary Medicine, Texas A&M University, College Station, Texas, USA

Tom W. deMaar, DVM
Fossil Rim Wildlife Center, Glen Rose, Texas, USA

Introduction

Prairie chickens are gallinaceous birds that occupy grassland habitats in semi-arid parts of North America. In North America, there are three species of grouse that inhabit prairie ecosystems, the sage grouse (*Centrocercus urophasianus*), the sharp-tailed grouse (*Pedioecetes phasianellus*) and the prairie chicken (*Tympanuchus cupido*). There are three distinct subspecies of prairie chickens in North America, the greater (*Tympanuchus cupido pinnatus*), the lesser prairie chicken (*T. cupido pallidicinctus*) and the Attwater's (*Tympanuchus cupido attwateri*).

Historically, Attwater's prairie chickens were found in the coastal prairies of Louisiana and Texas. Prior to 1937, Attwater's prairie chicken populations were estimated at over 1 million birds. In 1937, the Attwater's prairie chicken population was determined to be about 8700 birds, with the range of the birds restricted to Texas. By 1993, the distribution of Attwater's prairie chickens in Texas was limited to 5 counties in Texas and the total number of birds was estimated at 456.

The current Attwater's prairie chicken populations are geographically isolated from other prairie chicken populations and separated into three distinct subpopulations. The population has been declining at about 5% per year since 1937. The Attwater's prairie chicken was listed as endangered by the US Fish and Wildlife Service as well as the Texas Department of Parks and Wildlife in 1983. A recovery plan for the subspecies was written in 1992 with the goal to restore and maintain a genetically viable, self-sustaining free-living population.

A Population and Habitat Viability Workshop for the Attwater's Prairie Chicken was held in January 1994; the Attwater's prairie chicken is predicted to be extinct within 7 years. Despite initial efforts at habitat improvement and acquisition, and predator control, free-ranging populations of Attwater's prairie chickens continued to decline. In 1991, the recovery team elected to initiate a captive rearing program for Attwater's prairie chicken using Greater prairie chickens as a model for developing techniques for the rearing of Attwater's. In 1992, the first Attwater's prairie chickens were introduced into the captive rearing program and the first chicks were hatched.
This presentation will summarize the information gathered to date on rearing techniques and the major causes of morbidity and mortality in the captive propagation efforts.

Materials and Methods

Captive Breeding Facilities

Currently, three facilities in Texas are engaged in captive rearing of Greater Prairie Chickens (GPC) and Attwater’s Prairie Chickens (APC). These three facilities include The Fossil Rim Wildlife Center in Glen Rose, Texas; the Wildlife and Fisheries Science Department of Texas A & M University, College Station, Texas; and the Houston Zoological Gardens in Houston, Texas. Currently, the captive populations of birds at these three facilities consist of 72 Greater and 36 Attwater’s prairie chickens. All three facilities have the capability for egg incubation, chick rearing, housing of juvenile birds, and housing of adult breeding birds.

Captive populations of GPC were started with either wild trapped adults or acquisition of fertile eggs from private individuals with captive prairie chickens. Captive populations of APC were started from eggs collected from nests of wild prairie chickens in each of the three subpopulations.

Captive Rearing Techniques

Rearing techniques are unique to each facility, although some techniques are common to all facilities. Adult birds are generally kept in pairs, trios or small groups during the breeding season. Eggs are collected daily and incubated in standard poultry incubators using standard temperature and humidity settings. Chicks are removed from the incubator at hatching and placed into a hatcher/brooder for 24-48 hrs until the down is dried and the bird is active. Ideally, entire clutches of birds hatch together and the clutch is kept together in small pens or wire cages. As the chicks get older and larger, they are moved to larger cages and/or small pens indoors. Chicks are moved outdoors near the time of fledging and kept in groups until the next breeding season.

Diets for the captive birds vary with the facility. Standard poultry or game bird rations provide the basic adult ration, supplemented with fresh fruits and vegetables. Chicks are started on live insects, poultry or game bird mash, and fruits and vegetables. Water is provided ad lib.

The amount of intervention in the rearing of the birds is variable. All chicks are leg banded and weighed on a regular basis at Fossil Rim and Houston Zoo. Adults are handled more frequently at Texas A&M than at the other facilities.
Veterinary Care

Each captive rearing facility has a resident veterinarian, although the extent of veterinary involvement varies among the facilities. Basic record keeping and laboratory analysis practices vary as well. Dead prairie chickens are usually necropsied by the veterinarian at each facility. Diagnostic specimens or entire carcasses are shipped under refrigeration to the Texas Veterinary Medical Diagnostic Laboratory in College Station, Texas or to the Schubot Avian Disease Center at the College of Veterinary Medicine, Texas A & M University.

Results and Discussion

Captive rearing of GPC and APC has been successful, but increases in the captive population have been slow due to high incubation losses and high chick mortality. Egg production has been good and eggs have been collected from all birds represented in the captive population. Egg hatchability is variable both between facilities as well as between years and ranges from 50-95%.

Initial efforts at rearing GPC in captivity were successful in the first year of chick rearing at each facility. The second year was usually accompanied by high chick mortality, with the predominant loss of chicks associated with salmonellosis. A variety of diseases and parasites have been found in captive reared GPC and APC chicks. The major disease categories are summarized below. Mortality in APC tended to follow that of the GPC.

Trauma

Due to the flighty nature of prairie chickens, trauma occurs with frequency and precautions must be made to prevent or reduce injuries. Most trauma occurs either when birds are being caught for management purposes or in response to startling from a variety of causes. Head injuries, scalping and damage to the cere, and wing injuries were the most common. Fatal injuries due to spinal fractures or dislocations have occurred as well.

Prevention involves catching birds quickly, having a holder or cloth bag for each bird during procedures, wing clipping to minimize flight, minimizing human traffic, and the use of soft cloth netting on the sides and top of pens.

Intraspecific trauma has occurred during brooding of chicks with vent picking and eye abrasions being the most common manifestations. These vices seem to be encouraged by lack of space in small brooder pens and lack of activity. Provision of cage furniture and live insects in large brooder pens have diminished intraspecific trauma.
Salmonellosis

Salmonellosis was identified as the cause of the majority of chick mortality and has been identified in all three captive rearing facilities. A variety of serotypes have been isolated with *Salmonella newport* being predominant at TAMU and *S. typhimurium* at Fossil Rim. Clinically affected birds present as early as 3-5 days of age to as late as 30 days of age. Clinical signs include lethargy, anorexia, decreased activity, yawning, weight loss and ataxia. The most common necropsy findings were caseo-necrotic duodenitis and/or typhlocolitis, and enteritis. Most cases have been non-responsive to antibiotic therapy. Surviving birds have been checked repeatedly for fecal shedding of *Salmonella* spp. and to date; none have been identified.

Caseonecrotic typhlocolitis syndrome

During 1992 and 1993, a large number of chicks at Fossil Rim died with gross and histological lesions of caseonecrotic typhlocolitis. Affected chicks ranged from 6-75 days of age and most died after a sub-acute or acute clinical course. Laminar caseous cores were seen in the caeca on gross necropsy and consisted of feed material, cellular debris, fibrin and many bacteria. Multifocal superficial epithelial necrosis was evident on histologic evaluation. A layer of degenerating epithelial cells, predominantly mononuclear inflammatory cells, and some heterophils lined the luminal surface. Antibiotic therapy was unsuccessful in preventing mortality; supportive care prolonged survival, but did not alter the outcome.

To date, no definitive etiological agent was been identified. Bacteria cultured included *E. coli, Pseudomonas aeruginosa, Enterococcus* spp., *Enterobacter* spp. and *Clostridium perfringens*. Toxins of *Clostridium difficile* were detected in some samples. No virus, fungal or protozoal agents have been identified.

No cases were seen at Fossil Rim in 1994 after numerous management changes were made, including furnishing obstacles to induce exercise and large numbers of live insects as a food source.

Chick enteritis

Two other identifiable enteric conditions were identified in chicks manifested as soft stools or mortality. A peracute enteritis in 3-5 day old chicks occurred at Fossil Rim in 1992 and 1993. Bacteria cultured included *E. coli, Pseudomonas aeruginosa, Enterococcus* spp., *Enterobacter* spp. and *Clostridium perfringens*. Toxins of *Clostridium difficile* were detected in some samples. These cases appeared to respond to amoxicillin in the drinking water in 1992 and 1993, and no mortality occurred in 1994 when all chicks were placed on oral amoxicillin from 3- to 8-days of age.

An enteritis also occurred in chicks from 10-70 days of age with similar bacteria being isolated. This condition occurred during antibiotic therapy and all bacteria cultured were resistant to the antibiotic currently being used in the birds.
Antibiotics used in these birds included amoxicillin, bacitracin, tetracycline, sulfamethazine and gentacin. Antibiotic therapy did not stop morbidity, but did decrease mortality. Each time antibiotics were discontinued, clinical signs of enteritis appeared.

**Respiratory disease**

A few chicks have died of pneumonia or tracheitis, however, no flock outbreaks have been seen in any of the facilities. Disease appeared to be related to drastic weather changes and heavy precipitation. Symptomatic therapy with antibiotics, oral and subcutaneous fluids and exogenous heat aided recovery and minimized clinical signs in chicks that had contact with affected birds.

**Parasites**

*Dispharynx nasuata* was found in several greater prairie chickens that died at 5-8 months of age. Severe proventricular thickening was seen on necropsy. Control of this parasite has been done with environmental changes that discourage the intermediate host (sowbugs or pillbugs) and anthelminthics, especially ivermectin and fenbendazole.

Eggs of *Capillaria* spp. and *Ascaridia* spp. have been found on routine fecal flotation, however, the low numbers of oocytes have not been of concern. Periodic and regular worming schedules may help to minimize the numbers of these parasites.

Coccidiosis has been a concern for the captive rearing program due to the susceptibility of gallinaceous birds to this parasite. Several chicks have died with clinical signs suggestive of coccidiosis. The addition of amprolium at prophylactic levels in the drinking water has been found to prevent excessive infections, minimize morbidity and limit mortality.

Lice and chiggers have been seen in low numbers on several birds, but no morbidity or mortality has been associated with the presence of these parasites. Routine application of sevin dust to sand in dust bath areas within the pens is being used as a control measure.

**Developmental anomalies**

Three types of developmental anomalies have been seen in chicks. Wry neck or curvature of the cervical vertebrae in recently hatched chicks was seen in two of the three facilities. All birds died despite symptomatic and supportive care.

Deviated toes were seen in several chicks. Corrective splints applied for 2-3 days usually improved the positioning of the toes.

Tibiotarsal rotation was observed in several chicks between 10-20 days of age. No etiologic agent could be identified, although a few individuals with tibiotarsal rotations also had severely deformed feet or toes. The rotations were usually unilateral, although a few chicks
had bilateral rotations. Prosthetic devices did not improve the rotation and most chicks with tibiotarsal rotations died of bacterial infections.

**Miscellaneous conditions**

Yolk sac infections were seen in a small number of chicks. No specific etiologic agent was identified.

Two chicks died with cardiomyopathy of unknown etiology. Both birds were from the same subpopulation of APC, but the link may be spurious as apparently healthy birds have been raised from eggs collected from the same clutches as these two birds.

Histomoniasis was tentatively diagnosed in one GPC chick based on gross necropsy findings of discoloration of the liver. The organism was not definitely identified and the actual cause of the disease and its importance to captive rearing is unknown.

Proventricular impactions with grass have been identified in several young chicks and at least two adult wild-caught APC at TAMU. The impactions are presumed to be stress related and may indicate an unfamiliarity with feed offered in captivity.

Mortality of adult GPC and APC is has occurred, but only in low numbers. Mortality has occurred due to trauma, coccidiosis, and avian pox. Clinical reticuloendotheliosis has been identified in birds of one facility.

**Summary**

The captive propagation effort for these birds has not been in effect long enough to work out all of the problems that have been encountered. Mortality has been higher that expected and from unexpected diseases. However, as problems are defined, control strategies have been developed and appear to be working for the control of certain diseases and syndromes.

If the wild populations continue to decline, extinction of the subspecies is inevitable. If the decline is caused or hastened by similar disease conditions as seen in the captive birds, control of these diseases in wild populations is not possible at this time.

The fact that several diseases seem to be present within the captive population makes release of captive reared birds tenuous, at least until birds can be raised in disease free facilities or be tested and declared disease free prior to release. Releasing diseased birds into marginal habitats with small remnant populations is not acceptable. The challenge now is to develop methods to raise large numbers of birds with minimal disease problems for release into the wild in as short a time span as possible.
ACKNOWLEDGEMENTS

Many people have contributed to the captive rearing program. Steve Labuda and Mike Morrow of the USFWS, Nova Silvy, David Drake, Elizabeth Osterndorf, and Marcus Peterson of TAMU, Wildlife and Fisheries Sciences, Evan Blumer, Bruce Williams, Bob Smith, and Kelly Snodgrass of Fossil Rim, Bob Flase and Bill Wige of TVMDL, Joe Flanagan and Chelle Plasse of Houston Zoo have been intimately involved in many aspects of the program. Many other individuals in many disciplines have contributed their expertise.
BLUETONGUE AND AFRICAN HORSE SICKNESS VIRUSES INFECT CARNIVORES

K. A. Alexander, DVM*, P.W. Kat, PhD, N. J. MacLachlan BVSc, PhD, B. L. Osburn, DVM, PhD
Department of Pathology, Microbiology & Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

C. House, PhD
Foreign Animal Disease Diagnostic Laboratory, U.S. Department of Agriculture, Greenport, NY 11944, USA

African horse sickness (AHS) and bluetongue (BLU) are diseases of ruminants caused by orbiviruses, and are principally transmitted by Culicoides midges. AHS viruses mainly infect equids, but the host spectrum is known to include a number of other mammals like camels, boids, African elephants, and domestic dogs. AHS viruses were confined to Africa south of the Sahara, but during the last fifty years have spread to the Middle East, central Asia, and the Indian subcontinent as well as northern Africa and the Iberian peninsula. This has caused understandable concern within the European Community about the possibility of further spread to adjacent geographic areas, and calls have been made for a better understanding of the epizootiology of the disease. African horse sickness has long been known to infect and cause mortality among domestic dogs that ingest virus contaminated meat. We determined widespread natural AHS infection among a diversity of African carnivore species with a blocking enzyme-linked immunosorbant assay (ELISA) and microtiter virus neutralization tests. Neutralizing antibodies to five of nine AHSV serotypes were identified in carnivore sera. We hypothesize that such infection resulted from ingestion of meat and organs from AHS-infected prey species; highest seroprevalence levels were noted among large carnivores like lions and hyenas that regularly prey on zebras. The effect of AHS on the carnivores is unknown, as is their role in the maintenance cycle of the disease.

Bluetongue is an International Office of Epizootics List A disease described as the century’s most economically devastating affliction of sheep. Bluetongue viruses (BLU) were thought to infect only ruminants, shrews, and some rodents, but recently, inadvertent administration of BLU contaminated vaccine resulted in mortality and abortion among domestic dogs. Sera from a diversity of African and Indian carnivore species with a blocking enzyme-linked immunosorbant assay (ELISA) and microtiter virus neutralization tests. Neutralizing antibodies to five of nine AHSV serotypes were identified in carnivore sera. We hypothesize that such infection resulted from ingestion of meat and organs from AHS-infected prey species; highest seroprevalence levels were noted among large carnivores like lions and hyenas that regularly prey on zebras. The effect of AHS on the carnivores is unknown, as is their role in the maintenance cycle of the disease.
cattle. The effect of BLU infection on endangered carnivores like the cheetah and African wild dog requires urgent investigation. Also, the role of carnivores in the epizootiology of this disease needs elucidation.


THE RETURN OF ARABIAN ORYX, Oryx leucoryx, TO THE SAUDI ARABIAN EMPTY QUARTER: DISEASE MONITORING FOR THE REINTRODUCTION PROCESS

Marc Ancrenaz, DVM*, Stéphane Ostrowski, DVM, Alain Delhomme, Technical Breeder

National Wildlife Research Center, P.O. Box 1086, Taif, Saudi Arabia

Introduction

The Arabian oryx, Oryx leucoryx, became extinct in the wild in 1972. An intensive captive breeding program was established at the National Wildlife Research Center (NWRC), Taif, Saudi Arabia, in 1986 for the propagation and reintroduction into the wild of this antelope. Following successful reintroduction into the Mahazat as-Sayd reserve, the National Commission for Wildlife Development and Conservation decided to release Arabian oryx into the desert of Rub al Khalib or Empty Quarter. Thirty-three animals were translocated and released in 'Uruq Bani Ma'arid protected area at the beginning of 1995.

This paper presents the evaluation, the monitoring, and the control of disease agents with the potential to jeopardize the reintroduction process. Disease is here defined as "any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxicants, and climate; infectious agents; inherent or congenital defects, or combinations of these factors."

Monitoring and control of infectious agents

The translocation and reintroduction of captive Arabian oryx bred at the NWRC carries with it the risk of introduction of new diseases into the release area by the translocated oryx, and the risk that the released animals themselves will be vulnerable to diseases normally present at the release site.

Sanitary care of the captive Arabian oryx bred at the NWRC

Shortly after the captive breeding of oryx was established at the NWRC, an outbreak of tuberculosis (TB) caused by Mycobacterium bovis occurred in the founder stock (called A-generation). All the oryx present in the breeding were considered infected and were treated with isoniazid (10 mg/kg body weight), ethambutol hydrochloride (15 mg/kg BW), and rifampicin (10 mg/kg BW). The treatment was given in drinking water every day for 9 months. Since the outbreak of TB, the calves (called B-generation) born from the founder animals are removed from the dam immediately after birth, and are hand-reared. The B generation oryx in turn produce C-generation, mother-bred animals, suitable for reintroduction into the wild. Indirect and comparative ELISA tests are carried out regularly on the whole captive herd. Results of the serological tests show that the sanitary and medical measures undertaken at the NWRC have been successful in eradicating tuberculosis (Table 1). The releasable C-generation animals are free of tuberculosis.
Serological surveys carried out on the captive oryx showed that *Brucella abortus*, *Pasteurella multocida* (types B and D), and *Akabane virus* occurred with an extremely low prevalence in the captive-breeding, while *Coxiella burnetti*, *Chlamydia psittaci*, *P. multocida* (type A), and *Parainfluenza 3 virus* occurred with a low but significant prevalence (Table 2). Infected animals are isolated from the reproductive herd, for as long as sero-conversion is associated with clinical symptoms.

The relatively high number of sero reactions against *P. multocida* and the absence of clinical symptoms confirms the possible free carriage of this bacterium in Arabian oryx. Stressful situations related with capture operations, crating, and transportations tend to produce a clinical recrudescence of a latent pasteurellosis in the captive oryx. In 1991 and 1992, four oryx died of acute pasteurellosis within 10 days following translocation. All the captive born animals are now injected with an inactivated vaccine (Lysopast, virus strains A and D, Rhône-Mérieux, France) at birth and once every year thereafter.

Lumpy skin disease (LSD) was first identified in Saudi Arabia in a captive Arabian oryx in 1989 (Ethiopia 1 strain). One adult female showed a symptomatic infection and spontaneously recovered. Sheep freshly imported from African countries with LSD, could have been carriers of the virus and the origin of this infection. Arabian oryx appears to be fairly resistant to Capripoxviridae infection and since 1989, no symptoms of LSD infection have been recorded at the NWRC.

In order to avoid contact or even close proximity between the domestic stock and the oryx held at the NWRC, a fenced cattle-free area of 40 square kilometers has been established to isolate the captive breeding from the surrounding grazing range.

In addition, coprologic analyses are frequently carried out and show that a very low level of internal and external parasitism occurs in the breeding. All the animals are injected with 0.2 mg/kg BW SC ivermectin (Ivomec, MSD AGVET, Paris, France) at least once a year.

**Diseases encountered by translocated animals at the release site**

The Arabian oryx appear to be susceptible to most pathogenic bacteria and viruses which may affect domestic ungulates. According to the data available from the FAO Animal Health Yearbook and from the Saudi Ministry of Agriculture, the main infectious diseases present in the Kingdom are brucellosis, rinderpest, peste des petits ruminants, foot and mouth disease, bovine enzootic leukosis, and rabies. All captive oryx are vaccinated annually against rinderpest (Kabete strain type 0, Saudi Arabia Veterinary and Vaccines Institute, Saudi Arabia), rabies (Rabisin, Rhône Mérieux, France), foot and mouth disease (Aftovax, virus types O and C Asia 1, Rhône Mérieux, France), and pasteurellosis.

Anthrax and botulism occur sporadically in Saudi Arabia. Previous veterinary surveys failed to show that these diseases were enzootic in the proposed release sites of Arabian oryx within the Kingdom. So far, the oryx to be released have not been vaccinated against these
two diseases. In Oman, botulism caused the death of three adult zoo-bred Arabian oryx imported from USA and reintroduced into the wild.17

Sanitary preparation of the oryx to be released

Two months prior to the relocation, the oryx are isolated from the rest of the herd, kept in pre-translocation enclosures, and are boma-trained. During this period, they are injected with a booster dose of rinderpest, rabies, foot and mouth disease, and pasteurellosis vaccines. They are also dewormed and treated against external parasites. All the animals are blood sampled. Serial sera are kept frozen in a sera bank for each individual. Tuberculosis ELISA tests are carried out: two successive negative results are required before the reintroduction of any oryx. During the period of boma training, the animals are under a close veterinary surveillance and only the clinically healthy animals are moved to the new protected areas.

Monitoring and control of genetic fitness

Genetic constitution of the oryx population to be released

The genetic management program held in Taif aims to maintain at least 90% of the genetic variation in the original population over a period of 200 years16 and to produce animals genetically fitted to survive in the wild. This goal is achieved in different ways.

- Maximization of the genetic diversity: Although the pedigree of most founders originating from the Late King Khalid Farm at Thumamah was unknown, a study of allozyme variation showed that the mean heterozygosity in the founder stock was relatively high and reflected the diverse origins of the animals.18 In order to maximize the genetic diversity of the founder stock, animals from Bahrein (2.1), Qatar (4.1), Abu Dhabi (1.1) and USA (4.0) were imported from 1990 to 1994. These animals were included in the A generation of oryx to guarantee a good spread of their gene pool. Today, the captive herd of oryx held in Taif is the most genetically diverse in the world.

- Equalization of the Founder Representation and maximization of the effective population size: Because of the management of three different generations, some founders are still under-represented in B- and C-generations. When groups of captive-bred oryx are reintroduced in a new protected area, the missing or under-represented genetic lineages are added via the importation of oryx originating from Europe or via the relocation of wild born animals caught in Mahazat as-Sayd reserve.

- Management of the inbreeding: In small captive populations of ungulates, inbreeding induces a reduction in survival and reproduction rates and a decrease in resistance to disease.15 The effects of the inbreeding depression become apparent when the inbreeding coefficient reach a value of 0.125 (one common grandparent). In Oman, survival of
wild-born juvenile Arabian oryx was reduced when the inbreeding coefficient exceeded 0.076;\(^{17}\) far below the 0.125 value. This might reflect the more severe selective pressures to which desert-born oryx calves are subject, compared to animals kept in captivity.

In Taif, since the pedigree of the founders coming from Thumamah is unknown, it has been assumed that two different founders had one common grandparent. The matings to produce B and C generation oryx are managed in such a way to as possible avoid any known genetic relationships between the sire and the dam. Inbreeding coefficients of B and C generation oryx are ranging between 0.016 and 0.031, 0.008, and 0.031 respectively.

**Management of the 17:19 Robertsonian Translocation**

Cytogenetic studies of the captive population held in Taif revealed the presence of a chromosomal Robertsonian Translocation resulting from the fusion of the chromosomes 17 and 19.\(^4\) The translocation spread out from oryx imported from Qatar, and is inherited according to a Mendelian co-dominant mode. A 17:19 translocation is not usually expressed phenotypically, but a reduced fecundity in heterozygotes has already been reported in cattle.\(^{12}\) Following a workshop of the International Wild Arabian Oryx Panel, held in 1990 in London, it was decided to not reintroduce the carriers of the translocation into the wild, but to reintroduce only individuals with the normal karyotype (2n=58). The translocated oryx do not take part in the captive breeding programs, with the exception of a few individuals that have a high genetic value. Before being reintroduced in protected areas, the oryx are systematically karyotyped.

**Environmental factors**

The aim of reintroducing oryx in Saudi Arabia is to re-establish the species in several populations within the Saudi Arabian range that was documented in the 1930's. Today, five protected areas totaling about 5000 square km and established within the former range of the species are managed by the NCWCD and are potential sites for the reintroduction of oryx.

**Previous reintroduction of Arabian oryx in Mahazat as-Sayd**

The first reintroduction of oryx in the Kingdom was carried out in 1990 in Mahazat as-Sayd, a 2200 square km protected area.\(^{10}\) A study carried out to assess the quantity and quality of the plants preferred by oryx showed that the area could meet the species' nutritional requirements.\(^3\) A fence was erected to preclude illegal hunting and domestic stock. Sixty-eight oryx representing from all the different blood lines of Arabian oryx found in the world, were released between 1990 and 1993. The population of Arabian oryx has shown a rapid constant growth and at the beginning of 1995, it numbered 180 animals. The sex and age structure reflected a good future reproduction potential with a predominance of young animals. Natural mortality rates have been very low and the main cause of mortality was injuries inflicted during fights, with at least 12 recorded instances. When reintroduced into
suitable protected habitats, captive born oryx have shown that they were able to survive and to generate self-sustainable populations. In Mahazat as-Sayd, the released animals have survived without drinking; there is no permanent water point in the reserve. They also learnt to graze the native vegetation with no apparent ill-effects.

**Reintroduction of Arabian oryx in Uruq Bani Maʿarid**

Wildlife vanished from Uruq Bani Maʿarid as a result of over-hunting. The reserve is a complex linear system of reddish sand dunes dissected by numerous eastward-draining wadis. The wadis are covered by a rich and varied vegetation suitable for the reintroduction of Arabian oryx. Thirty-three oryx (28 captive born animals, bred at the NWRC; 5 wild born animals caught in Mahazat as-Sayd) were translocated and released in early 1995 into the unfenced reserve. The two main threats to the operation are the occurrence of illegal hunting and the heavy overgrazing associated with large numbers of camels found in the reserve. Camel surveys have shown that between 100 and 300 camels graze in the 2000 square kilometer core area of the reserve where the oryx have been released. This may result in strong competition for food between the released animals and the camel herds.

**Conclusion**

When illegal hunting and competition for food with domestic animals were prevented, previous reintroductions of Arabian oryx in Saudi Arabia have shown that healthy captive-born animals are able to establish self-sustaining, free-ranging populations. The new reintroduction process of Arabian oryx carried out in the Empty Quarter will determine if animals are still able to survive in the wilds of Saudi Arabia, where environmental conditions have been extensively modified as a result of the tremendous increase in domestic animal numbers, since the extirpation of the oryx.

**LITERATURE CITED**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>48</td>
<td>55</td>
<td>45</td>
<td>42</td>
<td>43</td>
<td>44</td>
<td>-</td>
<td>47</td>
<td>49</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>17</td>
<td>31</td>
<td>61</td>
<td>85</td>
<td>-</td>
<td>109</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>Low risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>37 (77%)</td>
<td>25 (45%)</td>
<td>11 (24%)</td>
<td>30 (72%)</td>
<td>39 (91%)</td>
<td>38 (86%)</td>
<td>-</td>
<td>15 (31%)</td>
<td>45 (92%)</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>-</td>
<td>8 (50%)</td>
<td>17 (100%)</td>
<td>29 (94%)</td>
<td>52 (85%)</td>
<td>77 (91%)</td>
<td>-</td>
<td>100 (92%)</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15 (100%)</td>
<td>11 (100%)</td>
<td>-</td>
<td>-</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>Doubtful</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>9 (19%)</td>
<td>22 (40%)</td>
<td>25 (56%)</td>
<td>6 (14%)</td>
<td>3 (7%)</td>
<td>6 (14%)</td>
<td>-</td>
<td>29 (61%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>-</td>
<td>7 (44%)</td>
<td>0</td>
<td>2 (6%)</td>
<td>9 (15%)</td>
<td>8 (0%)</td>
<td>-</td>
<td>9 (8%)</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>High risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2 (4%)</td>
<td>8 (15%)</td>
<td>9 (20%)</td>
<td>6 (14%)</td>
<td>1 (2%)</td>
<td>0</td>
<td>-</td>
<td>4 (8%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>-</td>
<td>1 (6%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE 1. Progression of tuberculosis infection in the N.W.R.C. Arabian oryx herd from 1986 to 1994.
### TABLE 2. Prevalence of antibodies to bacterial and viral pathogens in the captive herd of Arabian oryx held at the NWRC.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pasteurella multocida</em> (type A)</td>
<td>5/19</td>
<td>4/38</td>
<td>5/58</td>
<td>6/78</td>
<td>-</td>
</tr>
<tr>
<td><em>Pasteurella multocida</em> (type B)</td>
<td>0/19</td>
<td>0/38</td>
<td>1/58</td>
<td>0/78</td>
<td>-</td>
</tr>
<tr>
<td><em>Pasteurella multocida</em> (type D)</td>
<td>0/19</td>
<td>0/38</td>
<td>4/58</td>
<td>1/78</td>
<td>-</td>
</tr>
<tr>
<td>Lumpy skin disease</td>
<td>1/19</td>
<td>1/38</td>
<td>1/58</td>
<td>2/78</td>
<td>-</td>
</tr>
<tr>
<td><em>Parainfluenza</em> 3 virus</td>
<td>6/19</td>
<td>6/38</td>
<td>10/57</td>
<td>12/78</td>
<td>0/13</td>
</tr>
<tr>
<td><em>Coxiella burnetti</em></td>
<td>0/18</td>
<td>4/35</td>
<td>7/50</td>
<td>6/72</td>
<td>2/13</td>
</tr>
<tr>
<td><em>Chlamydia psittaci</em></td>
<td>1/18</td>
<td>0/35</td>
<td>2/51</td>
<td>5/74</td>
<td>0/13</td>
</tr>
<tr>
<td><em>Brucella abortus</em></td>
<td>0/19</td>
<td>1/38</td>
<td>0/56</td>
<td>1/78</td>
<td>0/13</td>
</tr>
<tr>
<td>Bluetongue virus</td>
<td>0/19</td>
<td>0/38</td>
<td>0/58</td>
<td>1/78</td>
<td>-</td>
</tr>
<tr>
<td>Akabane virus</td>
<td>0/19</td>
<td>0/38</td>
<td>0/58</td>
<td>0/77</td>
<td>-</td>
</tr>
<tr>
<td><em>Paratuberculosis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/13</td>
</tr>
<tr>
<td><em>Leptospirosis</em> (13 serovars)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/13</td>
</tr>
<tr>
<td>Bvine viral diarrhea</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/13</td>
</tr>
<tr>
<td>Leucosia</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/13</td>
</tr>
</tbody>
</table>
A SURVEY OF CAUSES OF MORTALITY IN CAPTIVE, CAPTIVE BRED RELEASED, AND WILD BORN HOUBARA BUSTARDS (*Chlamydotis undulata*) IN SAUDI ARABIA

Stephane Ostrowski, DVM* and Olivier Combreau, PhD
National Wildlife Research Center, PO Box 1086, Taif, KSA

Introduction

The houbara bustard (*Chlamydotis undulata*) is a medium-sized bustard of slender appearance, measuring 55-65 cm and weighing from 900-2400 g. The breeding distribution of the species range from Canary Islands though North-Africa, the Arabian peninsula east into Mongolia. Of all the bustards the houbara is the most adapted to desert environment. In the zone of distribution of the species, annual rainfall rarely exceeds 200mm.

In the 1980 summary of the world status of the houbara bustard, it was listed excessive hunting, overgrazing, agricultural development as probable reasons for the houbara bustard population decline. For the past twenty years, the species has significantly declined in at least 15 of the 20 countries in its range.

Beginning in 1986, a houbara bustard captive breeding program was undertaken by the National Commission for Wildlife Conservation and Development in order to return this extirpated species to the Saudi Arabian land. Previous experiments have shown that hand-reared houbara bustards are most suitable for captive breeding. Therefore, it was decided that the breeding flock should mostly comprise hand-reared birds. For this purpose, five expeditions to different houbara breeding areas were undertaken. Eggs and one-day old chicks were collected in 1986, 1987 and 1988 in Algeria (*undulata* subspecies) and Pakistan (*macqueenii* subspecies). Additionally, a releasing program was set up in Mahazat As-sayd, a fully protected fenced area of 2,300 km² from where domestic livestock have been totally excluded in 1989. First release of captive-bred houbara bustards were conducted in the area in 1991 by the NWRC.

Material and methods

Survey data included individual animal records, pathology records and population data from the NWRC computer database system. Pathology records were reviewed, and pathological findings were tabulated by etiological findings. From 1989 to 1994, 370 (167 subadult/adult birds and 203 neonate/juvenile birds) carcasses or remains of captive-born and wild-born birds were collected, and full post-mortem examinations were conducted at the National Wildlife Research Center of Taif. Additionally, 58 carcasses or remains of captive bred released birds were also examined.

Results

Post-mortem results on captive birds Analysis of post-mortem examinations performed between 1989 and 1994 on captive-born birds showed a marked decrease of mortality rate
both in sub-adult/adult (>1 yr-old) birds and neonates/juveniles (<1 yr-old). The mortality rate among sub-adult/adult birds dropped from 22.1% in 1989 to 4.3% in 1994 (Fig.1), and among juveniles/neonates, from 56% in 1989 to 19.8% in 1994 (Fig.2). No statistical differences of mortality rate were observed neither between males and females nor between undulata and macqueenii sub-species. Among juveniles/neonates, 28% (n=57) of deaths were due to infectious diseases, 29% (n=59) to accidental traumas, 7.9% (n=16) to ventriculus impaction and perforations, 14.4% (n=29) to neonatal diseases, 14.4% (n=29) were other cases and 6.4% (n=13) were of unknown etiology (Fig.3). Among sub-adult/adult captive-bred and wild-born birds, 43.7% (n=73) of deaths were due to accidental traumas, 28.1% (n=47) to infectious diseases, 16.8% (n=28) were of unknown origin, 8.4% (n=14) were other cases, and 3% (n=5) were due to foreign bodies and ventriculus perforations (Fig.4). Evolution of etiologies showed a marked drop of infectious diseases.

Infectious syndromes

Main infectious syndromes (Tab. 1) encountered in juvenile, immature and adult birds were:

- Multifactorial enteritis/peritonitis syndrome involving adult/subadult birds (n=27) associated with several pathogens such as: Chlamydia psittaci, an Herpesvirus, Pseudomonas aeruginosa and Klebsiella pneumoniae bacteria.

- Respiratory tract infections, with sinusitis, bronchitis and pneumonia (n=30), associated with several viral agents such as: Newcastle disease virus, a poxvirus and surinfecting bacteria, Escherichia coli, Proteus mirabilis.

- Upper digestive tract infections due to poxvirus (n=8) and trichomoniasis (n=3).

- Hepatitis and cholangiohepatitis (n=26) with disseminated necrosis of hepatocytes secondary to sepsis from herpesvirus infection and Chlamydia psittaci.

- Interstitial nephritis and glomerulonephritis (n=5) associated with combined bacterial infection.

- Generalized neoplasia (n=3) associated with reticuloendotheliosis virus.

Traumas

It was often difficult to prove the traumatic origin of deaths, as lesions could be very discrete. Complete epidemiological and pathological analyses were usually required. Traumas never occur in birds less than one month old. The youngest bird dead of trauma was 32 days old. After one month of age all birds are vulnerable to traumatic accidents. Those particularly susceptible are: stressed birds; non-domesticated birds; birds that have not had their wing feathers cut; birds with only one wing cut (they flip over when jumping). The particular events that precede trauma are: visit of "unknown" people; attempts to catch
the bird; manipulation. Necropsy rarely yield obvious indications. Ecchymoses in the calvarium were frequently observed (86% of all cases), but they were most frequently agonal pooling of blood within the skull and were rarely indicative of head trauma. Sometimes concomitant and associated lesions (wing and leg fractures, luxations) could lead to traumatic-origin conclusion. Most frequently observed lethal traumas were thoraco-lumbar vertebral lesions inducing a medullar compression (n=51), cervical luxations (n=28) and head trauma (n=11). When death was delayed, differential diagnosis had to be done to discriminate between nervous manifestations of some infectious diseases and toxic effects.

Neonatal deaths concerned birds of less than one month of age. They included, anoxic chicks, maternal neglect, yolk sac retention/ infection, dehydration/ emaciation syndrome, infectious related diseases (Fig.5). Yolk sac infection and anoxia were responsible of the majority of deaths during the first week of life.

Incidental findings in wild caught houbara bustards (n=21) included a high infestation with gastrointestinal nematodes (Harteria rotundata, Histiocephalus choristidias, Subulura brumpti), cestodes (Raiiilletina subgenus parionella sp. and Idiogenes oitis) and evidence of subcutaneous larvae of Ascaridia or Spirurida.

Post-mortem results performed on captive-bred released birds II

Houbara bustards released in Mahazat as-Sayd reserve were all captive-bred in Taif breeding center. Between 1991 and 1993, four different experimental release techniques were tested: hard release directly into the wild, feather-cut release in a pre-release enclosure, covey release and subadult release. Results of mortality during the experimental releases performed between April 1992 and November 1994 are presented in Table 2. A total of 47 houbara out of 82 (57%) released in good condition and which did not die for other reasons after release were killed by predators either inside or outside the pre-release enclosure. Out of 47 deaths related to predation, six (12.6%) were due to raptors, two (4.2%) to ravens and 39 (82.9%) to red foxes (Vulpes vulpes arabica). Despite all birds were radio-fitted and checked daily, post-mortem examination was often difficult to carry out, as part or all the carcasse can already be eaten. Frequently only feathers and radio-transmitter remained. When fox-predated, carcasses were generally found completely eaten, part of the body could be found buried, transmitter damaged with tracks of bites noticed on it. Footprints were often observed around the remains of dead bird. The way feathers were always cut rather than pulled out of the body was another argument for mammalian predation. Raven predation was observed on very young flightless birds. It occurred inside the pre-release fox-free enclosure. Eyes and visceral organs were always eaten first. Raptors predation seemed to occur on debilitated (feather cut) birds. Feathers were pulled out instead of being cut. Distinct beak tracks were visible on feathers.

Diseases have killed directly, or have led to the removal from release experiments, 11 houbara bustards during 1992-94. Five cases were due to a respiratory tract disease (sinusitis, and deep ulcerative bronchitis) caused by a combined infection of a pox virus and opportunistic bacteria. One case was due to a debilitating ocular pox lesion (the bird was...
unable to feed). In addition, four birds suffered from an unidentified respiratory tract disease prior to release in 1994. Paramyxovirus type 2 (Yucaipa strain) was isolated from feces. However, as it is often the case, there was no substantial evidence that it was this infectious agent that affect primarily the birds.

Discussion

In the initial planning stages of reintroduction project, a disease and medical problem preventive medicine program should be developed. Diseases and pathology of otidiformes were poorly documented. Many pathological events occurred during the first years of captivity in this species allowing the collection of data about infectious agents, traumatism therapy and predation impact. Definition of the diseases in captivity of the species, allowed us to develop a preventive medical program. High rate of traumatic-origin deaths was paradoxically linked to the sanitary effort accomplished during six years. Necessity to handle very stressful birds for medical prophylaxis, construction of non-permissive enclosures to prevent access of wild birds in the breeding unit, and erection of "non-smooth" fences to avoid neighboring wild predators (red foxes, feral cats, eagle owls...) to enter the rearing facilities were responsible of the majority of traumatic deaths. Attempts to captive-breed under intensive conditions and following strict sanitary rules a very fragile species inevitably led to a degree of environmental unadaptation.

Poxvirus infection and subsequent bacterial infection was common in translocated houbara bustards in mahazat as-Sayd reserve. Fifteen cases of confirmed cutaneous pox were observed. This caused 10 (67%) of the birds infected to die either directly from infection (5 cases) or from predation (5 cases). However, 5 birds preyed upon by carnivores out of 10 survivors is not significant when compared to non-infected birds and suggests that pox infection when subsequent bacterial infection was not directly lethal, was not a major problem to captive-bred houbara bustards. Since 1994 vaccination of birds with a different strain (canary strain instead of fowlpox strain) has proved to be more efficient.

Assessment of the primary cause of death in released animals can be very difficult to carry out with certitude, as many predators are known to be also scavengers. Careful attention should be made not to overestimate the proportion of primary predation deaths. In the present reintroduction program, a predator control experiment (trapping and translocation of carnivores) around the pre-release enclosure showed a 36% decrease of general mortality within the controlled area. Comparative mortality with and without predator control was significantly (Chi2=14.4, d.f.=1, P<0.001) decreased during predator control experiment. This results suggests that predation by carnivores was the major primary cause of death among released birds found already eaten.

Conclusions

An effort was made in reviewing the necropsy reports to determine the most significant pathologic findings leading to death. There were many cases that had multiple organ system involvement, making the determination difficult. The incidence of traumatic deaths (29%
of juvenile deaths and 43.7% of immature/adult deaths) may warrant a review of husbandry in rearing situations. In the planning stages of a reintroduction project, the goal of a good preventive medicine program should be to prevent the introduced species from contracting disease from animals indigenous to the reintroduction area. In the present case satisfactory survival rate among captive-bred released birds tend to prove that medical preparation of the houbara bustards released was successful. Although all released birds were carefully checked for different infectious agents to avoid introduction of diseases into the area's indigenous animal population, regular disease monitoring of the released population will be carried out in the next years. Furthermore, continuous evaluation of the diseases of other species in the release area will be of great benefit to the overall knowledge of infectious agent exposure to the released houbara bustards.

LITERATURE CITED

Fig 1: Histogram showing evolution of mortality rate (%) among adult/sub-adult captive birds between 1989 and 1994 (number of bird deaths per year divided by the average number of birds in the captive breeding unit each 12 month period).
Fig 2: Histogram showing evolution of mortality rate (%) among juvenile/neonate captive birds between 1989 and 1994 (number of bird deaths per year divided by the average number of birds in the captive breeding unit each 12 month period).
Fig. 3: Summary of causes of death on 203 necropsies of juvenile/neonate captive houbara bustards performed at the NWRC between 1989 and 1994

![Pie chart showing causes of death for juvenile/neonate captive houbara bustards.](chart)

- Infectious: 28.00%
- Trauma/stress: 29.00%
- Hernia of the yolk: 3.50%
- Dehydration: 4.00%
- Unknown: 6.40%
- Ventriculus impaction: 7.90%
- Others: 14.30%

n = 203

Fig. 4: Summary of causes of death on 167 necropsies of adult/sub-adult captive houbara bustards performed at the NWRC between 1989 and 1994

![Pie chart showing causes of death for adult/sub-adult captive houbara bustards.](chart)

- Infectious: 28.10%
- Trauma/stress: 43.70%
- Unknown: 16.80%
- Ventriculus impaction/Perforation: 3.00%
- Others: 8.40%

n = 167
Fig 5: Summary of mortality of captive neonate Houbara bustards between 1990 and 1994, showing etiological distribution during the first month of life.
Table 1: Summary of pathology and microbiology findings on 104 necropsies performed at the National Wildlife Research Center and related to infectious syndromes. Each case may have had multiple pathologic findings. A primary finding is defined as either the cause of death or the most significant finding present in a particular case. Findings are listed in order of frequency of primary findings.

<table>
<thead>
<tr>
<th>PATHOLOGY</th>
<th>INFECTIOUS AGENT</th>
<th>NEONATES (0 - 30 DAYS)</th>
<th>JUVENILES (1 - 12 MONTHS)</th>
<th>IMM. / AD (&gt;1 YEAR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory tract infection</td>
<td>Pox virus</td>
<td>7</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>(Sinusitis, bronchitis, pneumonia)</td>
<td>Newcastle disease virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Proteus mirabilis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other gram- bacteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entritis / peritonitis syndrome</td>
<td><em>Chlamydia psittaci</em></td>
<td>0</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Herpes virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas aeruginosa</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis and cholangiohepatitis</td>
<td>Herpes virus</td>
<td>5</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Adenovirus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Chlamydia psittaci</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper digestive tract infections</td>
<td>Pox virus</td>
<td>10</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>(Oral mucous membrane lesions)</td>
<td><em>Trichomonas sp.</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interstitial nephritis and glomerulo nephritis</td>
<td>Combined gram-bacteria</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Generalised neoplasia</td>
<td>Reticuloendotheliosis virus</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>suspected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenal parasitic impaction</td>
<td><em>Railletina sp.</em></td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>36</td>
<td>11</td>
<td>57</td>
</tr>
</tbody>
</table>
Table 2: Results of the experimental release of Houbara bustards conducted between 1991 and 1994 in Mahazat As-Sayd reserve.

<table>
<thead>
<tr>
<th></th>
<th>HARD</th>
<th>FCS</th>
<th>COVEYS</th>
<th>SUB</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>5</td>
<td>14</td>
<td>17</td>
<td>74</td>
</tr>
<tr>
<td>Failure before release</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Injury</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Disease</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Actually released</td>
<td>5</td>
<td>12</td>
<td>14</td>
<td>59</td>
</tr>
<tr>
<td>Failure after release</td>
<td>5</td>
<td>9</td>
<td>9</td>
<td>31</td>
</tr>
<tr>
<td>Disease</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Predation</td>
<td>5 (100%)</td>
<td>8 (89%)</td>
<td>6 (66%)</td>
<td>28 (90%)</td>
</tr>
<tr>
<td>Unknown cause of death</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

HARD= Hard release, COVEYS=Covey release, FCS= Feather cut sub-adult release, SUB= Sub-adult release.
RINDERPEST EPIDEMIC IN THE TSAVO NATIONAL PARK KENYA 1994-5

Richard A. Kock* MA, VET MB, MRCVS, John Wambua BVM, Jacob Mwanzia MVPH, BVM
Kenya Wildlife Service (KWS) P.O. BOX 40241 Nairobi, Kenya

Paul Rossiter BVETMED, MSc, PhD
Pan African Rinderpest Campaign Nairobi (PARC), Kenya

Henry Wamwayi BVM, MSc, PhD
National Veterinary Research Centre, Muguga, PO BOX 32 Kikuyu

Nancy Kock DVM, PhD
Department of Paraclinical Studies, University of Zimbabwe PO BOX MP 167 Harare, Zimbabwe

Introduction

Rinderpest is caused by a morbillivirus and affects wild and domesticated ruminants and pigs.1 The disease historically in Africa, Asia and Europe was devastating and prompted national and international control strategies to eradicate it. This has been achieved to a great extent with a few foci left in Asia and Africa. The last outbreak to significantly affect wild animal populations in East Africa was in 1982 in Tanzania2 and in Kenya in 1960-62.3,4 An epidemic disease in Tsavo National Park was monitored from April 1994 until the present and was definitively diagnosed in January 1995. There is evidence for the virus infecting lesser kudu (Tragelaphus imberbis) and buffalo (Syncerus caffer) in Tsavo East and buffalo, kudu, impala (Aepyceros melampus), eland (Taurotragus oryx) and Kongoni (Alcelaphus buselaphus cokei) in Tsavo West.

Disease history clinical and pathological findings

On 14 april 1994 buffalo deaths were reported near Voi on the river, 3 old carcasses a 2 month old calf and 2 adults and the herd were examined; there was evidence for loss of condition and diarrhoea in 20% of the animals. A further 4 animals were immobilised in the herd of 100 animals and one sacrificed for sample collection. Leucopaenia, lymphopaenia and neutropaenia were seen in an adult male which was sacrificed, post mortem examination showed intestinal mucosal haemorrhages with watery diarrhoea. The histopathology showed small intestinal crypts to be widely separated in areas due to oedema and crypt loss with a moderate mixed type inflammatory cell infiltrate with mainly lymphocytes in the lamina propria. There was an associated periportal mild subacute hepatitis. The pathology was indicative of a primary virus infection. Relative lymphocytosis was recorded in 2 other cases and neutropaenia in 1 - otherwise haemograms were within normal limits for buffalo (ZSL - Lynx 1991, Drevemo 1974). Foot and mouth disease virus antibody was found against serotype SAT2 in all cases but due to an administrative error the samples were not screened for rinderpest although this was requested.

In early June 1994 reports of blind kudu in the area of Ithumba in Tsavo East were investigated. The area is remote and difficult to work in due to the dense commiphora
bushland. The kudu are extremely secretive and are only seen from the air and on occasions from the road at distances usually greater than 40 meters. Over a period of weeks it was possible to obtain fresh or recent tissues, taken at post mortem from 8 individual kudu. The population in the sector (North of the Tiva river and East of the Yatta plateau) was considered to be in the order of 4-500 animals. The most obvious pathology was in the eye and associated with the joints - epiphora, severe ulcerative keratoconjunctivitis, uveitis, ulcerative arthritis, synovitis and tenosynovitis. In 3 fresh cases where complete post mortem examinations were possible there were gross pneumonic lesions, lymphadenopathy, intestinal (including caecal ulceration and zebra striping but in only one case diarrhoea), liver and kidney pathology. Gross and histopathological findings are summarised in table 1. The epithelial necrosis and syncytial formation are characteristic of a primary infection with an epitheliotrophic virus. The animals were in remarkably good general bodily condition.

Joint and ocular swabs were negative for bacteria except in one case (joint) with *Pseudomonas auriginosa*, 2 ocular swabs with *Staphylococcus albus* and 1 swab (infra orbital sinus - purulent material) where *Proteus* was isolated. Bacterial colonies were observed in eye and lung lesions presumably secondary to the viral infection.

*Mycoplasma* cultures on solid and liquid broth were negative as were serological tests using bovine and caprine antigen for contagious bovine and caprine pleuropneumonia. Attempts at virus isolation were unsuccessful. Hematology in 1 case had lymphopaenia and moderate red cell parasitemia, and 2 cases showed mild to moderate lymphocytosis and in one case severe dehydration with a packed cell volume of 75%. Electron microscopy demonstrated morbillivirus like particles in a lymph node.

Over a period of 3 months the estimated 4-500 kudu in the area (400 km squared) virtually disappeared but only 25 carcasses or skeletons were actually discovered the remainder probably hidden in dense bush or removed and scattered by carnivores. No other ruminants were observed to be affected at the time. There were several hundred buffalo, gerenuk (*Litocranius walleri*), dikdik (*Madoqua kirkii*), gazelles (*Gazella* sp.), oryx (*Oryx gazella callotis*) and bushbuck (*Tragelaphus scriptus*). Healthy kudu were observed in September in an area South of the Tiva river near Lugards falls and no further reports of mortality were obtained until October 1994 in an area 100+ kilometers away.

Sick buffalo were noticed late October at Salt Lick Lodge, Taita Hills after a movement of buffalo out of the lodge area to Mwatate, a town some 20 km away, where other herds of buffalo had also congregated from other areas of Tsavo. As many as 1500 buffalo may have been present at this time. This was a rare occurrence possibly reflecting the improved vegetation with the good rains in September and October after a protracted drought. In one herd of approximately 100 animals in the lodge vicinity 43 died with over 90 per cent affected, many other ranches in the vicinity recorded buffalo deaths. The first cases were examined from the 20.11.94. Symptoms included weakness and depression, watery diarrhoea with tenesmus and death. Post mortem examination showed gastroenteritis, oral, abomasal and intestinal ulcers including ulcers on the ileo-caeco-colic junction, lymphadenopathy and severe dehydration. Histopathology confirmed hepatitis, nephritis, enteritis and...
lymphadenitis (with haemosiderosis). Nematode, trematode and coccidial parasitism were observed in a number of cases. Epithelial cell necrosis and crypt necrosis were prominent and characteristic of a viral infection. The symptoms, morbidity and mortality and pathological lesions were suggestive of clinical rinderpest. There were approximately 1500 buffalo in the region at the time of the outbreak. Soon after (early December) the animals began to move away to the West and by 20 December a few unaffected older buffalo remained in the area. The only other species observed to be affected were eland and some kudu.

Carcasses were observed in Tsavo West national park in early December from Mzima springs - Ngulia and by the 8.12.94, 20 km south east of Kamboyo some 100 km from the Taita hills outbreak. By the week of 12.12.94 cases were reported all over northern Tsavo West towards the Chyulu hills. The pathology was similar to the Taita Hills cases. By 23.12.94 cases were appearing at Kitani and the Kuku ranch area West of the Chuyulu hills. Species involved were mainly buffalo but also impala, kongoni, (zebra Equus burchelli deaths were also coincidentally reported) and odd reports continue most recently (1.3.95) of eland in this area. The zebra mortality was most likely to have been anthrax.

By late December after extensive diagnostics it was accepted by the authorities to be a full blown epidemic of rinderpest and cattle vaccination measures were undertaken around the Tsavos. Fortunately, there had been a rinderpest and cattle vaccination campaign completed in January and February 1994, so only a small cohort of the population was susceptible and this explains why the virus failed to enter the cattle population on the west side which amounted to 10's of thousands of animals.

Two herds of buffalo (3-500) in the area of Kitani were closely observed between December and February 1995 and morbidity was close to 100%. One relatively normal looking but depressed individual was immobilised and samples taken. It was febrile; 105°F, dehydrated 10% and had diarrhoea. There were healing ulcers on the margins of the lips. Clinical and post-mortem examination of animals over the period confirmed some had recovered from the initial gastroenteritis but still showed lymphadenopathy, healing oral and gastrointestinal ulcers and in the eyes - keratoconjunctivitis, corneal ulcers, uveitis, iritis and cataract were seen. In a number of animals numerous skin lesions were noted 1-2 cm diameter plaque like with some verrucose and keratinized. In one herd sampled in late January in the Kitani area, 3 sick and 2 randomly chosen animals, all had eye involvement with 2 virtually blind. The Tsavo buffalo population was counted in June 1994 and although some animals had already died around Voi from the disease was found to be increased from previous surveys at 11800 animals. This will be repeated in June 1995 to assess overall mortality.

The disease was specifically confirmed in early February as classic rinderpest by laboratory diagnosis. The virus was not isolated from frozen buffalo tissue but all sera from affected herds were positive. Finally at the Pirbright Laboratory, England RNA from morbillivirus were recognised by F gene primers and sequenced. The virus is most closely related to the RGK/1 strain which was isolated from a sick giraffe shot in Northern Kenya in 1962 during
the wildlife epidemic that swept from Ethiopia to the Tana River. Kudu and kongoni tissues were subsequently also shown to be positive for rinderpest virus antigen.

Epidemiology

Retrospective monitoring of cattle, buffalo and other species was initiated in the areas around Tsavo West (to Tanzania and Amboseli) within Tsavo East and West and east of the Tsavos (To east of the Tana River). All animals within the park were seropositive. The animals west near Amboseli were negative. The animals east of Tsavo east and the Tana were positive. A large number of cattle skeletons were recorded along the Tana river in the rangelands occupied by the Orma ethnic group. There were numerous herds of cattle which moved into this region during the drought of 1993-4 and even into the park. Since the virus strain most probably originated from Ethiopia, an outbreak in 1994 in the Bali and Boran regions of Ethiopia (the latter bordering on Kenya) may have been the source. The Boran pastoralists are related to the Orma and trade in cattle.

Discussion

Pathology - with limited resources and logistic difficulties attending acute cases in an ecosystem like Tsavo is problematic and the vast majority of animals examined were too putrefied or consumed for useful diagnostics or were in the recovery phase of the disease. The visibility of buffalo make them an exception but even in this case they are shy and can almost completely disappear for weeks after disturbance. The gross pathology of the disease has been recorded in a number of species and is reviewed. In this outbreak comprehensive data was obtained from Kudu and the details are reported above and in table 1. The eye lesions are particularly significant as even in animals recovering from the acute phase, the effect of the blindness results in abnormal behaviour and death through inanition or predation. Diarrhoea was not an obvious sign and intestinal lesions were relatively mild, a situation perhaps not dissimilar to impala. The tenosynovitis and arthritis were also notable and a consistent finding. Histopathological lesions attributable to an epitheliotrophic virus with cell necrosis and syncytiae formation in a number of areas were consistent with other reports.

The buffalo cases reported showed similar changes to those reported in previous outbreaks but the predominance of eye and skin lesions in the retrospective survey of affected herds were of interest.

Hematology - except for as few acute cases which were examined and sampled from the leukopaenia was not a feature in the buffalo or the kudu. Most animals sampled from at varying times after the initial infection showed relative lymphocytosis.

Specific diagnosis - the major delay in obtaining diagnosis was a result of the insidious spread and initial sporadic mortality from April until October 1994 in the kudu and buffalo and lack of reported involvement in domestic cattle surrounding the Tsavos perhaps coincidental to the effects of drought at that time. Once large numbers of buffalo became
infected with the concentration of 1500 at Taita in October the true epidemic nature of the
disease became clear. In the kudu the more chronic lesions in the eye and joints were not
initially suggestive of rinderpest. Once clinical rinderpest was observed, the laboratory
diagnosis was relatively swift but in this outbreak came from serology and the processing
of fixed as opposed to fresh frozen tissues.

**Epidemiology** - a retrospective survey proved to be useful in this case to trace both the
extent and possible routes of infection. The pattern was complicated by the probable
introduction of disease through cattle transportation. The area from which it was most
probably introduced, east and north east of Tsavo is poorly administered involves pastoral
migrant communities and lacks active veterinary services. It clearly shows the danger of the
disease travelling considerable distances over a long time period (1000 km over 1 year) even
before diagnosis, in these circumstances. The value of the ongoing vaccine campaign in the
cattle population was also well demonstrated as no cattle deaths were confirmed. The
benefits of cattle vaccination to wildlife were also shown with the disease prevented from
entering the Loita Mara Serengeti system which was until relatively recently an enzootic area
for rinderpest.

An aerial census for buffalo is available from June 1994 (11,800 in Tsavo East and West and
surrounding areas) and will be repeated in June 1995 to establish a reasonable estimate of
mortality in this species.

**Implications for rare ruminants** - previous outbreaks in Kenya were implicated in the
decline of rare species such as the Bongo and to have affected the present day distribution
of many others such as the greater kudu (*Tragelaphus strepsiceros*) (1973). The situation in
the 1990's is more critical for a number of ruminants and a disease like this could be
catastrophic and lead to their extinction. The rare animals potentially exposed in this
outbreak were the hunter's hartebeest *Damaliscus hunteri* and the Roosevelt sable
(*Hippotragus niger roosevelti*). There is some evidence that the hunter's have declined over
this period.
Table 1. Summary of gross and histopathological findings in the lesser kudu with rinderpest.

<table>
<thead>
<tr>
<th>SYSTEMIC PATHOLOGY KUDU</th>
<th>K1</th>
<th>K2</th>
<th>K3</th>
<th>K4</th>
<th>K5</th>
<th>K6</th>
<th>K7</th>
</tr>
</thead>
<tbody>
<tr>
<td>EYE</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>NS</td>
</tr>
<tr>
<td>Bilat keratitis ulcer uveitis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>neovascularization melanisation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>epiphora</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>conjunctival epi. cell necrosis and syncytiae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SKIN</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>+++</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>- hair folcle -</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sebaceous gland: sq metaplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>para-hyper keratosis intracorneal pustule</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIT epithelial cell: necrosis</td>
<td>NS</td>
<td>NS</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>mineralized</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>syncytiae crypt - necrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cecal ulcer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pancreas (duct) cell necrotic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>zebra striping mucosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>enteritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>salivary gland duct cell necrosis and syncytiae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIVER</td>
<td>NS</td>
<td>NS</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>enlarged gall bladder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kupffer cell hyperplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bile duct cell necrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIDNEY</td>
<td>NS</td>
<td>NS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>renal tubal cell necrosis and syncytiae lymphocytic foci</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLANDS</td>
<td>NS</td>
<td>NS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>adrenal pigment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>medulla brown purple</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LYMPH</td>
<td>NS</td>
<td>NS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>gross enlargement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lymphoid depletion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPLEEN</td>
<td>NS</td>
<td>NS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>hyalinization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSCLE</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>sarcosporidiosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>heart sarcocystis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JOINTS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>teno-synovitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>articular erosions arthritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LUNG</td>
<td>NS</td>
<td>NS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>bronchopneumonia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>necrotic epithelial cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bronchiolo lymphoid hyperplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Not sampled
ACKNOWLEDGEMENTS

In an investigation of this kind many people are involved. We are grateful to the Directors of KWS, PARC and the Government Veterinary Service, the laboratories, all the wardens, rangers and interested individuals; ranch owners and local people who assisted. Particular thanks to the Chief Pilot Mr Phil Matthews whose assistance was crucial and the technicians Dennis Mudakha and John Ongeza who work in the veterinary unit at KWS. A special thanks to Mike Loomis of North Carolina USA who gave us the first clues that we were dealing with rinderpest from EM studies on one kudu case which he kindly arranged for us. Thanks also to Alexandra Dixon and her staff at the Conservation Consultancy Division of the Zoological Society of London for material support during this outbreak.

LITERATURE CITED

AN OUTBREAK OF SARCOPTIC MANGE IN FREE LIVING CHEETAH (*Acinonyx jubatus*) IN THE MARA REGION OF KENYA

Jacob M. Mwanzia, BVM, MVPH
Veterinary Unit, Kenya Wildlife Service, P.O. Box 40241, Nairobi Kenya

Richard A. Kock, MA.VET.MB., MRCVS
Veterinary Unit, Kenya Wildlife Service and Zoological Society London, Regent's Park, London and International Wildlife Veterinary Services Inc., 1850N. Main Street, Salinas, CA 93906 USA

John M. Wambua, BVM
Veterinary Unit, Kenya Wildlife Service, P.O. Box 40241, Nairobi Kenya

Nancy D. Kock, DVM, PhD
Department of Paraclinical Studies, University of Zimbabwe, P.O. Box MP167, Harare, Zimbabwe

Oswald Jarrett, BVM, PhD, MRCVS, FRSE
Department of Veterinary Pathology, University of Glasgow, Bearsden, Glasgow G61 1QH Scotland

Introduction

Cases of alopecia in the wild dog (*Lycaon pictus*) and cheetah (*Acinonyx jubatus*) have been reported in Masai Mara Kenya since the mid-1980s, with one confirmed case of sarcoptic mange in the wild dog (Richardson, pers. comm.). However there were no confirmed cases of Sarcoptes mange in Cheetahs. Mange is a debilitating condition which justifies intervention to establish causal agents and with rare species possible treatment. The paper documents a study of a skin disease found in cheetahs from Aitong/Lemek group ranches adjacent to the Masai Mara game reserve in Kenya.

Materials and methods

Investigations were carried out by a veterinary team from the Kenya Wildlife Service over a two and a half year period (26.7.92 to 31.12.94). The rationale was to investigate the nature, extent, cause and possible means of treatment or control of the skin disease reported in this species. The study was carried out in the Lemek/Aitong area group ranches in Kenya (35 13'E, 1 12'S). The area is delineated to the south and east by the Masai Mara game reserve, to the north by the Aitong hills and to the west by the Mara river, an area of approximately 400 km. sq. This region forms part of the Mara - Serengeti ecosystem in East Africa.

Capture and anesthesia

A total of eight adult cheetahs (six females and two males) and four cubs (two males and two females) were captured among an estimated total population of approximately 15 adult, two juvenile and eight cub, cheetahs in the area. The adults were captured by chemical immobilization while the cubs were captured by physical restraint. One female cheetah was immobilized on three different occasions during the study period. Three other individuals
immobilized on three different occasions during the study period. Three other individuals (one adult female, with severe alopecia and two juveniles, the latter cubs to an affected female) were observed with skin lesions but not immobilised as they were too shy to approach in the case of the cubs and the adult was seen when immobilising equipment was unavailable. Three females with a total of two, three and one cub respectively were observed at close range without any sign of mange. One of the females was immobilised and her cubs were restrained to confirm this.

Cheetah were located by searching the study area with a Land Rover 110, or relying on information received from tour drivers during game drives. The search was conducted in the early morning (6 to 10 a.m) or late afternoon (4 to 6 p.m). The technique involved cutting transects across the study area or following routes of known individual cheetah. Once a cheetah was spotted the vehicle was driven as close as the animal would allow for closer observation. Lesions suggestive of mange were looked for with the naked eye and binoculars.

Animals were then darted from the vehicle using either a rifle (Palmer Cap-chur, Palmer Chemical and Equipment Co., Inc. P.O. Box 867 Palmer Village, Douglasville, Georgia, 30133 U.S.A.) or a blow pipe (Dan-inject, Dan-inject Smith GmbH Special Vet. Instruments, Roy Smith. Friesenstratebe 5. 4650 Gelsenkichen, Germany). Cap-chur darts (three millilitre (ml), one and half inch needle) or pneumarts (Pneu-Dart Inc. P.O. Box 1415, Williamsport, Penn., 11708 U.S.A.) were used with the rifle. Daninject (Daninjekt, Daninjekt smith GmbH Special Vet. Instruments, Roy Smith. Friesenstratebe 5. 4650 Gelsenkichen, Germany) plastic darts (two or three ml volume and one and half inch long, two millimetre (mm) diameter needle) were used with the blowpipe. Xylazine (500 milligram (mg)) (Crystalline Rompun, Bayer U.K. Ltd., Agrochem Division, Eastern Way, Bury St Edmunds, Suffolk IP32 7AH U.K.) was mixed with five ml Ketamine 10% (Vetalar, Parke Davis and Company, Usk Rd., Pontypool, Gwent, NP4 OYH, U.K.) to make a 1:1 ratio of ketamine and xylazine. This drug cocktail was then placed in the dart and the projectile fired at the animal’s shoulder or rump from a distance ranging from five meters (m) to 30 m.

Average doses for seven adult cheetah darted were: ketamine (mg) 221.4 ± 19.8 (S.E.) and xylazine (mg) 214 ± 16.6 (S.E.) In one animal 200 mg ketamine and 1 mg Medetomidine (Domitor, Smithkline and Beecham Animal Health Ltd., Cavendish Rd, Stevenage, Hertfordshire SG1 2EJ, U.K) was used.

After darting, anesthetic induction times were 9 ± 1.2 (S.E.) minutes. Ophthalmic ointment (Orbenin, Beecham Animal Health, Brentford, Middlesex, England.) was applied to protect the eyes from drying due to loss of the blink reflex. A cloth was then placed over the head. Each animal was clinically examined and samples taken.

**Sampling**

Alopecic or crusted areas of skin were scraped until bleeding using a scalpel blade (no. 22), concentrating at the junction of the lesion with the normal skin. The wound was treated with
antibiotics. Blood samples were taken from the jugular vein and placed into plain tubes or tubes with anticoagulant (serum in plain glass tubes (10 ml), heparinised tubes (five ml), and ethylenediaminetetraacetic acid (EDTA) tubes (five ml) were used) for routine serology, biochemistry and hematology. Smears were made to check for hemoparasites and to examine blood morphology. Biopsy tissue samples measuring approximately five mm by two mm were taken from skin lesions using a scalpel blade (no.22). Specimens were immediately fixed in 10% phosphate buffered formal saline.

Treatment and reversal of anesthetic

Some positively infected animals were treated with ivermectin (Ivomec - Merck and Co., Rahway, New Jersey 07065, USA) (0.3 ug/kilogram (kg) of body weight) administered subcutaneously over the chest wall. Each animal was ear notched for future identification.

Xylazine effect was reversed using an alpha2 receptor agonist antagonist Rx821002a (Reckitt and Colman, Research Division, Kingston-upon-Hull, U.K.) which was administered intravenously at an average total dose of 1.75 mg ± 0.15 (S.E.). The animal was then left and observed from a distance until recovery.

Laboratory procedures

Samples from the scrapings were examined in a field lab within 24 hours. Two drops of 10% potassium hydroxide (KOH) were added to a proportion of the skin scraping in a test tube and heated gently over a flame for one minute. The residual material was placed on a microscope slide and covered with a cover slip. This was examined with a lens x 100 for the presence of sarcoptic mange mites or eggs using a field microscope. Skin scrape samples remaining from KOH tests were placed in insect pots with moist cotton wool and holes for ventilation then transported to Nairobi and on to a laboratory (Department of Biological Sciences, Dayton, Ohio 45435 USA) at 10-15 °C for further analyses. Formalized skin biopsies were embedded in paraffin, sectioned at 10um, and stained with hematoxylin and eosin (H&E).

Blood samples were examined using a commercial laboratory for haematology and biochemistry. Serum was stored frozen and transported in dry ice for serological investigations. Western blotting using lysates of FIV infected cells was carried out as described previously.9
Results

Skin scrapings, biopsies and serological tests

Of the 12 cheetahs whose skin scrapings and or biopsies were processed nine were confirmed to have sarcoptic mange mites (Table 1). Animals of all ages and sex were affected.

The mite affecting the cheetahs was identified as *Sarcoptic scabiei*. The parasite was roughly circular in outline. The posterior legs were short and did not project beyond the margin of the body. The pedecles of the suckers on the legs were not segmented and the anus was terminal. The variety of *Sarcoptes scabiei* remains unknown. Attempts to identify the mite variety by infesting New Zealand white rabbits were unsuccessful after allowing the infestation to progress for two months. This probably rules out the possibility of the mite being var canis since the mite did not colonize the rabbit hosts.

Histopathological findings from the adult female MARACHE4 showed the epidermis was largely intact, though overlain with thick, sero-cellular, exudate in which cross sections of ectoparasite are present. The dermis was intensely infiltrated by mixed type inflammatory cells including many eosinophils. The diagnosis was diffuse subacute severe parasitic dermatitis.

Sera from the cheetah were tested for antibodies cross, reactive with FIV by ELISA and western blotting. Serum from seven cheetah were confirmed to have antibodies. The pattern of reactivity of antibodies in cheetah plasma with the FIV core proteins, p17 and p24, and the lack of cross-reactivity with the envelope protein, gp120, was similar to previous findings in cheetahs.\(^\text{18}\) The results are shown in Table 1. Hematologic analyses were unremarkable except for a tendency for higher lymphocyte counts in infected cheetah, Table 2.

Other domestic and wild animals sampled in the study area appeared not to have sarcoptic mange. These included one lion, (*Panthera leo*), six Thomson’s gazelle (*Gazella thomsoni*), eleven domestic cats, twelve domestic dogs and one cow with skin lesions. The problems were associated with trauma, ticks or, in the case of the cow, fungal infection.

Several hundred animals of a variety of carnivores and herbivores (domestic and wild) were observed and appeared free of skin disease. No cheetah observed in the Mara Reserve at the time were effected but in november 1994 one animal in the Keekerok area was seen with similar lesions. A single case was also confirmed in the Serengeti in 1994 (Roelke pers.comm.).

The female cheetah immobilized on three different occasions over a period of five months was negative for sarcoptic mange on the third immobilization after treatment with ivermectin (on the first two immobilizations). It was also seronegative to FIV on the last sample. The two cheetah cubs of this animal were also treated but died within a month. The carcasses were not recovered.
Two males given one dose of Ivermectin were seen seven months later with no signs of sarcoptic mange and lesions previously seen had disappeared. Four females infected were also treated but resolution was not individually confirmed on these animals except for MARACHE8 which was seen 17 months later with two sub adult cubs. No other effected animals were seen as of December 1994 and it is assumed their recovery was complete.

Of the three untreated effected animals, one was presumed to have died as it was no longer seen and two juveniles appeared to recover spontaneously although this was not definitely confirmed.

Discussion

*Sarcoptes scabiei* is an ectoparasitic mite that either burrows into the epidermis of mammalian skin or, in more severe infestations where there is strong host reaction, will live in the fissures and chambers of crusts formed from the hosts exudates. Mites of the genus *Sarcoptes* are parasitic on man and on a number of domestic and wild mammals including sheep, goats, cattle, pigs, horses, dogs, jackal (*Canis mesomelas*), foxes (*Urocyon spp.*), warthog (*Phacochoerus aethiopicus*) and rabbits. There are also reports in jackal and warthog. Other confirmed cases are in jaguar (*Panthera onca*), lynx (*Felis lynx*), puma (*Felis concolor*), buffalo (*Syncerus caffer*), blue wildebeest (*Connochaetes taurinus*), impala (*Aepyceros melampus*), springbuck (*Antidorcas marsupialis*), sable antelope (*Hippotragus niger*) (the outbreak was associated with human cases) and red hartebeest (*Alcelaphus buselaphus*) were also reported to be affected in the Kruger National Park, Republic of South Africa and the Kalahari. Noteodric mange has been confirmed in cheetahs. Some investigators believe that sarcoptic mites found on different hosts belong to different species, but others believe that they are biologic or physiologic races of the same species, *Sarcoptic scabiei*. However most investigators agree that these mites are host specific. Long survival off the host coupled with host seeking behaviour of *Sarcoptic scabiei var canis* mites raises the possibility that environmental contamination is a source of scabies in domestic and wild mammals. Sarcoptic mange in foxes occurs as an epizootic in local populations. A recent outbreak was recorded among Arctic foxes (*Alopex lagopus*) in 1986. Opportunistic infections have been associated with FIV in cats and sarcoptic mange was also recorded in a cat which was presumed to be immunocompromised. Other cases in domestic cats have been confirmed in Japan from animals with FIV infection. A study on the seroprevalence of FIV in captive and free ranging felid species confirmed the presence of antibodies in cheetah.

This paper confirms the presence of *Sarcoptes scabiei* in wild cheetah in the Mara - Serengeti ecosystem. The fact that the mite appeared to affect only cheetahs among the wild and domestic carnivores in the same area posed a number of questions. Either the cheetah had an underlying problem (e.g immunosuppression) which led to a clinical manifestation of an endemic disease or the mite infected the host from another source. The most common contact is between predator and prey and the positive finding of this mite in Thompson's gazelle (*Gazella thomsoni*) suggests this as a possible source. There have been previous
reports of mite infestation in this species of gazelle in the Serengeti (Roelke pers.comm.) but there was no evidence for infection in gazelles at the time.

Morphologically the mite was similar to other sarcoptic mange mites but could not be propagated in New Zealand white rabbits which was notable. *Sarcoptes scabiei var canis* usually grows well on this host so perhaps this suggests physiologic differences between this mite and var canis and possibly others. Permanent transfer of *Sarcoptes scabiei var canis* from one host species to another does not occur readily even among animals that may interact closely. *Sarcoptes scabiei var canis* has been reported not to establish permanent infestations on sheep, goat calf or cat hosts. This reduces the possibility that the mite affecting the cheetahs could have originated from the Masai dogs. The exact source of infection with mange mites could not be confirmed.

The possibility of the cheetah having a predisposing factor was evinced when sera from the cheetah tested positive for the presence of antibodies cross-reactive with FIV proteins. Previous surveys of cheetah in the Serengeti showed a prevalence of 29% seropositive which is lower than this survey (70%). The immune system of animals in the area may have been compromised thus exposing the cheetahs to opportunistic infections such as sarcoptic mange but this data is not conclusive as one infected and one uninfected animal were negative FIV and positive FIV respectively.

In this particular study various factors could be identified which could cause stress to the cheetahs and perhaps affect their ability to resist various diseases. This is in addition to the inherent factors such as a lack of genetic diversity which has been reported in the cheetah. The sharing of local communal lands between wild animals, the local Masai people, their livestock and tourism is stressful to cheetah, as these animals are highly visible and attract attention. Large numbers of tourists visit and use the area without proper enforcement of rules which apply to National Parks and reserves. This led to frequent and persistent off road driving to areas where the cheetahs are resting or hunting.

It is interesting to note that no cases were observed in the Mara reserve area at the time which might reflect a lower density of animals in that area or perhaps less stress, but the one possible case seen later was in a highly visited area similar to Aitong.

The effects of competition from other predators may also be relevant. It has been noted that the Masai lands adjacent to the Masai Mara game reserve in Kenya provided a better nursery area for female cheetahs and their cubs than the reserve itself due to the scarcity of lions. Another author noted that high cheetah density was associated with low hyena density. With the establishment of the group ranches which promote eco-tourism, there has been an apparent increase of lions and hyenas in this area around Aitong. Lions and hyenas feed on cheetah cubs and chase adults from their kill. So the previous observations although probably still holding true in some areas, are not pertinent and the density remains high and breeding continues perhaps as the cheetah has little choice left. The disturbance associated with all the above may affect the animals inducing anxiety and chronic adrenal responses but must remain speculation.
The histopathological findings confirmed the severe nature of the disease. Exudation had lifted the epidermis off the dermis, thus removing it from sustenance. Epidermal necrosis at the edges of ulcers and the mouths of hair follicles. This was observed in the cheetah which had been treated with Ivermectin.

Ivermectin has potent activity against numerous immature and mature nematode and arthropod parasites. In dogs ivermectin has been shown to be a highly effective and safe treatment for sarcoptic mange. Ivermectin treatment has also been reported to be effective against Sarcoptes scabiei and Otodectes cynotis in dogs. In domestic cats toxicity has been documented. However there has been no evaluation to determine efficacy of ivermectin treatment of cheetahs with Sarcoptic mange.

The present study attempted treatment of cheetahs with sarcoptic mange with ivermectin. Ivermectin 1% w/v given at a dose rate of 0.3ug/kg bw appeared to be effective against the mites in the cheetah as proved by negative samples obtained after repeat immobilization in one treated individual and from subsequent observations on other individuals.

ACKNOWLEDGEMENTS

The authors would like to thank other staff of KWS and the veterinary unit, wardens, rangers and the technician, Denis Mudakha who were essential to the work. Prof. L. Arlian kindly carried out further investigations on the mites. The cooperation of the manager, Mr Willy Roberts of the Ol-Choro-Oirosa Ranch, the Maasai residents and the Lodge drivers and staff ensured the work was carried out efficiently and comprehensively. Finally we would like to thank the former Director KWS Dr. Richard Leakey for supporting this study, Friends of Conservation for material support and the International Distillers of Kenya who provided financial assistance.

LITERATURE CITED

12. Keep, M.E. 1990. Lice (Phthiraptera), fleas (Siphonsapera) and mites (Trombidiformes, sarcoptiformes and mesostigmata) recorded from the larger game species in Natal. Lammergeyer 41: 23-29.


Table 1

RESULTS OF SKIN SCRAPINGS, BIOPSIES AND SEROLOGY

<table>
<thead>
<tr>
<th>CHEETAH I.D.</th>
<th>SEX</th>
<th>AGE</th>
<th>LESIONS</th>
<th>KOH +VE</th>
<th>FIV ANTIBODIES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>MARACHE1</td>
<td>M</td>
<td>7d</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MARACHE2</td>
<td>F</td>
<td>7d</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MARACHE4</td>
<td>F</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MARACHE5</td>
<td>F</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MARACHE6</td>
<td>M</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>MARACHE7</td>
<td>M</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MARACHE8</td>
<td>F</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MARACHE9</td>
<td>F</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MARACHE10</td>
<td>M</td>
<td>14d</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MARACHE11</td>
<td>F</td>
<td>14d</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MARACHE12</td>
<td>F</td>
<td>A</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>MARACHE13</td>
<td>F</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MARACHE4*</td>
<td>F</td>
<td>A</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

KOH +VE - mites visible by microscopy after treatment of skin scraping by KOH.
M - male F - female A - adult
7d - week old cubs of MARACHE4 14d - 2 week old cubs of MARACHE9
Table 2

HAEMATOLOGICAL DATA FROM CHEETAH COMPARED TO A REFERENCE RANGE

<table>
<thead>
<tr>
<th>HAEMATOLOGICAL PARAMETERS</th>
<th>CHEETAH AITONG</th>
<th>NORMAL RANGE LYNX DATABASE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total red cell 10^12/l</td>
<td>7.94 ± .71 N=9</td>
<td>5.55 - 9.08 N=35</td>
</tr>
<tr>
<td></td>
<td>(0 - 0.36)</td>
<td>31 - 49 N=36</td>
</tr>
<tr>
<td>Packed Cell Volume l/l</td>
<td>42.22 ± 1.56 N=9</td>
<td>31 - 49 N=36</td>
</tr>
<tr>
<td></td>
<td>(40 - 45)</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin g/dl</td>
<td>14.21 ± 0.80 N=9</td>
<td>10.68 - 17.09 N=36</td>
</tr>
<tr>
<td></td>
<td>(13.30 - 15.90)</td>
<td></td>
</tr>
<tr>
<td>Mean Cell Volume fl</td>
<td>54.00 N=1</td>
<td>45.54 - 63.99 N=35</td>
</tr>
<tr>
<td>Mean Cell Hg pg</td>
<td>19.10 N=1</td>
<td>16.19 - 21.84 N=35</td>
</tr>
<tr>
<td>Mean Cell Hg Conc. g/dl</td>
<td>35.50 N=1</td>
<td>32.36 - 37.11 N=36</td>
</tr>
<tr>
<td>total white cell 10^9/l</td>
<td>10.03 ± 4.94 N=9</td>
<td>4.78 - 15.25 N=36</td>
</tr>
<tr>
<td></td>
<td>(3.2 - 19.1)</td>
<td></td>
</tr>
<tr>
<td>neutrophil 10^9/l</td>
<td>5.54 ± 2.94 N=9</td>
<td>2.36 - 11.56 N=36</td>
</tr>
<tr>
<td></td>
<td>(1.76 - 10.07)</td>
<td></td>
</tr>
<tr>
<td>lymphocyte 10^9/l</td>
<td>4.08 ± 4.13 N=9</td>
<td>0.48 - 3.59 N=36</td>
</tr>
<tr>
<td></td>
<td>(1.08 - 13.11)</td>
<td></td>
</tr>
<tr>
<td>eosinophil 10^9/l</td>
<td>0.46 ± 0.64 N=9</td>
<td>0.08 - 3.07 N=36</td>
</tr>
<tr>
<td></td>
<td>(0.00 - 1.58)</td>
<td></td>
</tr>
<tr>
<td>monocyte 10^9/l</td>
<td>0.08 ± .12 N=9</td>
<td>0.00 - 0.88 N=36</td>
</tr>
<tr>
<td></td>
<td>(0.00 - .36)</td>
<td></td>
</tr>
<tr>
<td>basophil 10^9/l</td>
<td>0.00 - 0.00 N=9</td>
<td>0.00 - 0.00 N=36</td>
</tr>
<tr>
<td>fibrinogen g/l</td>
<td>2.75 ± 0.96 N=4</td>
<td>1.53 - 3.72 N=27</td>
</tr>
<tr>
<td></td>
<td>(2.00 - 4.00)</td>
<td></td>
</tr>
<tr>
<td>total protein g/l</td>
<td>10.55 ± 1.11 N=4</td>
<td>60.81 - 78.59 N=27</td>
</tr>
<tr>
<td></td>
<td>(9.00 - 11.60)</td>
<td></td>
</tr>
</tbody>
</table>

Comments: lymphocytes in cheetah affected with sarcops (N=6 mean 5.06 ± 4.85) were generally higher than normal animals (2.10 ± 0.91 N=3) and the reference range but without statistical significance.

* Lynx - Blood Database - Department of Veterinary Science, Zoological Society of London 1991
DISEASE EPIDEMIC IN LESSER FLAMINGOS (*Phoeniconaias minor*) IN KENYA

Nancy Kock, DVM, PhD  
*University of Zimbabwe, po Box MP167, Mount Pleasant, Harare, Zimbabwe*

Richard Kock, MA, Vet MB, MRCVS  
*Kenya Wildlife Service, PO Box 40241, Nairobi, Kenya*

An outbreak of disease in free-ranging Lesser Flamingos (*Phoeniconaias minor*) in Kenya resulted in more than 30,000 deaths over six months in 1993-1994. Disease spread between lakes along the Rift Valley, concentrating primarily on Lake Bogoria. Coincidental to the outbreak was a bloom of algae, toxins from which were suspected to be causative. Discrete necrotic lesions were noted in spleen and liver, and a variety of bacteria were recovered in pure culture from visceral organs, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus* spp. Histopathological evaluation of the tissues disclosed chronic and often life-threatening lesions of mycobacteriosis (tuberculosis), primarily involving spleen and liver, with rare pulmonary lesions. Typical hepatic lesions associated with algae toxicosis were not seen in any of the birds. It is possible that the algae bloom allowed for the proliferation of potentially pathogenic bacteria in such numbers that the birds were unable to mount appropriate immune responses and were overwhelmed by infections. Those affected and debilitated by mycobacterial infections were undoubtedly most susceptible. Attempts are being made at the time of submission of this abstract to further characterize the mycobacterial organism responsible.
POLYMERASE CHAIN REACTION: A REVIEW OF THE TECHNIQUE AND ITS APPLICATIONS

Nancy P. Lung, VMD, MS
Fort Worth Zoo, 1989 Colonial Parkway, Fort Worth, TX 76110, USA

Introduction

The polymerase chain reaction (PCR) is a remarkably simple process that results in the in vitro enzymatic synthesis of millions of copies of specific DNA sequences. Because of its ability to detect a very small quantity of a particular gene sequence in a heterogeneous sample mixture, it provides a powerful diagnostic tool with both high specificity and sensitivity. The power and limitations of this tool in the characterization of genetic defects, prenatal diagnosis, carrier testing, HLA typing, identifying activated oncogenes, characterizing neoplasias and identifying micro-organisms are just now being realized. As zoo veterinarians, we can most benefit from the advances PCR has provided in diagnostic microbiology. This is especially true for the diagnosis of stubborn pathogens such as chlamydia and mycobacterium. PCR can be used to detect actively replicating, latent, or dead organisms from virtually any sample including hair, nails, bodily fluids, tissue homogenates, feces, and formalin-fixed, paraffin-embedded tissues. The beauty of this technology in the diagnosis of diseases of non-domestic animals is that amplification and identification of DNA by PCR is the same regardless of the species involved, eliminating the need for expensive and cumbersome species-specific reagents. This paper outlines the principles of the PCR, a brief review of its current applications, and a summary of the technology's strengths and weaknesses.

Refresher terminology

amplicon--the term used to describe the product of PCR, i.e., the DNA template sequence of which millions of copies have been made.
annealing--the process by which complimentary nucleotide sequences on single stranded DNA join to produce a double stranded molecule.
denaturation--the process of reducing the native double stranded DNA into its complimentary single strands. This is traditionally accomplished through brief heating of the sample.
deoxyribonucleotide triphosphates (dNTPs)--this group of nucleotides includes the four standard components of DNA: adenine (A), guanine (G), cytosine (C) and thymine (T). In double stranded DNA, adenine is always found opposite (complementary to) thymine, and cytosine is always complimentary to guanine (A to T, C to G).
oligonucleotide-- a scientist-defined, chemically synthesized nucleotide sequence, usually produced in an automated DNA synthesizer.
polymerase enzyme--DNA synthesizing enzyme that is capable of accurately copying a single strand of DNA by placing the appropriate nucleotide in sequence complimentary to that of the opposite single strand.
primer—in PCR technology, primers are oligonucleotides, usually 20-70 nucleotides long, that are specifically synthesized to be complimentary to the areas that flank the DNA target sequence. They are called primers because they serve as a starting point for the polymerase enzyme to begin building the DNA chain.

The polymerase chain reaction

PCR was first introduced in 1985. It provides a method for amplifying specific nucleic acid sequences through repeated cycles of DNA synthesis, rather than through time consuming DNA cloning. Researchers have claimed that PCR has made DNA research boring by removing the need for subtle deduction, clever manipulation, and special insight from DNA research! All PCR applications have a similar design of repeated DNA amplification cycles followed by a method for detection of the amplified DNA. The three steps of each amplification cycle include: 1) denaturation of the double stranded DNA, 2) annealing of primers to the single strands, and 3) polymerization of nucleotide sequences complementary to the single strand sequence of interest. Each of these steps, described below, are initiated by temperature changes, and have been greatly simplified by the use of automated thermocyclers. As PCR makes its way into infectious disease, genetics, and cancer research laboratories, the methodology is manipulated and molded to optimize its usefulness for that purpose.

The PCR Cycle

Denaturation—The DNA region of interest, (the target), is likely to be a small component of all DNA present in a test sample, and will be present in double stranded form. Before this region can be copied by the polymerase enzyme, it must be denatured into its two complementary single strands. This is readily accomplished by raising the sample temperature above 90°C for approximately 30 to 120 seconds.

Annealing—PCR primer pairs are constructed such that the nucleotide sequence of one primer is complementary to the sequence flanking one end of the target DNA. The other primer is complementary to the other end of the target sequence. See Figure 1. Primers are added to the sample mixture in excess prior to the denaturation step. When the sample is cooled, primers rapidly anneal to their complementary regions on the target DNA. They align in a 5'-3' direction, such that the primers' 3' end is oriented toward the target sequence.

Polymerization—The polymerase enzyme is triggered by the bare 3' ends of the primers. The enzyme sequentially lengthens the primer sequence by placing the appropriate complementary nucleotide across from the single stranded DNA, using the DNA target region as its template. The enzyme will continue building in a 5' direction until the reaction is stopped.

Following one PCR cycle, the number of copies of the DNA target region in the test sample is doubled, as each single strand has been built into a double strand. The cycle, which lasts
only 2-3 minutes, can be repeated multiple times, each time with the DNA created in previous cycles serving as templates for the next cycle. This gives an exponential increase in the number of target sequences. Although not 100% efficient, 20 cycles will magnify the number of template copies by $10^6$ to $10^8$ fold. The high specificity, high sensitivity, and short duration of most PCR assays make it very attractive to diagnostic laboratories. Often results can be available within 1 to 2 days.

**Components of the Reaction**

The PCR reaction requires a means of rapidly heating and cooling the sample preparation. This is most readily achieved with the use of an automated thermocycler. Before entering the thermocycler, the sample mixture must contain: 1) the test sample, which has been processed for DNA access, 2) excess quantities of two well defined primers which flank the area of interest, 3) a thermostable polymerase enzyme, and 4) excess quantities of the dNTPs, (A,T,G,C), the building blocks of the polymerase reaction. When the template, the primers, the polymerase, and the dNTPs are all present with the appropriate buffer system and co-factors, PCR is a rapid and efficient process. Identification of the appropriate primer sequences may be the most time consuming aspect a PCR assay design, or it may be as simple as searching the existing computer databases of known DNA sequences. Assay specificity requires the identification of a gene sequence that is unique to the organism of interest. Once the target gene sequence is identified, oligonucleotide primers which flank the region can be produced on an automated DNA synthesizer for use in the PCR reaction.

**Identification of Amplicons**

Once the PCR reaction is complete, a method is needed to detect the presence of amplified target sequences in the test sample. If the test sample did not contain the organism of interest, no amplified DNA will be detectable. Amplicon detection methods are as varied as the scientists designing them. The simplest methods utilize radiolabeled or chemically labeled primers. Which can be detected by radioactivity, colorometric analysis or chemiluminescence. ELISA and other immunological detection schemes are also in use. Four commonly used detection methods with description are illustrated in Figure 2.

**Applications of the polymerase chain reaction**

**Infectious Diseases**—PCR technology has broad application to infectious disease diagnosis and research. In the area of diagnosis, PCR permits the detection of quiescent or latent infections that cannot be detected by immunologic assays or culture. It is particularly useful in the detection of difficult to cultivate, dangerous, or untypeable organisms such as fungi, parasites, viruses, and bacteria, especially mycobacterium and the mycoplasmales. PCR has tremendous application in infectious disease research, epidemiologic and retrospective investigation. PCR has lead to the discovery of previously unknown and still uncultivatable organisms and is revolutionizing microbial taxonomy. Epidemiologic investigations are
assisted by the ability of PCR to identify subspecies or strain similarities between multiple bacterial or viral isolates. PCR can be used to detect environmental contamination with epidemic microorganisms such as Legionella pneumophila. The ability of PCR to amplify DNA sequences in paraffin-embedded or even archeological samples provides tremendous power to retrospectively investigate the biology of infectious disease. Table 1, although not comprehensive, lists many infectious agents that are currently being identified using PCR technology.

**Genetic Applications**—Because of its ability to amplify as few as one copy of a target sequence in a test sample and its rapid assay time, PCR is quickly revolutionizing genetic research and diagnosis. In the study of genetic disorders, PCR can be used to rapidly and inexpensively detect point mutations, deletions, insertions or rearrangements in as little as a single cell. This is invaluable to prenatal diagnosis, neonatal screening and carrier testing for such diseases as sickle cell anemia, phenylketonuria, and X-linked Duchenne muscular dystrophy. PCR has been crucial to the investigation of viral and genetic links to certain forms of cancer, as well as the study of the biology of oncogenes. Table 2 lists some of the medical applications of PCR based genetic investigation.

**Archival Studies**—The use of PCR in the evaluation of DNA from virtually any sample in which intact DNA can be found, opens worlds of opportunity for retrospective study. Linkage analysis of families going back several generations is possible, provided paraffin-embedded tissue blocks from deceased family members are available. PCR has created windows into archeological specimens, permitting investigation of ancestral humans, extinct animal species, patterns of disease, etc. PCR is applicable to retrospective and current forensic studies, as DNA evaluation can be performed on minute tissue samples or strands of hair, etc.

**Limitations of the polymerase chain reaction**

As with any diagnostic test, PCR assays have a large number of variables that must be optimized before results of the assay are valuable to the clinician or investigator. Enzyme selection, annealing temperature, cycle number, primer concentration and template length are only a few of the variables that must be controlled. This is the job of the PCR scientist. Once the scientist has achieved this, however, there are still potential problems and pitfalls that must be appreciated in clinical decision making.

**False Positivity**—The most critical pitfall encountered in PCR based diagnostics is PCR product contamination. Since PCR can be used to detect as few as one copy of a DNA sequence, the slightest contamination from sample to sample can lead to significant levels of false positivity. It seems that good laboratory practice could avoid this problem. However, lots and lots of copies of DNA product are released by aerosol every time sample test tubes are opened. After just a few days of PCR testing, pipettes, lab benches and clothing of personnel have significant levels of PCR product coating their surfaces. An excellent analogy is as follows: if the products of a 100µl PCR (10^12 target copies) were
diluted uniformly in an Olympic size swimming pool, a 100µl aliquot of the pool water would still contain 40 amplifiable products!\textsuperscript{2} False positivity cannot be completely eliminated. However, measures to reduce its incidence include the following:

1. Physical separation of workspace, equipment and personnel handling the PCR sample set up from anything to do with amplified PCR product evaluation or disposal.
2. Positive displacement pipettes or plugged pipette tips to reduce carryover from one sample to another.
3. Aliquoting small volumes of reagents, so that if contamination does occur, it will affect only a small number of samples.
4. Stringent use of negative controls, especially the no-DNA control that will detect any contaminating DNA sequences.

**Over-Interpretation**—Although PCR has the ability to render obsolete many standard culture techniques, the results of PCR-based diagnostic microbiology must be utilized within the context of broad clinical evaluation. The risk of over interpreting a positive result is great, as the technique may detect as few as 1-10 organisms in a sample. Interpretation of the presence of an organism as a commensal, a secondary invader, or the cause of clinical pathology must be made by the clinician. The monitoring of therapy via PCR techniques also has inherent risks. PCR has the ability to detect DNA from dead or antibiotic treated organisms, making evaluation of treatment and cure difficult. PCR positivity in culture-negative specimens must be evaluated carefully. A new push to follow RNA target sequences rather than DNA target sequences may help to circumvent this problem, as ribosomal RNA correlates directly with cell growth. Over-interpretation is equally possible when evaluating samples for genetic disease or cancer detection. For example, PCR evaluation for residual disease following lymphoma chemotherapy may detect remaining cells with the specific genetic translocations, but these cells are not necessarily malignant.

**Limitations of Sample Preparation**—Although PCR seems like the answer to our prayers for the diagnosis of stubborn pathogens like mycobacterium and fungi, PCR has not yet proven universally applicable to the diagnosis of these infectious agents. The DNA present in these pathogens must be accessed before they can be amplified. Products such as harsh detergents are needed to break down the tough exterior wall of fungal and mycobacterial cells. Such detergents are direct inhibitors of the PCR process. Hemin is a chemical in red blood cells that inhibits the activity of some of the thermostable polymerase enzymes. Samples containing significant amounts of blood can give false negative results if not prepared properly.

Polymerase Chain Reaction methodology is still a relatively new science. PCR assay development and optimization is an ongoing process. As sample preparation methods are improved, laboratory methods are standardized, and new reagents are discovered, PCR will provide us with our most powerful tool in genetic and infectious disease diagnostic medicine. In exotic animal diagnostic medicine, PCR is currently being investigated, or is in use, for
the diagnosis of mycobacterial infections (including Johne's disease), Bluetongue virus, Malignant Catarrhal Fever, Chlamydiosis, Avian Polyomavirus, Psittacine Beak & Feather Disease virus, Giardiasis, Mycoplasmosis, and probably others as of this printing. The reader is referred to the suggested reading list below for an overview of the strengths and limitations of PCR and for a summary of its uses in diagnostic microbiology.

Suggested reading

Table 1. Infectious Agents which PCR technology has been successfully used to identify

<table>
<thead>
<tr>
<th>Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human enteroviruses</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td>Rotaviruses, Groups A, B and C</td>
</tr>
<tr>
<td>Retroviruses</td>
</tr>
<tr>
<td>Hepadnaviruses</td>
</tr>
<tr>
<td>Adenoviruses</td>
</tr>
<tr>
<td>Herpesviruses</td>
</tr>
<tr>
<td>Papillomaviruses</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Treponema pallidum</em></td>
</tr>
<tr>
<td><em>Borrelia burgdorferi</em></td>
</tr>
<tr>
<td>Mycobacterium (genus-specific primers and species-specific for <em>M. tuberculosis</em>, <em>M. avium</em>, <em>M. fortuitum</em>, <em>M. leprae</em>, <em>M. paratuberculosis</em>, <em>M. intracellular</em>, <em>M. bovis</em>, <em>M. simiae</em>, <em>M. kansasi</em>, <em>M. xenopi</em>, <em>M. marinum</em>, <em>M. microti</em>, <em>M. gasri</em>, <em>M. scrofulaceum</em>, <em>M. gordonae</em>)</td>
</tr>
<tr>
<td>Enteric Pathogens (<em>Helicobacter pylori</em>, <em>Campylobacter cholerae</em>, <em>Clostridium difficile</em>, <em>Salmonella</em> spp., <em>Shigella</em> spp., <em>Echerichia coli</em>)</td>
</tr>
<tr>
<td>Rickettsia (<em>R. prowazekii</em>, <em>R. rickettsii</em>, <em>R. conorii</em>, <em>R. tsutsugamushi</em>, <em>Erhlichia chaffeensis</em>)</td>
</tr>
<tr>
<td>Chalmydia (genus specific primers and species-specific for <em>C. trachomatis</em>, <em>C. psittaci</em>, <em>C. pneumoniae</em>)</td>
</tr>
<tr>
<td>Mycoplasma (genus specific primers and species-specific for <em>M. genitalium</em>, <em>M. fermentans</em>, <em>M. pneumoniae</em>, <em>U. urealyticum</em>)</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
</tr>
<tr>
<td><em>Bacteroides gingivalis</em></td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parasites</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Giardia</em> spp.</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus</em> spp.</td>
</tr>
<tr>
<td><em>Candida</em> spp.</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
</tr>
</tbody>
</table>

(Compiled from Ehrlich & Greenberg, 1994)
Table 2. Medical Applications of the Polymerase Chain Reaction in Human Genetics

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Medical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle cell anemia</td>
<td></td>
</tr>
<tr>
<td>β-thalassemia and hemoglobin H disease</td>
<td></td>
</tr>
<tr>
<td>Phenylketonuria</td>
<td></td>
</tr>
<tr>
<td>Diabetes (insulin-gene mutation)</td>
<td></td>
</tr>
<tr>
<td>Cystic fibrosis (allele linked)</td>
<td></td>
</tr>
<tr>
<td>Hemophilia A (allele linked)</td>
<td></td>
</tr>
<tr>
<td>Hemophilia B (gene mutation)</td>
<td></td>
</tr>
<tr>
<td>Hemophilia B (allele linked)</td>
<td></td>
</tr>
<tr>
<td>Clotting factor VIII mutation</td>
<td></td>
</tr>
<tr>
<td>α1-Antitrypsin deficiency (allele linked)</td>
<td></td>
</tr>
<tr>
<td>Leber's hereditary optic neuropathy (mitochondrial mutation)</td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein mutations</td>
<td></td>
</tr>
<tr>
<td>Duchenne's muscular dystrophy</td>
<td></td>
</tr>
<tr>
<td>Lesch-Nyhan syndrome</td>
<td></td>
</tr>
<tr>
<td>Huntington's disease (allele linked)</td>
<td></td>
</tr>
<tr>
<td>Redidual leukemia (Philadelphia chromosome)</td>
<td></td>
</tr>
<tr>
<td>Lymphoma dissemination</td>
<td></td>
</tr>
</tbody>
</table>

Pathogenesis (allele linked diseases)

<table>
<thead>
<tr>
<th>Disease</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td></td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
<td></td>
</tr>
<tr>
<td>Myasthenia gravis and multiple sclerosis</td>
<td></td>
</tr>
<tr>
<td>Oncogene-linked cancers</td>
<td></td>
</tr>
</tbody>
</table>

(reproduced from Desforges, 1990)
Polymerase chain reaction (PCR) and its limitations. One cycle of PCR is shown in detail; subsequent cycles lead to further geometric increases in DNA copies. At right, potential problems are indicated near the point that they affect.

1. Clinical samples containing PCR inhibitors
2. Contamination of sample with amplicons made in previous assay
3. Cycles of temperature changes requiring an automated equipment item
4. Detection of amplified DNA

(Reproduced from Engleberg, 1994)
Four methods for detecting specific amplified DNA after polymerase chain reaction (PCR). Amplification process is suggested by the central diagram; the four detection methods are illustrated from left to right, counterclockwise. **Reverse dot-blot assay:** PCR is performed using primers labeled with a chemical substance that will create a signal, S (e.g., luminescence, enzyme activity, fluorescence). Because all amplified DNAs (amplicons) have primers at their ends, they all carry this signal-producing label at the end of PCR cycling. These PCR products are incubated with a solid support (filter membrane, microtiter plate) onto which unlabeled probes have been fixed at separate sites. One of these probes is directed at the specific DNA region targeted by the PCR. When the labeled amplicon hybridizes to the complementary probe, the signal will be localized and detected at the specific probe site. **Oligonucleotide ligation assay:** No label is added to the PCR primers; instead, unlabeled amplicons are added to a solution containing two adjacent probes, one labeled for signal production, S, and the other labeled with biotin, B. When the probes bind to the amplicons, their ends come into close proximity and are covalently joined by the reagent enzyme DNA ligase. The ligated molecule is then captured on a solid support coated with streptavidin, SA (which binds biotin). Subsequent washing will remove any of the signal-labeled probe that is not covalently bound to the biotin-labeled probe. Detection of the signal on the solid support constitutes a positive test. **Product capture, probe immunoassay:** In this assay the PCR primers are labeled with biotin and the detection probe is labeled with an antigen (e.g., digoxigenin) for which a specific antibody is available. After hybridization of amplicons with the probe, complexes are captured on a solid support coated with SA. The presence of the probe is detected with the specific labeled antibody, S. **Hybridization protection assay:** A probe labeled with an acridinium ester is added to sample containing amplicons. When the probe binds to a target nucleic acid, the ester is sterically protected from hydrolysis. If the probe remains unbound, the ester bond then is hydrolysed and the labeled probe loses its chemiluminescent property. In a positive sample, the bound probe is detected by the emission of light in a luminometer.

(Reproduced from Engleberg, 1994)
TESTING METHODS FOR FELINE IMMUNODEFICIENCY VIRUS IN NONDOMESTIC FELIDS

Michael B. Worley, DVM
Virology Laboratory, Center for Reproduction of Endangered Species, Zoological Society of San Diego, P.O. Box 551, San Diego, CA 92112, USA

The control and management of an infectious disease in a zoological collection depends to a great extent on the development of methods to comprehensively identify and characterize the causative agent. The laboratory diagnosis of feline immunodeficiency virus (FIV) can be accomplished by serology, cell culture, or gene amplification. When considering appropriate assays for testing for FIV in nondomestic felids, two issues of importance are: 1) The specificity and sensitivity of the assays being used, and 2) The degree of genetic relatedness among the potential viruses to be detected.

At present, serological assays are the foundation for screening individual clinical patients as well as populations for prevalence of infection. These assays identify infected individuals by detecting antibodies to FIV proteins. Since the discovery of FIV in domestic cats, several research and diagnostic laboratories have detected serum antibodies to lentiviruses in a variety of species of captive and free-ranging nondomestic felids.\(^1\,^2\,^3\,^4\,^7\,^8\) The types of assays utilized to detect exposure to FIV have varied but the original domestic cat Petaluma strain or a molecular clone of it has, with few exceptions, been used as the target antigen.

The original test for detecting antibodies to FIV in domestic cats was an indirect immunofluorescence assay that utilized virus-infected, cultured peripheral blood lymphocytes (PBL) as the target.\(^9\) Because of an unacceptable level of non-specific staining associated with this assay, it has been abandoned for routine use. Soon thereafter, IDEXX Corporation produced a commercially available microdilution plate format enzyme-linked immunosorbent assay (ELISA) with manufacturer reported sensitivity and specificity values of 98.5% and 99%, respectively, for FIV antibody in domestic cats.\(^7\) This assay requires a microtiter plate reader for optimal interpretation of the colorimetric results. IDEXX also produced an ELISA known as the CITE test that could evaluate samples individually and be conducted easily in the veterinarian’s hospital. According to the manufacturer, this assay has sensitivity and specificity values of 98.3% and 99%, respectively, for FIV antibody in domestic cats.\(^7\)

When the prevalence of FIV infection is low, even highly specific tests will yield a large number of false-positive results. Therefore, specimens that are repeatedly reactive by either commercial ELISA should be confirmed with additional techniques. In addition, these assays may not be sensitive enough to detect the low levels of virus-specific antibodies that are present in some infected cat sera.\(^10\) Recently, recombinant proteins have been used in an ELISA to detect antibodies directed against FIV p17 and p24.\(^10\) Although not commercially available, this assay allowed the identification of seropositive cats following infection with FIV and possessed greater sensitivity and specificity than the commercially available plate ELISA.
Antibodies to specific viral proteins can be detected with the Western blot, in which electrophoretically separated proteins adsorbed to a solid support are the target antigens. When compared to the plate ELISA and the CITE test for detecting FIV antibody in domestic cats, the Western blot possesses superior sensitivity and specificity. Some cats that test negative or are equivocal with the plate ELISA are antibody positive by the Western blot. In contrast, some cats that are CITE test positive are subsequently found to be false positive when tested by Western blotting (Barr, M., personal communication). As a result of these types of results, the Western blot has become the test of choice in most research laboratories and in an increasing number of regional diagnostic centers.

The challenge of accurately detecting FIV antibody in nondomestic felids is further complicated by the heterogeneity between viruses of different nondomestic species as well as between nondomestic and domestic cat viruses. Not only are certain animals CITE test negative and Western blot positive, there is also a concern that a Western blot utilizing a domestic cat virus as the target may not detect FIV antibodies in all infected individuals of the many species of nondomestic felids being tested. Although partial or complete nucleotide sequence has been obtained from lentiviruses from the puma, African lion, and Pallas cat, only the Pallas cat virus has been propagated in cell culture in sufficient quantity to be used in Western blots. Therefore, at the present time, antibodies to FIV can be detected in nondomestic felids with the following methods: 1) commercial ELISAs that utilize domestic cat FIV antigens as the target and produce a certain number of false-negative and false-positive results, and 2) Western blot utilizing domestic cat FIV proteins as the target, the preparation of which is as variable as the number of laboratories performing the assay.

A potential alternative to testing for FIV antibody is the detection of the viral DNA intermediate (proviral DNA) in peripheral blood lymphocytes (PBL) with the polymerase chain reaction (PCR) test. Despite the extreme sensitivity of PCR, the DNA primers required to initiate the reaction may need to be made from the DNA sequence of domestic cat virus or from conserved regions shared by several nondomestic cat viruses. However, recent attempts in the CRES virology laboratory to PCR amplify viral products from PBL DNA of Western blot-positive cheetahs with primers whose sequences were shared by lion, puma, and domestic cat viruses were unsuccessful.

The Felid Taxon Advisory Group (TAG) has recommended that all zoological institutions have their felids tested for FIV antibody through one laboratory using one test. For the time being, this is a prudent decision. Clearly there is a need for the isolation and sequencing of lentiviruses from additional species. The information obtained from this research will bring us closer to the development of comprehensive antibody and antigen-detection assays for FIV infection in all felids.
LITERATURE CITED


DISEASE AND PARASITE SURVEILLANCE OF A HERD OF SCIMITAR-HORNED ORYX USING DOMESTIC SHEEP AS SENTINEL ANIMALS

James M. Jensen, DVM*, Thomas M. Craig, DVM, PhD
College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843, USA

Introduction

Tracer lambs were utilized to determine the helminth and contagious disease exposure of a herd of scimitar-horned oryx (Oryx dammah) in the eastern Edwards Plateau of Texas. The oryx herd was comprised of more than 70 adult animals ranging over 250 hectares of grassland. It was developed over several years through the cooperation of the ranch owner, David Bamberger, and the Species Survival Plan committee for the scimitar-horned oryx. The actual test herd of scimitar-horned oryx involved approximately 20 bachelor males which were rotated between two pastures. These grazing areas were roughly 50 hectares apiece.

The area allotted for the oryx herd was segregated from contiguous land by a 7' wildlife fence. Cross-fencing within the 250 hectare land mass was also wildlife fencing of the same dimension. Two fallow deer (Dama dama) and a red deer (Cervus elaphus) had continuous access to the 50 hectare grazing paddocks. A varying number of white-tailed deer (Odocoileus virginianus) were able to enter and exit the high fenced area due to their leaping ability.

Methods

Groups of 3 lambs were used to survey parasite and infectious diseases in this study. Prior to their introduction to the bachelor herd of oryx, the lambs were ascertained to be free of nematodes by fecal examination and anthelmintic treatment. Serum samples were also drawn to provide baseline titers for ruminant diseases. These diseases included Pasteurella hemolytica, Leptospira spp. (pomona, icterohaemorrhagiae, canicola, grippotyphosa, hardjo), Coxiella burnetii, Mycobacterium paratuberculosis, Toxoplasma gondii, Anaplasma marginale, Chlamydia psittaci, Bluetongue virus, Epizootic Hemorrhagic Disease virus, and Caprine Arthritis and Encephalitis/Ovine Progressive Pneumonia viruses.

Lamb trios grazed with the oryx herd for 4-6 weeks and then were replaced by the next group of sentinel sheep. After lamb groups were retrieved from the ranch, they were held on concrete for 2-3 weeks to allow the completion of infective cycles by nematodes. A paired serum sample was taken from each sheep within 2-3 days of their return from the ranch. Ultimately the sheep were slaughtered and a thorough helminth examination was performed.
Results

*Camelostrongylus mentulatus* and *Trichostrongylus colubriformis* were the predominant nematode species recovered from the tracer lambs. *Trichostrongylus axei*, *Cooperia punctata*, *Oesophagostomum venulosum*, and *Trichuris* spp. were recovered in lesser numbers. The summer of 1994 was unusually dry and virtually no nematodes were transmitted from July through September. Peak parasite transmission occurred during the periods of rapid grass growth following rains. Arrested 4th stage ostertaginae larvae were recovered during the late winter and early spring. *C. mentulatus* was the only species of ostertaginae identified in this survey.

All serum samples drawn from sentinel sheep before and after their ranch stay were negative for bluetongue, EHD, anaplasmosis, Johne's disease, CAE/OPP, toxoplasmosis, and all Leptospirosis. Chlamydia and Pasteurella titers either remained constant or decreased during the grazing period.

Discussion

Domestic sheep were used as tracer animals in this parasite/disease survey because they are small, grazing artiodactyl. This was important, since taxonomically related creatures tend to harbor similar diseases and parasites. Sheep are tractable and relatively inexpensive for such investigations. The sentinel sheep associated with the scimitar-horned oryx and foraged with the bachelor herd. This added to their merit as tracer animals, because it exposed them to the same environment, parasites, and pathogens as the oryx.

The two prevalent nematodes of this study, *C. mentulatus* and *T. colubriformis*, are commonly seen in African antelope species. *T. axei*, *O. venulosum*, and *Trichuris* spp. also have wide host ranges that include wild ruminants. It is most likely that these parasites were introduced when the oryx were placed on this region of the ranch. The sentinel sheep effectively confirmed that no serious nematode transmission occurred from July to September. Examination of their gastrointestinal tracts at slaughter also indicated that *C. mentulatus* is capable of hypobiosis in late winter and early spring. These two facts are valuable when formulating anthelmintic treatment schedules.

The negative serum titers obtained in this survey represent an apparent absence of those individual diseases in the oryx environment. Multiple susceptible sheep were cycled through the oryx pastures on a 4-6 week interval. It is, therefore, unlikely that these negative titers could represent a failure of individual animals to respond to disease organisms that were present.

The noticeable decline in some of the chlamydia and pasteurella titers during the period of commingling is noteworthy. The lambs were taken from a research farm where both organisms were obviously present. The drop in titers likely occurred due to absence or low incidence of these organisms in the oryx herd. It is noteworthy that the general dispersal
of animals over acreage, as compared to the confinement of a farm, may reduce the incidence of transmission of these organisms.

ACKNOWLEDGEMENTS

The authors would like to thank J. David Bamberger (owner) and Randy Lenz (wildlife manager) of the Bamberger Ranch, Johnson City, Texas, for their support and collegiality. Dale Tuttle (director) and Dr. Doug Page (veterinarian) of the Jacksonville Zoo, Jacksonville, Florida provided valuable assistance in gaining endorsement of this research project by the scimitar-horned oryx Species Survival Plan committee. Desiree McCollam, Sonja Hamner, and Allison Merrill (VM3) supplied important technical support for this project at the College of Veterinary Medicine, Texas A&M University.
DETECTION OF CIRCULATING MYCOBACTERIAL ANTIGENS IN *Mycobacterium paratuberculosis*-INFECTED NONDOMESTIC RUMINANTS

Barbara J. Mangold, DVM*, Bonnie L. Raphael, DVM, Robert A. Cook, VMD
Wildlife Health Sciences, Wildlife Conservation Society, Bronx, New York 10460, USA

Kris Huygen, PhD
Pasteur Institute of Brabant, 1180 Brussels, Belgium

Henry P. Godfrey, MD, PhD
Department of Experimental Pathology, New York Medical College, Valhalla, New York 10595, USA

Introduction

*Mycobacterium paratuberculosis* occurs worldwide and can infect cattle, sheep, goats, and a variety of wild and exotic ruminants to cause paratuberculosis (Johne's disease). Clinically, Johne's disease is a chronic wasting disease with diarrhea, weight loss and decreased productivity. It can be a devastating disease in a zoological collection because of its chronicity and the difficulty of diagnosing it until late in the disease process. Laboratory diagnosis of Johne’s disease (and mycobacteriosis in general) is often difficult. Although mycobacterial culture is definitive if positive, culture may take up to 16 weeks, and mycobacteria do not always grow in culture. Serological tests for mycobacterial antibodies are limited by low sensitivity or specificity.

Antigen 85 (Ag85) complex proteins are major secretory products of actively proliferating mycobacteria *in vitro*. These fibronectin-binding proteins have been detected in serum from human patients with active tuberculosis, suggesting that the presence of circulating Ag85 proteins may correlate with active mycobacterial infection (H. P. Godfrey, unpublished). This project was therefore undertaken to evaluate measurement of serum levels of Ag85 in exotic ruminants as a rapid diagnostic immunoassay for paratuberculosis. Ag85 levels were correlated with clinical signs, bacteriologic cultures, and histopathologic lesions in animals with and without *M. paratuberculosis* infection. Initial results indicate that animals actively infected with *M. paratuberculosis* have 10-fold greater median levels of circulating Ag85 than uninfected controls.

Methods

Two herds of Nyala (*Tragelaphus angasi*) and one herd of Impala (*Aepyceros melampus*) were tested. One of the Nyala herds was considered a negative herd on the basis of no clinical or pathologic evidence of *M. paratuberculosis* infection. Two animals from this herd were negative on radiometric fecal culture. The other two herds were a mixture of known *M. paratuberculosis*-infected and uninfected animals. Serum samples were submitted as unknowns and the assay was run as a single blind study.
Duplicate dilutions of serum samples and Ag85 standards were blotted to nitrocellulose. Positive and negative controls were used with each assay performed. Ag85 was detected by dot immunobinding using mouse monoclonal anti- \textit{M. bovis} Ag85 with limited cross reactivity to \textit{M. avium} Ag85 as primary antibody.\textsuperscript{1,2} Blots were developed using horseradish peroxidase-conjugated goat anti-mouse immunoglobulin and luminescent substrate (ECL, Amersham). Luminescence was detected by standard x-ray film. Ag85 levels in serum samples were assessed by comparison with purified \textit{M. bovis} Ag85 standards.

Results

Five samples from the \textit{M. paratuberculosis}-negative Nyala herd were tested. Serum levels of Ag85 were 5-25 µg/ml in these animals (median, 15 µg/ml). Ten samples were tested from each herd of Nyala and Impala. In each herd, 5 were from \textit{M. paratuberculosis}-infected animals and 5 were from ostensibly uninfected animals. In the Nyala, 9 of 10 samples correlated with clinical and laboratory findings of disease and non-disease states. Serum levels of Ag85 ranged from 50-100 µg/ml for the non-diseased animals (median, 65 µg/ml), and from 80-320 µg/ml for the infected animals (median, 160 µg/ml). In the Impala, ten of ten samples correlated with clinical and laboratory findings. Serum levels of Ag85 ranged from 5-50 µg/ml for the non-diseased animals (median, 5 µg/ml) and from 50-210 µg/ml for the infected animals (median, 170 µg/ml).

Discussion

Mycobacteriosis can be a significant health problem in many zoological species including mammals, birds, reptiles, amphibians, and fish. Immunoassays for the detection of serum antibodies to mycobacterial antigens have not been widely accepted. Low sensitivity of these assays may be due to low number of organisms present in the host and resultant low levels of anti-mycobacterial antibody formation. Poor specificity may be due to the extensive cross reactivity of mycobacterial antigens.

Dot immunobinding was used to quantitate circulating Ag85 levels in three captive herds of non-domestic ruminants. Animals actively infected with \textit{M. paratuberculosis} had more than 10-fold greater median levels of circulating Ag85 than uninfected controls. Serum levels of Ag85 correlated well with clinical and laboratory findings of mycobacterial disease. These initial results suggest that measurement of circulating Ag85 levels may predict active infection with \textit{M. paratuberculosis}. Ag85 was detected in the present study by a monoclonal anti-\textit{M. bovis} Ag85 antibody reactive with determinants common to Ag85 from several mycobacterial species (but not \textit{M. avium}).\textsuperscript{2} A more specific diagnostic assay would be one based on monoclonal antibodies directed to antigens present only on \textit{M. paratuberculosis} Ag85. However, the use of a broadly reactive anti-Ag85 antibody is advantageous for general detection of other mycobacterial diseases of veterinary importance in captive populations. Ongoing studies in our laboratories in fact suggest that circulating Ag85 levels detected by this assay may be elevated in ruminants infected with \textit{M. bovis}.
The dot blot immunobinding assay used in this study detects mycobacterial antigens, not antibodies produced in response to these antigens. Therefore it does not depend on an immunocompetent host to be positive and is a direct indication of active mycobacterial disease. It therefore offers great promise in the early, rapid, antemortem diagnosis of \textit{M. paratuberculosis}, and possibly other mycobacterial infections, in both domestic and non-domestic animal species.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Freed Foundation (B.J.M.) and by NIH grants AI37014 and CA34141 (H.P.G.).

LITERATURE CITED


CANINE DISTEMPER IN WILD FELIDS

L. Munson, DVM, PhD•
Department of Pathology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901, USA

M.J.G. Appel, Dr. Med. Vet, PhD
James A. Baker Institute for Animal Health, Cornell University, Ithaca, N.Y. 14853, USA

Margaret A. Carpenter, PhD and Stephen J. O'Brien, PhD
Laboratory of Viral Carcinogenesis, Frederick Cancer Research and Development Center, Frederick, MD 21702, USA

Melody Roelke-Parker, DVM
Serengeti Wildlife Research Institute, Tanzania National Parks, Arusha, Tanzania

When three lions in the Serengeti National Park, Tanzania were observed with grand mal seizures and two other lions were observed with myoclonus in early 1994, a network of investigators specializing in carnivore diseases was immediately mobilized to assist in identifying the causative agent. The recent appointment of a Chief Veterinary Officer and establishment of the Veterinary and Wildlife Disease Investigation Unit in the Tanzania National Parks facilitated the collection of key data and diagnostic material that resulted in a rapid and definitive diagnosis. The epidemic, affecting lions, hyenas, and bat-eared foxes in the Serengeti Ecosystem, was determined to be caused by canine distemper virus (CDV) through a multidisciplinary collaborative approach using traditional and contemporary diagnostic procedures.

Tissues and sera were obtained from spontaneous deaths, animals euthanized in a moribund state, and live lions anesthetized during the disease investigation. Necropsies were performed on accessible carcasses, and tissues were fixed in 10% formalin for shipment. Selected tissue samples, sera, buffy coats, and lymph node aspirates were stored and transported in liquid nitrogen. Fixed tissues were processed routinely for histopathology; then tissues with lesions compatible with canine distemper infection (such as encephalitis, interstitial pneumonia with Type II pneumocyte hyperplasia, or lymphoid depletion) or with intracellular viral inclusions were selected for immunohistochemical procedures. The presence of intrallesional viral antigens was confirmed on duplicate sections using a mouse monoclonal antibody to a CDV-N protein (MAb N3.991), a commercial avidin-biotin kit, and Gill’s hematoxylin as a counterstain.

High titers of serum neutralizing antibodies to canine distemper were measured in the majority of the lions from the Serengeti using microtiter methods and both the Ondersteoport strain of CDV adapted to Vero cells and a Vero cell-adapted CDV isolate (A92-27/20) from a lion. Canine distemper virus was isolated from cerebral spinal fluid and tissues in a canine blood lymphocyte coculture system. DNA sequences homologous to the "P" gene of canine distemper also were obtained from blood lymphocytes by polymerase
chain reaction procedures. Phylogenetic analysis of the derived sequences in comparison to closely related Morbilliviruses indicated that the causative agent in the Serengeti epidemic was a close relative of, if not identical to, canine distemper virus.

ACKNOWLEDGMENTS

This research was supported in part by The Messerli Foundation, Zurich, Switzerland.
THE USE OF PCR TO DIAGNOSE MCF AND DETECT VIRUS CARRIERS

Robert B. Kliefforth, BS*, Stacy M. Sutton, BA, and Werner P. Heuschele, DVM, PhD
Infectious Diseases Division, Center for Reproduction of Endangered Species (CRES), Zoological Society of San Diego, P.O. Box 551, San Diego, CA 92112-0551, USA

Introduction

Malignant catarrhal fever (MCF) is at present associated with two different infectious agents. Alcelaphine herpesvirus 1 (AHV-1) is well established as the causative agent of wildebeest-derived (Connochaetes spp.) MCF, having been first isolated in 1960.25 Ovine herpesvirus 2 (OHV-2), while it has not been propagated reliably in cell culture or fully characterized molecularly, has been linked to "sheep-associated" cases of MCF in the United Kingdom, Asia, and the United States.17,36 OHV-2 has also been shown to possess regions of DNA both homologous to and distinct from AHV-1.1,2

It has been proposed that species-specific ruminant gammaherpesviruses exist.27 In addition to AHV-1 and OHV-2, at least two other viruses have been isolated which may fit this category. Alcelaphine herpesvirus 2 (AHV-2) has been isolated several times23,28,31 from topi (Damaliscus lunatus or D. korrigum) and hartebeest (Alcelaphus buselaphus) and has been shown to be distinct from AHV-1.31,33 AHV-2 is believed to be non-pathogenic in domestic bovines.23 A virus termed hippotragine herpesvirus (HiHV) has been isolated from a roan antelope26 (Hippotragus equinus).

MCF, whether attributed to AHV-1, OHV-2, or of unknown etiology, has been reported worldwide4 and has caused documented losses in zoological institutions. Clinical signs and histopathologic lesions have been reviewed.6,27 Recovery from clinical disease is rare but has been recorded.10,13,21 A serologic survey of artiodactyls in U.S. zoos by AHV-1 virus-neutralization assay and immunofluorescence found significant levels of antibodies in most bovid subfamilies as well as in the Cervidae.31

The virus-neutralization assay has been employed as a method of detecting possible latent infections by MCF agents4,8 to assess the risk of disease transmission to other susceptible species. Immunofluorescence and immunoperoxidase tests have also been used for this purpose.29,31 More recently, a competitive-inhibition enzyme-linked immunosorbent assay (CI-ELISA) has been established15 that employs a monoclonal antibody. This assay is reported to produce positive results using serum from suspected carriers of either AHV-1 or OHV-2, and allows rapid processing of specimens for herd surveillance.

Several groups have developed reagents or assays for MCF testing based on the detection of viral DNA.12,19,20,34 The polymerase chain reaction (PCR) is a general method30 which has been applied to specimens from experimentally or naturally infected animals. The first such assay for MCF14 was developed at the USDA's National Veterinary Services Laboratory using DNA sequence from a portion of the AHV-1 genome originally cloned32 at the
The Zoological Society of San Diego's (ZSSD's) Center for Reproduction of Endangered Species (CRES). The same clone was used independently at CRES to derive another set of primers first applied to the post-mortem confirmation of clinical disease in a naturally infected Indian gaur (*Bos gaurus*) at the San Diego Wild Animal Park. In this paper we define another set of primers, based on cloned cDNA sequence originating in a different region of the AHV-1 genome, that was developed to provide greater specificity of amplification. A PCR assay for OHV-2 has also been described recently that amplifies DNA obtained from sheep and domestic goats, but not DNA from AHV-1 or HiHV-1 (H.W. Reid, pers. comm.).

MCF agents appear to be lymphotropic. Consequently, purified leukocyte DNA from anticoagulated blood samples is an appropriate PCR test substrate and is easily collected during pre-shipment or quarantine examinations with minimal risk of adverse effects.

The purpose of this paper is to communicate our most recently developed PCR assay and to provide recommendations for collection and processing of specimens, PCR amplification and product detection, and interpretation of results.

**Materials & Methods**

As previously described, an AHV-1 cDNA library was constructed and screened (using rabbit anti-AHV-1 serum) to identify a clone designated 8'a which was fully sequenced. PCR primers and a probe internal to the primer sites were chosen from within the 8'a sequence by computer analysis, using the OSP oligonucleotide selection program. Primers were chosen based on the following criteria: balanced GC content, random base distribution, lack of significant complementary sequences between the primers, similar Tm values for both primers, and lack of internal complementary sequence in either primer. The 8'a-derived primer and probe sequences are:

- primer 8'a-R, 5'-CAGCCAGATAATTCACCC-3'
- primer 8'a-S, 5'-ACGAATTGCACGTGTCAAC-3'
- probe 8'a-K, 5'-GTAGACAAGATTCTCTTTTG-3'

For comparative purposes, PCR primers (herein designated H3D-A and H3D-B) and an internal probe (H3D-PROBE) derived from the earlier-cloned HindIII fragment D were also employed in separate reactions using the same DNA specimens and controls.

Leukocytes were prepared by subjecting Buffy-coat cells to hypotonic lysis for removal of contaminating red blood cells as previously described. DNA was extracted from leukocytes either as previously described or by use of the QIAamp Blood Kit according to the manufacturer's instructions (QIAGEN Inc., Chatsworth, California 91311, USA).

PCR reactions were set up and conducted essentially as previously described, using one microgram (mg) of purified leukocyte DNA in each test reaction and 10 nanograms (ng) of purified AHV-1 isolate WC11 DNA as the positive control. Detection of amplification products was also performed as previously described by electrophoresis in agarose gels,
capillary transfer to nylon membranes, and Southern blot analysis using non-radioactively labeled probes.

For comparison with PCR results, virus-neutralization assays were conducted using serum specimens obtained at the same time as whole blood for DNA extraction.\textsuperscript{16}

Results

A total of 39 animals were tested by both sets of PCR primers and by virus-neutralization assay. Results are shown in Table 1.

Table 1. Serum virus-neutralization assay (VNA) compared with PCR using two different sets of primers (designated H3D and 8’a) derived from alcelaphine herpesvirus 1 (AHV-1). Results are shown as number positive / number tested. Titers of 4 or greater are considered positive in the VNA. PCR is positive if binding of amplicon-specific probe is detectable by Southern blot hybridization.

<table>
<thead>
<tr>
<th></th>
<th>PCR using H3D primers</th>
<th>VNA</th>
<th>PCR using 8’a primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wildebeest (\textit{Connochaetes} spp.)</td>
<td>9/11$^a$</td>
<td>6/11</td>
<td>5/11$^b$</td>
</tr>
<tr>
<td>Other ruminants</td>
<td>22/28</td>
<td>2/28</td>
<td>13/28$^c$</td>
</tr>
</tbody>
</table>

$^a$ Three specimens positive using the H3D primers and negative by VNA were also negative using the 8’a primers.

$^b$ All specimens positive using the 8’a primers were also positive by H3D.

$^c$ Two specimens positive using the 8’a primers were negative by H3D.

Discussion

Previously, we reported that PCR using primers H3D-A and H3D-B amplified AHV-1 or similar sequences in approximately half of the opportunistically obtained ruminant blood samples tested, while virus-neutralization assay results were positive for less than 20\% of the samples.\textsuperscript{16} However, these primers were known also to amplify DNA from AHV-2.\textsuperscript{22} Further refinements in PCR testing were undertaken to determine whether primers could be found that would distinguish among known ruminant gammaherpesviruses and thus provide a clearer indication of the disease-transmission risk posed by infected animals.

Specimens from thirty-nine animals have now been tested by PCR and Southern blot analysis using the H3D primer set\textsuperscript{16} and the 8’a primer set along with their respective internal probes. In this group, which included a higher percentage of specimens that were positive in the earlier assay, the 8’a primers and probe produced positive results for fewer specimens than did the H3D primers and probe, as shown in Table 1. Among wildebeest,
which are known asymptomatic reservoirs of AHV-1, four of nine animals positive by H3D were negative by 8'a; of these, three were also negative by virus-neutralization assay. Among the other ruminant species, 11 animals positive by H3D were negative by 8'a, while two animals negative by H3D were positive by 8'a. Taken together, these results are not inconsistent with the hypothesis that the 8'a primers have restricted specificity relative to the H3D primers and may indicate the presence of more-virulent, AHV-1-like agents.

The potential value of PCR for providing sensitive and specific identification of MCF agents suggests that it be applied in the zoological setting in conjunction with serologic methods. Evidence of latent infection may be helpful in making decisions regarding animal accessions, breeding loans, or movements between enclosures.

Currently, PCR testing for MCF agents is not being done routinely by commercial or government-operated diagnostic laboratories. While this situation may change in the future, it is likely that PCR assays for MCF agents as well as other infectious agents and genetic disorders affecting exotic species will need to be performed by the institution's own laboratory staff. The proliferation of published methods and decreasing costs for necessary equipment have made in-house testing more readily justifiable. Based upon our own experience, we offer the following recommendations:

1. **General considerations.** Separate, dedicated work areas and equipment such as pipettors and microcentrifuges should be used for specimen processing, PCR reaction set-up, and further analysis of amplified products. All reagents used for specimens and reactions should be prepared using ultrapure water (United States Biochemical [USB], Cleveland, Ohio 44128, USA) and disposable containers. A benchtop PCR workstation equipped with an ultraviolet (UV) lamp (C.B.S. Scientific, Del Mar, California 92014, USA) allows decontamination of supplies and equipment by timed UV irradiation before use. Autoclavable and positive-displacement pipettors (Brinkmann Instruments, Westbury, New York 11590, USA) further reduce the risk of false positives by cross-contamination, as do aerosol-barrier pipet tips for conventional pipettors. Containers of ultrapure water, mineral oil (to prevent evaporative loss during thermal cycling), and reaction tubes can also be autoclaved before UV irradiation to provide an extra measure of safety.

2. **Specimen collection and processing.** Whenever possible, tissues (including blood) and serum should be collected at the same time so that results from different test methods can be compared. DNA is prepared from anticoagulated blood as described in Materials & Methods. Prior to DNA extraction, we also divide each lymphocyte preparation into multiple aliquots for long-term storage at -70°C so that backup specimens are available in case of sample loss or contamination during subsequent processing. For post-mortem specimens obtained at necropsy, we suggest direct involvement in tissue collection to reduce cross-contamination. The prosector should provide relatively large tissue pieces which can then be transferred to disposable worksheets and sub-sampled by a second individual who uses disposable forceps (Nalge Co., Rochester, New York 14610, USA) and scalpels to remove pieces of
approximately one cubic millimeter (mm$^3$) beginning with a fresh incision on a surface uncontaminated by contact with gross-dissection implements. These tissues can be stored at -70°C in screw-cap microcentrifuge tubes. DNA can be efficiently extracted from such specimens using the QIAamp Tissue Kit (QIAGEN, Inc.) with much lower risk of contamination than by preparing and processing homogenates. If necessary, the quality of extracted DNA can be confirmed in a functionally relevant manner by conducting PCR amplification using primers derived from the bovine growth-hormone (bGH) gene, which is conserved among ruminants. For the bGH assay, it is sufficient to visually verify amplification by UV illumination of ethidium bromide-stained electrophoretic gels containing PCR reaction products.

3. **PCR reactions, detection of amplification, and interpretation of results.** Appropriate positive and negative controls must be included in every set of PCR reactions. We strongly advise amplification using a single pair of primers followed by Southern blot analysis of electrophoretically separated reaction products. Use of the AHV-1-derived primers with ruminant genomic DNA as described in Materials & Methods sometimes results in the appearance of bands in the same area of the electrophoretic gel as the diagnostic band amplified from purified AHV-1 DNA. If Southern analysis is done, an oligonucleotide probe specific for the region of sequence between the amplification primers generally provides unambiguous identification of the virus-related product, if present. Non-radioactive labeling of the probe sequence gives far greater sensitivity than ethidium bromide-based visual detection, while avoiding the storage, handling, and disposal problems of radioisotope use. An alternative PCR method such as the use of nested primers for two-stage amplification, while it can be used to advantage in some circumstances, greatly increases the likelihood of specimen cross-contamination as tubes containing products from the first-stage amplification must be opened in order to set up the second-stage reaction. Regardless of the precise methods employed, results from PCR testing for MCF infection should be interpreted cautiously. Although we expect that primers derived from AHV-1 will correctly identify AHV-1-infected animals in most instances, other ruminant gammaherpesviruses are likely to be found in exotic species. These agents may or may not be capable of causing disease resembling MCF and may or may not contain DNA sequences amplifiable by primers derived from either AHV-1 or OHV-2. Consequently, a negative PCR result does not guarantee the absence of a pathogenic ruminant gammaherpesvirus, nor does a positive result necessarily imply a high risk for disease transmission. Increased experience with PCR should eventually clarify the predictive value of MCF testing by this method. At the present time, both serologic testing and PCR should be done if possible in any situation where MCF disease risk must be considered. Examples would include movement of animals within and between institutions, multi-species exhibits, breeding groups of endangered species, and adjacent exhibits where virus transmission to susceptible ruminants is possible by aerosols, direct contact, or surface water flow.
In conclusion, we believe that PCR using multiple sets of primers, initially in conjunction with virus-neutralization assays (or another antibody-based method) using serum samples obtained at the same time as whole blood for DNA extraction, will ultimately permit greatly improved surveillance of ruminants in zoos for latent infection by potential agents of MCF. Devastating MCF losses in the small herds typical of zoos can be avoided by supplementing low-cost serologic screening with PCR, which is not only potentially more sensitive but provides direct detection of virus.

LITERATURE CITED


NEW DEVELOPMENTS IN THE DIAGNOSIS OF JOHNE'S DISEASE IN DOMESTIC AND ZOOLOGICAL SMALL RUMINANTS

Michael T. Collins, DVM, PhD
Dept. Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706-1102, USA

Background

One hundred years ago, Johne and Frothingham described a disease in a dairy cow which has become known as Johne's (Yo-nees) disease. The disease was first recognized in the United States in 1935. Mycobacterium paratuberculosis, the cause of this chronic intestinal infection, has become firmly established in dairy cattle herds in almost every country of the world. Approximately 5 to 15% of dairy cattle in the USA are infected. A herd survey in Wisconsin indicated one-third of herds are seropositive. Johne's disease seems to be endemic in beef cattle as well, although far fewer surveys have been done to document the extent of the problem. Similarly, herds of domestic sheep and goats in many countries are also infected, but few surveys have been done to establish the magnitude of the problem.

The epidemiological history of M. paratuberculosis in animals resembles that of tuberculosis. Domestication and confinement rearing of animals facilitates transmission of the pathogen. Because of the long latency period, 2 to 8 years, the pathogen has ample opportunity for transmission to a new host before its original host becomes debilitated and dies. In the wild, even if the organism is present in a population, it has very limited opportunity for spread and so will not likely become epidemic. On the herd level, some populations of animals are free of paratuberculosis. Unless a M. paratuberculosis-infected animal is added to the population, the population will remain free of the infection.

Confinement rearing of wild animals makes transmission of M. paratuberculosis more likely. Once introduced to a population, this fecal-orally transmitted pathogen slowly penetrates herds reaching very high infection rates. While the steadily increasing rate of infection truly represents an epidemic in the population, it is often not perceived as such because of the glacial-like rate of disease spread.

The host range of M. paratuberculosis is not fully known. Intestinal infections caused by M. paratuberculosis are predominantly found in ruminants, but based on recent experience at several zoological institutions in the USA, a wide range of wild and domestic ruminants are apparently susceptible. Experimental challenge studies suggest that non-ruminants, including birds, are susceptible to the infection. Naturally occurring infections have been reported in humans and non-human primates as well.

Control of paratuberculosis in domestic animals is based on a two-pronged attack on the organism: 1) limit opportunities for transmission of M. paratuberculosis by altering animal
management practices, and 2) identify and remove from populations the *M. paratuberculosis*-infected sources of infection. In captive populations of wild animals, options for changing management are very limited. Consequently, test and removal is the primary means of paratuberculosis control. The focus of this article is to review tests available for detection of *M. paratuberculosis* infections in nondomestic animals.

The bottom line is that through research, we have many new tests for diagnosis of paratuberculosis, far more than available for many other disease including tuberculosis and brucellosis which have nearly been eradicated from cattle populations. Adaptation of paratuberculosis diagnostic technology to both free ranging and captive populations of wild animals is feasible. Some new tests work now, others require more research and development. However, the disease is spreading and veterinarians and keepers should begin using the available tests for paratuberculosis.

**Introduction**

Decades of research effort and millions of public and private money has been expended in development of better diagnostic tests for paratuberculosis. Presently, there are three methods for detection of the organism in clinical samples, a test for serum antibodies to *M. paratuberculosis*, and an in vitro assay of cell-mediated immunity (CMI) to *M. paratuberculosis* based on gamma interferon (IFNγ) detection. While these tests have been developed principally for used in cattle, several can be used in nondomestic animals as well. Each type of test will be briefly discussed relative to its accuracy and applicability to wild and zoological animals. A more comprehensive review can be found in the Proceedings of the Fourth International Colloquium on Paratuberculosis.7 For test comparison purposes, estimates of diagnostic sensitivity and specificity of each method may be mentioned, however, these are not absolute values. Both parameters of diagnostic test accuracy vary considerable among published studies, reflecting differences in study design and *M. paratuberculosis* infection severity in the populations of animals studied. Moreover, sensitivity and specificity estimates have only been done on cattle or sheep and should be regarded only as rough estimates for nondomestic animals.

**Organism detection tests**

There are three methods available for detecting *M. paratuberculosis* in clinical samples; conventional culture (test tube media), radiometric culture (commercial radioisotope-containing broth culture system called BACTEC), and a gene probe (PCR-amplified DNA probe sold by IDEXX Laboratories, Inc.). Detection of *M. paratuberculosis* in clinical samples is considered definitive for diagnosis of the infection. Thus, all of these tests are 100% specific. Largely because of differences in how samples are processed, the tests have different *M. paratuberculosis* detection limits. This translates to different levels of diagnostic sensitivity. The sensitivity of conventional culture, BACTEC culture, and DNA probe on subclinically infected cattle is roughly 50%, 70%, and 30%, respectively.19
Conventional culture is the most widely available test for paratuberculosis. However, proficiency at culturing this very fastidious mycobacterial pathogen varies widely among laboratories. Test cost is usually $10 to $15 per sample and isolation of *M. paratuberculosis* requires roughly 16 weeks. Seldom do laboratories do confirmatory testing on mycobacterial isolates. Rather, by convention laboratories assume that all slow-growing, acid-fast, and mycobactin-dependent bacterial isolates are *M. paratuberculosis.*

BACTEC (radiometric) culture for mycobacteria is among the most common methods used for diagnosis of tuberculosis (*M. tuberculosis*) and *M. avium* infections in human hospital laboratories. Without modification of the commercial BACTEC 12B medium, however, isolation of *M. paratuberculosis* is not possible. At the University of Wisconsin, we have adapted the BACTEC system for detection of *M. paratuberculosis* and, coupled with filter concentration of samples, find it to be more sensitive and faster than conventional culture. Most positive samples are detected after 4 to 6 weeks and samples are declared negative after 7 weeks of incubation. Samples that are positive for mycobacteria are tested using RNA and DNA probes and high performance liquid chromatography (HPLC) analysis of cell wall extracts to define the species of *Mycobacterium* isolated. The current cost for attempted isolation of mycobacteria is $16 and the cost for identification of the isolate is $25.

A genetic element called insertion sequence 900 (IS900) unique for *M. paratuberculosis* was discovered in the late 1980s. Primers for sections of IS900 were converted into a commercial kit which is sold by IDEXX Laboratories, Inc. Westbrook, ME. The kit uses polymerase chain reaction (PCR) enzymes to amplify the target genetic sequence after extraction from a clinical sample and an enzyme-labeled (visual interpretation) indicator system. The probe is intended for use on fecal samples to permit detection of fecal shedders of *M. paratuberculosis* quickly. A problem for this and many other gene probes is the presence of unknown substances in clinical samples that inhibit PCR enzymes. To circumvent this problem the kit uses sample dilution and special proprietary resin columns to remove PCR inhibitors. The consequence of this essential sample processing procedure is that the detection limit is >10<sup>4</sup> *M. paratuberculosis* per gram of sample. As a result, only relatively heavy shedders can be detected by the probe. The advantage of the probe is that the assay only requires 3 days to complete making it much faster than either of the culture-based methods. Several veterinary diagnostic laboratories offer this probe and the charge varies from $5 to $25 depending on level of state subsidy. The probe can be used on mycobacterial isolates obtained by culture as well as on clinical samples. Isolate identification is the preferred application for the probe at the University of Wisconsin.

**Serum antibody detection**

An enzyme-linked immunosorbent assay (ELISA) for serum antibodies to *M. paratuberculosis* was devised in the mid-1980s. The assay uses a novel strategy to enhance its specificity. Serum samples are mixed with antigens from a common
nonpathogenic soil mycobacterium, *M. phlei*, before being tested for antibodies to *M. paratuberculosis*. By this technique, nonspecific, crossreactive antibodies are removed from serum prior to testing. The ELISA for *M. paratuberculosis* is thus more correctly called the absorbed ELISA. ELISAs not using the absorption procedure lack specificity. The assay most widely used today was invented by workers at the National Animal Disease Center, Ames, Iowa, converted into a commercial kit by a company in Australia, and the kit was subsequently purchased by IDEXX Laboratories, Inc. and then licensed by the USDA. Comments on the accuracy of the ELISA pertain only to the commercial USDA-licensed kit.

The *M. paratuberculosis* ELISA is the most sensitive means of detecting serum antibodies. Antibodies levels rise as the infection progresses and are very high in late stage disease. Generally, animals with a high level of serum antibody (high ELISA optical density reading) have a high likelihood of shedding the organism in feces. However, exceptions to this generalization are not uncommon. The diagnostic sensitivity of the ELISA is dependent on the stage of disease in the individual or the population tested, but in subclinically infected cattle it is roughly 50%. The ELISA can be completed in 2 hours, is easily automated, and hundreds of samples can be processed in a day at a cost of $4.00 per sample. A disadvantage of the ELISA, as currently sold, is that it uses a species-specific antibody detection system (conjugate). Consequently, the kit only can be used on bovids. By changing the conjugate, the kit has also been shown to work well in sheep and goats. Until such time as conjugates for zoological animal species are devised, or a nonspecies-specific conjugate is developed, the ELISA will have limited application in zoological animals.

Agar-gel immunodiffusion (AGID) and complement fixation (CF) are alternative methods of detecting antibodies to *M. paratuberculosis*. Studies in cattle indicate that these tests are less sensitive than the ELISA and useful, if at all, only on animals in late stage, clinical Johne’s disease.

**Cell-mediated immunity**

Detection of cell-mediated immunity (CMI) can be done by inoculation of small amounts of antigens intradermally and then observation of swelling and induration 48 to 72 hours later. This procedure is commonly called skin testing. The swelling is caused by influx of macrophages and lymphocytes at the injection site and indicates T-lymphocyte-mediated immune reactivity to the inoculated antigen. Skin testing using antigens of *M. bovis* called PPD is the age-old method for diagnosis of tuberculosis. Skin testing does not work well for diagnosis of paratuberculosis. High rates of false-positive and false-negative tests are reported.

CMI reactions result from complex interactions between macrophages and T-lymphocytes that involve communication between cells via chemical substances known as cytokines. One of the first discovered and critically important cytokines in a CMI response is gamma interferon (IFNγ). An Australian, Dr. Paul Wood, created a monoclonal antibody
to bovine IFNγ and an ELISA-based assay to detect it. Then, using purified protein derivative (PPD) antigens from *M. bovis* and *M. avium* he adapted the concept of comparative skin testing to an in vitro assay system. This assay was bought by IDEXX Laboratories, Inc. and produced as a kit for diagnosis of tuberculosis (not yet approved by USDA) and for diagnosis of paratuberculosis (USDA-licensed).

To perform the IFNγ assay for paratuberculosis, heparinized whole blood is collected and divided into three 1 ml portions in separate tubes. One tube serves as a nontreated control. To the second tube is added PPD from *M. bovis*. To the third tube is added PPD from *M. avium* (antigenically similar to *M. paratuberculosis*). All three tubes are incubated at 37°C overnight. If the animal's lymphocytes have experienced the antigens in either PPD preparation, meaning the animal either is or has been infected, the lymphocytes will respond by production of IFNγ. The amount of IFNγ in the plasma from each tube of blood is then detected using an IFNγ-ELISA (results are in optical density (OD) units). As presently designed, the assay is interpreted based on the ratio between the OD for the *M. bovis* PPD-stimulated blood sample, and the OD for the *M. avium* PPD-stimulated blood sample, referred to as the bovis/avium ratio. High bovis/avium ratios, eg >1.8, indicate the animal has been exposed to *M. bovis* or *M. tuberculosis*, since the PPD antigens of these two species cross react. Low bovis/avium ratios, eg <0.7, indicate the animal has been exposed to *M. avium* or *M. paratuberculosis*. Because recovery from infection is considered an uncommon event for mycobacteria, any positive immunological test is interpreted to indicate the animal is infected.

In theory, animals infected with *M. bovis* respond stronger to *M. bovis* PPD, animals infected with *M. paratuberculosis* respond stronger to *M. avium* PPD, and noninfected animals respond to neither PPD. The paradigm of immunity to mycobacteria further suggests that animals should develop CMI before producing serum antibodies. Few studies have been reported that test this theory or evaluate the diagnostic accuracy of the IFNγ kit for diagnosis of paratuberculosis. For bovine tuberculosis, the IFNγ kit has a sensitivity and specificity equivalent to that of the skin test.

Few studies have reported the sensitivity and specificity of the IFNγ test for paratuberculosis. Three such studies are in progress and near completion in my laboratory. A 2.5 year longitudinal study on 115 adult cattle in 6 Wisconsin dairy herds found that the sensitivity of the IFNγ kit for paratuberculosis was comparable to that of the serum antibody ELISA. Testing of 500 cattle in certified paratuberculosis-free herds demonstrated the IFNγ assay has a specificity of 97%. Monthly testing of 10 experimentally-infected Holstein calves indicated that the assay may perform best in young animals where it detected *M. paratuberculosis* infection earlier than did the serum antibody ELISA.

Because of similarity of IFNγ among cattle, sheep and goats, the assay theoretically can work equally well in all three animal species. Recently, we successfully used the IFNγ assay to detect *M. paratuberculosis* infection in a herd of pygmy goats. To what extent
this assay can be adopted for use in other ungulates has yet to be determined, but there is reason for optimism.

Conclusion

Armed with better diagnostic tools, paratuberculosis diagnosis and control is now achievable. Infection prevention is the most cost-effective way to combat the disease. Herds acquire the infection by introduction of infected animals. The prolonged subclinical but infectious stage of the *M. paratuberculosis* infection in animals mandates use of laboratory tests to detect them. Trade of animals between captive collections without first testing the animals, preferably the entire source herd, for paratuberculosis is the surest way to spread the infection.

ACKNOWLEDGEMENTS

Recent advancements in diagnosis and control of paratuberculosis in zoological animals has been a direct result of the hard work and dedication by Dr. Jim Oosterhuis to control the infection in animals at the San Diego Wild Animal Park.

LITERATURE CITED


1995 JOINT CONFERENCE AAZV / WDA / AAWV 149
18. Rothel JS, Jones SL, Corner IA, et al. A sandwich enzyme immunoassay for bovine interferon-gamma and its use for the
19. Sockett DC, Carr DJ, Collins MT. Evaluation of conventional and radiometric fecal culture and a commercial DNA probe for
21. Vary PH, Andersen PR, Green E, et al. Use of highly specific DNA probes and the polymerase chain reaction to detect
22. Whipple DL, Callihan DR, Jarnagin JL. Cultivation of Mycobacterium paratuberculosis from bovine fecal specimens and a
23. Whipple DL, Kapke PA, Andersen PR. Comparison of a commercial DNA probe test and three cultivation procedures for
NEW METHODS FOR DIAGNOSIS OF *MYCOBACTERIUM AVIUM* INFECTION IN BIRDS

Susan L. Clark, DVM*
*National Wildlife Health Center, National Biological Service, Madison, WI 53711 and Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706, USA

Michael T. Collins, DVM, PhD
Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706, USA

Jessie I. Price, PhD
National Wildlife Health Center, National Biological Service, Madison, WI 53711, USA

Introduction

Avian tuberculosis (ATB) is a chronic disseminated granulomatous disease caused by the organism *Mycobacterium avium*. Once a scourge of the poultry industry, ATB is now less prevalent there due to changes in husbandry practices. However, it continues to cause mortalities in exotic birds kept as companion animals, captive wild birds in zoological collections, and free-ranging wild birds. ATB is reported to be more prevalent in certain avian species, for instance, wild populations of the endangered whooping crane. Certain species are thought to be more susceptible to infection with *M. avium*, for example the Micronesian kingfisher.

Epidemiologic evidence, including the isolation of *M. avium* from avian fecal samples, suggests ATB is transmitted by ingestion of food and/or water contaminated with the feces of infected birds. *M. avium*-infected birds, including those shedding the pathogen in their feces, usually do not show any clinical signs until late in the disease course and none of these signs is pathognomonic for ATB.

Diagnosis of ATB is most often made postmortem and based on observation of yellowish-white nodules 1 mm to several cm in diameter in multiple organs, and acid-fast organisms in tissue smears and histologic sections. Many other methods are employed in an effort to achieve an antemortem diagnosis however. Hematologic findings in ATB vary from normal to marked leukocytosis with or without a left shift, monocytosis and/or reactive lymphocytosis, polychromasia, and decreased hematocrit. Serum chemistry changes reported in severe disseminated disease include increased liver enzymes and bile acids, and hyperproteinemia with hypoalbuminemia and hyperglobulinemia. Radiography may reveal hepatomegaly, splenomegaly, pulmonary nodules, or bone involvement in ATB. Enlarged or granulomatous organs may be observed by endoscopy of the body cavity. Difficulty in finding a suitable site for skin testing in birds without wattles, the need to handle each bird being skin tested twice, and the poor agreement between tuberculin reactions and necropsy findings in avian species other than chickens.
all limit the usefulness of skin testing as a means of diagnosis of ATB. A whole-blood agglutination test for ATB reportedly has poor sensitivity in avian species other than domestic fowl. Enzyme-linked immunosorbent assays (ELISAs) have been described for detecting anti-mycobacterial antibodies in both experimentally infected chickens and naturally infected feral Barnacle geese. A lymphocyte transformation test has been investigated as a potential diagnostic tool for mycobacterial infections in birds, however there reportedly is poor correlation between the lymphocyte transformation response and necropsy findings. M. avium has been isolated from avian fecal and tissue samples using conventional culturing techniques, and there is a single report of isolation of M. avium from blood from a known M. avium-infected bird using a radiometric culturing method established for human samples.

The goals of our research have been to develop radiometric culture for isolation of M. avium from avian fecal and tissue biopsy samples, and to develop an ELISA for detection of serum antibody against M. avium as tools for antemortem diagnosis of M. avium infection in birds.

Radiometric Culture of Mycobacterium avium from Fecal and Tissue Biopsy Samples
Radiometric culture offers several advantages over conventional culture methods for the isolation of mycobacteria, including its speed, sensitivity, and suitability for quantitative studies. A method for culturing M. paratuberculosis from animal fecal and tissue samples using a modified BACTEC system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) has been described. Using this method as a starting point, we optimized radiometric culture for isolation of M. avium from avian fecal and tissue samples. Radiometric culture involves the following steps: (1) sample decontamination to prevent the overgrowth of normal microbial flora or other contaminating, nonacid-fast organisms; (2) filter concentration of mycobacteria to increase the culture sensitivity; (3) inoculation and incubation of the growth medium; and (4) mycobacterial growth detection and species identification. The growth medium used in our lab for the culture of M. paratuberculosis is Middlebrook 7H12 broth (BACTEC 12B) enriched with egg yolk and mycobactin-J, and supplemented with an antibiotic cocktail to control contamination. Pilot studies showed M. avium to be sensitive to a component of the cocktail used to isolate M. paratuberculosis and so alternatives were investigated. Most effective control of contaminants, with minimal inhibition of M. avium growth, was achieved using an antibiotic cocktail composed of bacitracin (40 µg/ml), amphotericin B (20 µg/ml), and nalidixic acid (30 µg/ml). Due to differences between M. avium and M. paratuberculosis in their tendency to aggregate, filter concentration of samples was not found to increase the sensitivity of the radiometric culture method for M. avium.

The protocol for radiometric culture of M. avium from avian fecal samples is presently as follows. Fecal samples are first decontaminated in a 1% hexadecylpyridinium chloride solution (HPC) (Sigma Chemical Co., St. Louis, MO) for 15 min or less, then filtered through two layers of gauze to remove large particulate material, and then inoculated directly into the enriched and antibiotic-supplemented growth medium. Inoculated growth vials are incubated at 37°C under 5% CO₂ without shaking for three weeks.
Growth is measured daily by a gas ionization detector instrument called the BACTEC 460 and reported as raw growth index (GI) value. When the cumulative GI exceeds 100, a few drops of the culture broth are plated on a blood agar plate (BAP) and observed for growth for 48 hrs. In addition, a drop of culture is placed on a slide and acid-fast stained. If no growth is detected on the BAP and acid-fast organisms are seen on the slide, then a *Mycobacterium* sp. is assumed to have been isolated, and identification is attempted using a DNA probe that hybridizes with *M. avium* 16S rRNA (Accuprobe, Gen-Probe, San Diego, CA). The protocol is the same for tissue biopsy samples, with the exception of the first step in which tissues are homogenized in saline before exposure to HPC.

This protocol has been used successfully to isolate *M. avium* from the feces of experimentally infected chickens and quail, starting as early as eight weeks after either a single intravenous or oral challenge with 10⁸ colony forming units of a field isolate of *M. avium*, and also from tissue samples collected postmortem. We are using this radiometric culture protocol to isolate *M. avium* from clinical samples from avian and some non-avian animal species, however, further refinements are necessary before it can be used extensively as a diagnostic tool in a clinical setting. The sensitivity of different strains of *M. avium* to the decontaminant HPC and the use of alternative decontaminants are current areas of investigation to optimize the radiometric culture technique.

**ELISA for Detection of Serum Antibody Against *Mycobacterium Avium***

An ELISA for detection of serum antibody against *M. avium* in chickens challenged intramuscularly with *M. avium*, reported in 1978,⁹ and another for detection of antibody in naturally infected feral Barnacle geese, reported in 1993,²³ served as the starting point for our ELISA work. We developed a solid-phase, antibody-capture ELISA for detection of antibody against *M. avium* in serum collected weekly from chickens and quail challenged either orally or intravenously with *M. avium*.

Assays are performed in polystyrene 96-well microtiter plates coated with a whole cell homogenate of a field isolate of *M. avium*. Diluted serum samples are added to the antigen-coated wells and antigen-bound antibody is detected using a commercially available goat anti-chicken IgG (H+L) horseradish peroxidase conjugate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD), in the case of chicken serum, and a goat anti-turkey IgG (H+L) horseradish peroxidase conjugate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD), in the case of quail serum. The substrate for the enzyme conjugates is TMB (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). Using this system, we were able to detect a specific antibody response to experimental challenge with *M. avium* in both chickens and quail. The extent of cross reactivity among IgGs of diverse avian species will determine the usefulness of ELISAs for diagnosis of *M. avium* infection in birds.
As diagnostic methods, radiometric culture of avian fecal and tissue biopsy samples for *M. avium* and an ELISA for detection of serum antibody against the pathogen are still primarily research tools. However, with further refinement, both have potential clinical applications as means of identifying birds infected with *M. avium* and excreting the pathogen in their feces. Early diagnosis of ATB is critical for control of this disease in captive avian populations.

**ACKNOWLEDGEMENTS**

Partial funding for this research was provided by the Smithsonian Institution, Grant #NBS TP3040660000.

**LITERATURE CITED**

5. Junge, R. E. (Personal communication).
EMERGING LYSSAVIRUSES: SURVEILLANCE AND DIAGNOSTIC ISSUES

Charles E. Rupprecht, VMD, MS, PhD,* Jean Smith, MS, Makonnen Fekadu, DVM, PhD, Lillian Orciari, MS, Pam Yager, BS, John Krebs, MS, James Childs, ScD
Centers for Disease Control & Prevention, 1600 Clifton Rd, Atlanta, GA 30333, USA

Rabies virus is the type species of the Family Rhabdoviridae, Genus Lyssavirus, a related group of enveloped, bullet-shaped, single-stranded, non-segmented, negative-sense RNA viruses of approximately 75 x 180 nm, that produce an acute encephalomyelitis among mammals. Of the genera, only rabies virus is found world-wide, with the exception of countries either without a history of natural establishment or having achieved costly secondary elimination, such as Australia and the United Kingdom, respectively. Other lyssavirus representatives, such as Mokola, Duvenhage, Lagos Bat, etc., are restricted in distribution to the Old World, where little is known concerning their basic epidemiology, beyond casual fatal disease associations with bats, limited other wildlife, humans, and domestic animals, notably regardless of rabies vaccination in the latter. Because of their obvious public health significance, most research emphasis has been placed upon those viral members with the potential for epizootic disease manifestation. In general, only a passive surveillance system exists for detection of lyssaviruses among those wild mammalian hosts directly involved in human or domestic animal exposure, usually via a bite. Lyssaviruses typically produce intracytoplasmic, acidophilic inclusions of viral ribonucleoprotein in neuronal cells, which may be detected by routine light or immunofluorescent microscopy. Due to diagnostic cross-reactivity, isolates may be further characterized by antigenic and genetic typing into viral variants compartmentalized to particular niches. Meaningful resolution of taxonomic issues among the known lyssaviruses and putative relationships with the many existing unclassified rhabdoviruses (as well as those awaiting discovery) will only likely occur through comprehensive collaborative analysis at the genome level.

How may global lyssavirus interchanges occur? Serious epidemiological risks are often associated with the improper translocation of wildlife for research, companion animal and hunting purposes. Intercontinental translocation of rabies virus-infected wildlife (gray foxes, insectivorous bats) from North America to Europe has been documented on at least two occasions over the last 12 months. To date, no non-rabies lyssaviruses have been diagnosed in the New World, although the potential for such introduction readily exists. For example, also during the past year, several thousand bats (primarily Egyptian fruit bats, *Rousettus aegyptiacus*, and several other related species) may have been imported and improperly offered for sale as pets in the USA. Sales of imported bats (and their offspring) to private collectors or as pets are strictly prohibited, under the provisions of the CDC's Foreign Quarantine Regulations, that limit the entrance of animal species which may be vectors of foreign diseases of public health concern into the USA. Such animals should only enter the USA for restricted use at accredited zoos or research institutions, where contact with the general public would be prevented. As with other non-indigenous animals, unwanted imported bats, legal or otherwise, should not
be released into the wild because, in theory, they may cause harm to agriculture, displace native species and establish exotic diseases.

Bats perform many critical ecological functions worldwide and generally avoid contact with humans. However, they may be infected with rabies, related lyssaviruses, and a variety of other etiological agents, albeit infrequently. Yet, when placed in a setting such as a private household or a pet shop, where contact with the public is unavoidable, the hazard of disease transmission may be greatly increased. Additionally, many mammalian species, including bats, may not always demonstrate obvious clinical signs related to infection with these agents. Thus, it may be difficult to determine which animals may be infectious without extensive and costly testing and quarantine. Moreover, to avoid the potential for false-negative results, reliable diagnosis for some agents, such as the neurotropic lyssaviruses, may only be available post-mortem. Clearly, bats and other wildlife are not suitable as pets.

While surveillance issues related to exotic lyssavirus introductions in the USA remain theoretical, regional indigenous rabies outbreaks have been initiated through direct human intervention. The current raccoon rabies epizootic in the Mid-Atlantic and Northeastern USA is the result of translocation of infected raccoons from the southeastern USA during the late 1970s. Similarly, in repeat fashion, with the recent proliferation of hunting pens in the Southeastern USA, there has been a parallel increase in the sale of live furbearers to supply these pens, including a substantial amount of illegal interstate importation. In kind, transport of infected coyotes from Texas has recently led to multiple cases of dog rabies in Alabama and Florida. One of the greatest dangers of the coyote rabies virus variant currently epizootic in south Texas and adjacent Mexico is its adaptability to a variety of canid species, with the capacity for interspecific propagation. Obviously, intentional movement of species from geographic areas where diseases are enzootic into other regions poses a needless threat to native wildlife, domestic animals, and humans. Because of the consequent health risks and the lack of feasible methods to certify that such animals are disease-free, enforceable federal and state regulations are necessary to prohibit the translocation of wildlife species with the documented potential for the introduction and spread of zoonotic disease.
ANTIBODY PREVALENCE TO MALIGNANT CATARRHAL FEVER VIRUS IN DOMESTIC AND WILD RUMINANTS BY COMPETITIVE INHIBITION ELISA

Li Hong,* David T. Shen, John R. Gorham
Animal Diseases Research Unit, USDA-ARS, Pullman, WA 99164, USA

David A. Jessup
California Department of Fish and Game, Rancho Cordova, CA 95670, USA

Tom Thorne, Donal O'Toole
Wyoming Game and Fish Dept, Laramie Lab and University of Wyoming, State Veterinary Lab, Laramie, WY 82070, USA

Timothy B. Crawford
Department of Veterinary Micro-Path, Washington State University, Pullman, WA 99164, USA

Malignant catarrhal fever (MCF) is a poorly characterized disease of certain ruminants that is caused by a group of gammaherpesviruses which exist in nature as inapparent infections in other ruminant species. A competitive inhibition ELISA (CI-ELISA), based on a monoclonal antibody to an epitope conserved among MCF virus (MCFV) strains of both wildebeest and sheep origin, was used to determine the prevalence of antibody to MCFV in certain domestic and wild ruminants, both free-ranging and captive. A total of 2796 sera from 14 species were examined. Among these, 1191 sera were from white-tailed deer, mule deer, elk, pronghorn antelope and bighorn sheep from 5 states, including California, Idaho, Montana, Washington and Wyoming. A high prevalence of antibody to MCFV was demonstrated in domestic sheep, goats, and some bighorn sheep herds, ranging from 33 to 62% and the presence of antibody was age-related. A low antibody prevalence to MCFV (1 to 13%) was detected in clinically susceptible species, such as domestic cattle, deer, elk and bison, confirming a significant rate of latent infection in these species. The CI-ELISA has potential as a tool to improve our understanding of the epidemiology of this disease. Studies such as this should lead to improvement in management of captive and free-ranging wild ruminants.
The animal management committee at your institution, located in Delaware, has just met: they wish to import a wild-caught tapir from Malaysia, ship a palm cockatoo to an institution in a neighboring state on breeding loan, receive a killer whale from a sister U.S. institution on a breeding loan and import a captive-bred gorilla from Canada on a breeding loan. It's your job to arrange the transportation for these animals and to ensure that all legal shipping requirements are met.

You have a new assistant veterinarian on staff and figure this would be a good learning opportunity for him/her. Your assistant figures this should be easy and does not understand why you are beginning to dread all the paperwork. You explain, shipping regulations are your worst nightmare. There are four or five different kinds of laws or requirements for shipping wildlife and unless you are quite familiar with them, things can get confusing. You begin to explain.

Lacey Act

In 1949, Congress directed the Secretary of the Treasury to prescribe requirements for the transportation of "wild animals and birds under humane and healthful conditions." The Lacey Act Amendments of 1981 transferred this responsibility to the Secretary of the Interior. Under the Lacey Act, it is unlawful for anyone to cause or permit wild animals or birds to be transported in inhumane or unhealthful conditions. The statute attempts to lay down a standard of proof. It says that if the vessel or conveyance contains a substantial ratio of dead, crippled, diseased, or starving wildlife, "that is prima facie evidence of a violation."

In 1982, the U.S. Fish and Wildlife Service (FWS) began receiving information and comments regarding the development of appropriate regulations for the humane and healthful transport of wild animals and birds to the United States. In 1987, FWS issued a final rule which was to become effective in February 1988.

Criticisms of the final rule came pouring in to FWS who decided to extend the effective date until August 1988 so that a thorough review could be conducted. In April, FWS was sued by animal rights groups seeking the rule to be enforced immediately. FWS began enforcing their regulations while continuing to work on new regulations. Finally in June 1992, FWS issued regulations for the humane and healthful transport of wild mammals and birds to the United States. At present, there are no regulations for fish, reptiles, amphibians and invertebrates.
The following are some general principles included in the Humane and Healthful Shipping Regulations of wild animals to the United States.

No wild bird or mammal can be transported unless it has been examined within 10 days prior to the transport. This examination must be by a veterinarian certified as qualified by the national government of the country from which the specimen is being exported.

The certificate of medical inspection must state that the animal has been examined, is healthy, appears free of any communicable diseases and is able to withstand the normal rigors of transport.

A nursing mother with young, an unweaned mammal unaccompanied by its mother, or an unweaned bird cannot be transported unless it's for medical treatment. Such animals must be accompanied at all times by and accessible to a veterinary attendant.

A sick or injured wild mammal or bird is only permitted to be transported for medical treatment.

Wild mammals and birds would not be accepted for transport to the U.S. by carriers less than two hours or more than six hours before the scheduled departure.

Container requirements are the same as those published in the International Air Transport Association (IATA) Live Animal Regulations. The interior of shipping enclosures should be free from any protrusions and no part of the wildlife can be exposed outside the enclosure, if it might cause injury to the wildlife or transport handlers. Handholds should be provided for lifting the enclosures.

Spacer bars must be fitted to the outside of all walls, the roof and the primary enclosure to ensure that ventilation openings are not obstructed.

Primary enclosures must be cleaned and sanitized prior to each shipment to destroy pathogenic agents and pests.

Primary enclosures or shipping containers shall have a solid bottom to prevent leaking fluids and shall contain unused litter or a suitable material to absorb and cover excreta unless the wildlife are on wire or other non-solid floors which are suspended above the solid bottom.

The primary enclosure must be clearly labeled (on the top and on one side in letters at least one inch in height) as to its contents.

Documents and care instructions must be attached to the outside of the primary enclosure.
The animal cargo space within the conveyance must be constructed and maintained to prevent the ingress of engine exhaust fumes and gases produced by the conveyance.

During a stopover or while still in custody of the carrier after arrival in the United States, each mammal or bird must be observed at least once every four hours. During air transportation where the animal cargo space is not accessible during flight, the carrier shall observe the mammals and birds whenever loaded or unloaded and whenever the animal cargo space is otherwise accessible.

Carriers are required to take adequate precautions to ensure that wild mammals and birds are not subjected to adverse temperatures.

Animals incompatible with one another shall not be crated together or held in close proximity.

Transport shall be by the carrier in the most expeditious manner, with the fewest stopovers possible.

Carriers must provide shelter to wild mammals and birds from sunlight and weather while they are being moved to and from animal holding areas and the primary conveyance.

These regulations include specific requirements for nonhuman primates, marine mammals, elephants and ungulates, sloths, bats and flying lemurs; other terrestrial mammals and birds.

**Animal Welfare Act**

The Animal Welfare Act (AWA) was enacted in 1970 and ensures that animals used in research facilities, for exhibition purposes, and for pets are to be provided with humane treatment and care. The AWA regulates aspects of transportation, purchase, sale, housing, care, handling and treatment. Current regulations cover dogs, cats, nonhuman primates, guinea pigs, hamsters, rabbits and most other warm-blooded animals. The regulations do not apply to cold-blooded animals, fish, birds, horses and farm animals.

The Animal and Plant Health Inspection Service (APHIS), a division of the US Department of Agriculture (USDA), has been challenged in the past two years to develop standards for rats, mice and birds. The Animal Legal Defense Fund challenged the APHIS policy of excluding rats, mice and birds from coverage under the Animal Welfare Act. A lower court judge ruled that the APHIS decision not to regulate certain animals was arbitrary, capricious, an abuse of discretion and a violation of the AWA. The Department of Agriculture appealed the decision. The Court of Appeals overturned the Lower Court's decision finding that the plaintiffs lacked standing to sue.
The central terms of the APHIS standards are primary enclosure and primary conveyance. Primary conveyance is "the main method of transportation used to convey an animal from origin to destination, such as a motor vehicle, plane, ship, or train." Primary enclosure is "any structure or device used to restrict an animal or animals to a limited amount of space, such as a room, pen, run, cage, compartment, pool, hutch, or tether." APHIS has specific requirements for nonhuman primates and marine mammals. The requirements for the other mammals are more general in nature.

Some of the key elements for nonhuman primates include:

The consignor must certify in writing to the carrier that the nonhuman primate was offered food and water during the 4 hours before shipment.

Only one nonhuman primate may be transported in a primary enclosure, except a mother and her nursing infant, an established male-female pair or female group, or a compatible group of juveniles of the same species that have not reached puberty.

Each nonhuman primate one year of age or more must be offered food at least once every 24 hours.

Some key elements for transporting marine mammals include:

Socially dependent animals must be allowed visual and olfactory contact.

Marine mammals shall not be transported for more than 36 hours without being offered food.

An employee or attendant of the shipper or receiver must accompany cetaceans, sirenians, pinnipeds and sea otters during transport. If the period of transport exceeds 24 hours, polar bears must be accompanied.

The Animal Welfare Act (AWA) and the Lacey Act shipping standards are compatible. The AWA covers transport within the United States and the Lacey Act shipping standards cover transport to the United States.

Convention on International Trade in Endangered Species of Wild Fauna and Flora

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) was signed in 1973. Its purpose and objective is "the protection of certain species of wild fauna and flora against overexploitation through international trade." The substantive articles of the treaty seek to regulate trading activities, as defined by CITES, through the listing of species on three Appendices. In addition to the other CITES permit requirements, before exports of Appendix I or II species are allowed, the Management Authority of the exporting country must be satisfied that any living specimen will "be so
prepared and shipped as to minimize the risk of injury, damage to health or cruel
treatment." A similar requirement applies for re-export and export, re-export or
introduction from the sea.

In 1980, CITES developed their own standards for shipping entitled the CITES Guidelines
for Transport and Preparation for Shipment of Live Wild Animals and Plants. The CITES
guidelines contain general information on the preparation and shipment of living specimens
carried by all forms of transport. CITES has officially endorsed air transport as the
preferred method for transporting many live wild animals and recognized that there are
special requirements necessitated by air transport. In that light, CITES adopted the Live
Animal Regulations of the International Air Transport Association (IATA) as the air
transportation regulations for CITES. The IATA guidelines are amended annually and
therefore are more quickly responsive to changing needs.

The development of the CITES guidelines served a useful purpose in directing the attention
of the CITES Conference of the Parties to the major problems encountered in the
preparation and shipment of living specimens. Throughout the years, a number of ad hoc
committees were established to assist with transport problems.

In 1989, CITES established a Transport Working Group to:

1. Assist Parties with the implementation of the Convention and its Resolutions
   pertaining to preparation for shipment and transport of live specimens;

2. Cooperate with the Secretariat in presenting training workshops to CITES
   exporting Parties on preparation for shipment and humane transport of live animals;

3. Seek information from the Parties on numbers of live specimens per shipment
   and mortalities and causes related to transport and on individual cases of high
   mortalities in transport for any CITES-listed species;

4. Obtain information from scientists, veterinarians, zoological institutions, and other
   experts to make recommendations to the Parties designed to minimize mortality;

5. Specifically track mortalities of shipment and transport of live birds listed in
   CITES;

6. Review the IATA Live Animal Regulations and make recommendations to the
   IATA Live Animals Board for changes to meet CITES requirements; and

7. Work on preparing/revising international transportation guidelines concerning
   terrestrial (road and rail) and marine transportation of live animals for consideration
   by the CITES Parties.
At the 1994 CITES meeting held in Ft. Lauderdale, Florida, the Animals Committee, a Standing Committee of CITES, met to undertake matters related to the transport of live animals. The Animals Committee recommended the repeal of a number of the old CITES transport regulations. The CITES Parties adopted these recommendations. The Animals Committee is now charged with the work plan outlined above and the CITES Transport Working Group has been disbanded.

International Association of Air Transportation Live Animals Regulations

IATA has a live Animals Board that meets twice a year to consider complaints, suggestions and recommendations for the upcoming year’s publication of the Live Animals Regulations. The IATA guidelines contain specific crate design requirements and directions for the use of persons actually handling consignment as well as for enforcement authorities.

Importation of Elephants, Hippopotami, Rhinoceroses and Tapirs

APHIS requires that elephants, hippopotamai, rhinoceros or tapirs cannot be imported into the United States without a special import permit. Although this regulation is not solely limited to transport, I mention it here because it involves aspects of transport. When importing elephants, hippopotamai, rhinoceros or tapir they need to be shipped with a health certificate signed by a salaried veterinarian of the national veterinary services of the exporting country where inspection and treatment occurred or by a national veterinarian of the exporting country. The elephant, hippopotamus, rhinoceros or tapir must have been inspected and found free of any ectoparasites within 72 hours of being loaded on the transport conveyance bound for the United States. The animals must have been treated between 3 and 14 days before transport for ectoparasites under the supervision of the person signing the health certificate. After treatment there can be no contact with other animals unless they are part of the same shipment. Upon arrival in the United States, hay, straw, bedding, feed and any material from the crate must be removed and sealed in plastic bags and incinerated. The shipping crate or the vehicle is then sealed. Once at the importer's destination, the animal is inspected and treated once if no ectoparasites are found. If ectoparasites are found the animal is dipped or sprayed until no ectoparasites are found.

Having explained the primary shipping laws and regulations to your new assistant veterinarian, here is what applies for these specific shipments:

Importing a wild-caught tapir from Malaysia -- This shipment will be covered by CITES as tapirs are listed on Appendix I. (Not your job, but you hope your institution has received both an import permit from the U.S. and a CITES export permit from Malaysia). Your tapir would also covered by the Lacey Act Humane and Healthful Shipping Regulations as it is in transit to the United States. The Lacey Act regulations are compatible with the IATA regulations so there is no cause for concern.
Once in the United States the tapir is covered under the Animal Welfare Act as it will be transported interstate from New York's Kennedy Airport to Delaware. Again, no worry as there is compatibility between the Lacey Act and the Animal Welfare Act and, therefore, between the Animal Welfare and the IATA Live Animal Regulations.

Tapirs are also covered under the special rule of APHIS regarding the importation of elephants, hippos, rhinoceros and tapirs. This means there must be a special import permit from APHIS, a health certificate and various treatments for ectoparasites.

Shipping the palm cockatoo from your institution to an institution in a neighboring state -- This shipment is not covered by any federal laws. Presently, there are no standards for birds under the Animal Welfare Act. The other shipping laws do not apply because they only apply to imports to the United States, not to interstate commerce. The receiving state agriculture departments may require specific testing and wording on the health certificate and they should be contacted in advance.

Receipt of a killer whale from a sister institution -- This shipment would be covered under the Animal Welfare Act. An attendant (either from your institution or the shipping institution) would have to accompany the animal from the sister institution to yours. There are specific requirements in the APHIS regulations that must be met. You hope your institution has notified the National Marine Fisheries Service at least 15 days before the transport will occur.

Import a captive-bred gorilla from Canada -- This shipment would be covered by both the Lacey Act Humane and Healthful Shipping Regulations and by CITES (IATA Live Animals Regulations). You hope your institution has its CITES/ESA import and CITES export permit and, oh, you need to find a Center for Disease Control (CDC) registered import facility to quarantine the animal for a minimum of 31 days after importation into the U.S. CDC also has several requirements for handling of the nonhuman primate cages in transit to the U.S. and once in the U.S.

The primary shipping regulations are implemented by the Lacey Act, the Animal Welfare Act and the Convention on International Trade in Endangered Species of Wild Fauna and Flora. There are other specific requirements for other shipments such as the one I mentioned for elephants, rhinoceros, tapirs and hippopotami and the requirements for shipping an animal covered under the Permanent Post Entry Quarantine Standards by the USDA. Shipping regulations can be your worst nightmare, but if you consult the primary regulations and consult the folks at APHIS, FWS or NMFS they don't have to be.
QUARANTINE PROCEDURES FOR AZA-ACCREDITED ZOOLOGICAL PARKS

R. Eric Miller, DVM*
St. Louis Zoological Park, 1 Government Drive, St. Louis, MO 63110-1396, USA

Quarantine is a basic component of preventive medicine programs in zoological parks. It is a fundamental step in the prevention of the spread of disease into an animal collection. Indeed, the word quarantine reflects that fact - it is derived from the Latin word for "forty." For, in medieval Venice, 40 days was the length of time that human immigrants were kept separate from the general population to limit the spread of plague. Although the prevention of disease is primary, quarantine also offers an opportunity to establish the baseline health status of new arrivals. The principles that apply to quarantine for interzoo and wild-to-zoo animal transfers, are also crucial for zoo animals moving to the wild in reintroduction projects, and may also apply to animal transfers between different wildlife areas.

Recognizing the importance of quarantine, in 1989 the American Zoo and Aquarium Association (AZA) requested that its Animal Health Committee (AHC) draft a written protocol that would be included as part of its requirements for accreditation. The protocol was completed in 1993 and adopted by the AZA Board in 1994. It represents the first specific, written standards that were incorporated into the accreditation process.

The original quarantine requirements covered terrestrial mammals, birds, reptiles and amphibians. Writing the regulations was the group effort of AHC members and numerous members of the zoological community. Separate sections were later added for marine mammals, an effort spearheaded by Dr. James McBain, and fish, written and submitted by members of the aquarist community. One challenge of the original process was attempting to create regulations that were meaningful and detailed, yet allowed the institutional veterinarian flexibility to use his/her judgement when exceptions might be advisable.

Additionally, the regulations had to reflect the reasonable realities of our institutions; for example, in the near future, not all zoological parks may have the ability to quarantine an adult elephant or an adult giraffe. However, pre-shipment testing and other procedures may assist in reducing the risk of disease transfer from those animals. A similar effort was the quarantine of great apes. Recognizing the limitations of some smaller institutions, the regulations allow for the isolation of a primate at either the shipping institution (if shipped without contact with other nonhuman primates) or at an AALAS approved institution.

These quarantine regulations have also been of value in assisting the AZA's pursuit of exemptions from disease testing when animals are transferred between AZA-accredited institutions. One example is the exemption from federal requirements for cervid tuberculosis testing. The AZA noted and the USDA accepted the fact that AZA-accredited zoos 1) identify and keep individual records on animals (critical to tracing them), 2) they have animal health programs, including quarantine of new arrivals, in place, and 3) all deaths are necropsied by a veterinarian. These exemptions are advantageous for AZA...
institutions and they behoove the zoological community to strictly monitor its own performance to both maintain our standards and not endanger these exemptions.

Finally, it is important to note that the regulations were written to be minimum standards and those that wish to exceed them are encouraged to do so. Following are the regulations as adopted by the AZA and now required for accreditation:

**Quarantine procedures recommended for AZA-accredited institutions**

Quarantine Facility: A separate quarantine facility, with the ability to accommodate mammals, birds, reptiles, amphibians and fish should exist. If a specific quarantine facility is not present, then newly acquired animals should be isolated from the established collection in such a manner as to prohibit physical contact, to prevent fomite transmission, and to avoid aerosol and drainage contamination. Such separation should be obligatory for primates, small mammals, birds, and reptiles, and attempted wherever possible with larger mammals such as large ungulates and carnivores, marine mammals and cetaceans. If the receiving institution lacks appropriate facilities for isolation of large primates, preshipment quarantine at an AAZPA or AALAS accredited institution may be applied to the receiving institution’s protocol. In such a case, shipment must take place in isolation from other primates. More stringent local, state or federal regulations take precedence over the recommendations of this report.

Quarantine Length: Quarantine for all species should be under the supervision of a veterinarian and consist of a minimum of 30 days (unless otherwise directed by the staff veterinarian). Mammals: If during the 30 day quarantine period, additional mammals of the same order are introduced into a designated quarantine area, the 30 day period must begin again. Birds, reptiles, amphibians or fish: The 30 day quarantine period must be closed for each of the above Classes. Therefore, the addition of any new birds into a bird quarantine areas requires that the 30 day quarantine period begin again on the date of the addition of the new birds. The same applies for reptiles, amphibians and fish.

Quarantine Personnel: A keeper should be designated to care only for quarantined animals or a keeper should attend quarantined animals only after fulfilling responsibilities for resident species. Equipment used to feed and clean animals in quarantine should be used only with these animals. If this is not possible, then equipment must be cleaned with an appropriate disinfectant (as designated by the veterinarian supervising quarantine) before use with post-quarantine animals.

Institutions must take precautions to minimize the risk of exposure of animal personnel to zoonotic diseases that may be present in newly acquired animals. These precautions should include the use of disinfectant foot baths, wearing of appropriate protective clothing and masks in some cases, and minimizing physical exposure in some species, e.g., with primates, by the use of chemical rather than physical restraint. A tuberculin testing/surveillance program must be established for zoo/aquarium employees in order to ensure the health of both the employees and the animal collection.
Quarantine Protocol: During this period, certain prophylactic measures should be instituted. Individual fecal samples or representative samples from large numbers of individuals housed in a limited area (e.g., birds of the same species in an aviary or frogs in a terrarium) should be collected at least twice and examined for gastrointestinal parasites. Parasiticide treatment should be prescribed by the attending veterinarian. Ideally, release from quarantine should be dependent upon obtaining two negative fecal results spaced a minimum of two weeks apart either initially or after parasiticide treatment. In addition, all animals should be evaluated for ectoparasites and treated accordingly.

Vaccinations should be updated as appropriate for each species. If the animal arrives without a vaccination history, it should be treated as an immunologically naive animal, and given an appropriate series of vaccinations. Whenever possible, blood should be collected and sera banked. Either a -70 degree C freezer or a -20 degree C freezer that is not frost-free should be available to save sera. Such sera could provide an important resource for retrospective disease evaluation.

The quarantine period also represents an opportunity to, where possible, permanently identify all unmarked animals when anesthetized or restrained (e.g., tattoo, ear notch, ear tag, etc.). Also, whenever animals are restrained or immobilized a complete physical, including a dental examination, should be performed.

Complete medical records should be kept and available for all animals during the quarantine period. Animals that die during quarantine should have a necropsy performed under the supervision of a veterinarian and representative tissues submitted for histopathologic examination.

Quarantine Procedures: The following are recommendations and suggestions for appropriate quarantine procedures for several animal groups:

<table>
<thead>
<tr>
<th>MAMMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primates</td>
</tr>
<tr>
<td><strong>Required</strong></td>
</tr>
<tr>
<td>1. Direct and flotation fecals as described above.</td>
</tr>
<tr>
<td>2. A minimum of 2 negative tuberculin tests using a tuberculin containing at least 1500 units/1ml (e.g., Mammalian Human Isolate, Coopers Animal Health, Kansas City, KS) or other appropriate regimen as necessary for the species in question (e.g., orangutans, New World primates, etc.)</td>
</tr>
<tr>
<td><strong>Strongly Recommended</strong></td>
</tr>
<tr>
<td>1. chest radiographs</td>
</tr>
<tr>
<td>2. Appropriate viral panels (SIV, retrovirus type D)</td>
</tr>
<tr>
<td>3. Urinalysis</td>
</tr>
</tbody>
</table>
3. CBC/sera chemistry panel

4. Culture of feces for *Salmonella/Shigella/Campylobacter*

5. For appropriate species; eg, Old World monkeys, serology for *Herpesvirus simiae* (Herpes B).

**Hoofstock**

1. Direct and floatation fecals
2. TB test whenever possible

1. CBC/sera profile
2. Appropriate serology, eg, leptospirosis, brucellosis, MCF, IBR, BVD, etc. Paired titers whenever possible.

3. Urinalysis
5. Coggins test for equids.

**Small Mammals/Carnivores**

1. Direct and floatation fecals
2. Vaccinate as appropriate.

1. CBC/sera profile
2. Urinalysis
3. Appropriate serology, FIP, FeLV, FIV)
4. Heartworm testing in appropriate species.

(See Fowler as under hoofstock recommendations, pp 800-881; and recommendations for small exotics in upcoming *Current Veterinary Therapy XI* WB Saunders Co., Philadelphia).
BIRDS

1. Direct and floatation fecals as above.
2. Evaluate for ectoparasites.
3. Appropriate serological tests for psittacosis, and if positive, confirmed by culture.

1. CBC/sera profile
2. Fecal culture for *Salmonella* sp.
3. Fecal gram stain.

REPTILES/AMPHIBIANS

1. Direct and floatation fecals
2. Evaluate for ectoparasites.

1. Veterinary examination
2. CBC/blood chemistries
3. Paramyxovirus titers for viperids, incoming after being quarantined for 30 days.
4. Full post-mortem examination and histopathology on all specimens dying while in quarantine.

FISH

General Comments: Quarantine standards for other zoo and aquarium animals cannot always be applied to fish, and adaptations must be made to the proposed procedures as they apply to fish populations. Proper and appropriate fish quarantine is a vital component of any successful health management program for fish. Quarantine procedures must be tailored to individual species and require greater variation than quarantine for other zoo and aquarium animals. It is in the interest of accredited institutions to carry out quarantine procedures that are both effective and practical, leading to improved animal health.

Fish are usually acquired as populations, not as individual specimens, and individual identity may be impractical to establish. Few aquariums have the facilities and/or space to properly maintain large fish specimens in separate life-support systems, making individual quarantine of these specimens difficult. Aquariums may operate as open or semi-open systems, and specimens acquired from the surrounding waters of these
institutions may not benefit from rigid quarantine procedures due to the constant introduction of potential disease organisms. Veterinarians may be part of the team supervising quarantine, but the institutions should appoint staff it feels has the best expertise to supervise and operate the quarantine program. It is appropriate to note that state and federal fish hatcheries do not often employ veterinarians, yet have well-established and internationally recognized fish health programs of which quarantine is an important factor.

Specific recommendations:

Quarantine Facility: Where appropriate, separate life support systems (LSS) with the ability to quarantine fishes should exist. The LSS should be operated in such a way as to preclude disease transfer from one system to another and/or introduction into natural waters. Quarantine tanks should have viewing that is adequate to observe fish for behavior and signs of pathology, the LSS should be adequate to maintain the health of the quarantine population. If an aquarium does not have a separate LSS, it should have the ability to divert flow through the quarantine systems, bypass the common filter, and discharge the water. Disinfection of the discharge water prior to release is advisable. In addition, discharge of this water must comply with federal, state and local environmental regulations.

Quarantine Length: A quarantine period of 30 days is an adequate standard; however, it must be recognized that certain species or disease problems may require more or less time.

Quarantine Personnel: The institution will appoint the staff it feels has the most expertise to supervise and operate the quarantine program. All equipment (boots, nets, cleaning equipment, etc.) should be confined to the quarantine area. Access to and from the quarantine area should be restricted so as to minimize cross-contamination. Precautions must be taken to minimize the risk of zoonotic disease to personnel.

Quarantine protocol: Each institution must have a written quarantine protocol. During quarantine, appropriate prophylactic measures should be instituted. Complete medical records should be maintained for the species during the quarantine period. Fish that die during quarantine, or a representative sample thereof, should be necropsied. Care must be taken that all equipment use with quarantined fish is separate from other systems (if this is not possible, adequate disinfection procedures must be employed before equipment is used for post-quarantine fish).

Required quarantine protocol: Due to the great diversity of fish, required quarantine procedures are difficult to establish. The institution should follow the guidelines stated in the above sections to fashion a quarantine program best suited to their needs.
MARINE MAMMALS

All AZA member zoological parks and aquariums should have a quarantine program for new marine mammal arrivals at the institution. A facility should be available which can provide for the isolation of newly acquired marine mammals in such a manner as to prohibit cross-contamination resulting from physical contact, disease transmission, aerosol spread, waste drainage, or the reuse of untreated water. Ocean pens must be located in a way that prevents the spread of any disease from animal to animal through natural water movement and at a distance from other penned animals deemed adequate by the supervising veterinarian. If a receiving institution does not have appropriate isolation facilities, the staff should arrange for quarantine at an acceptable alternate site or only receive animals that do not require quarantine. More stringent local, state or federal regulations relating to marine mammal quarantine take precedence over these recommendations.

Isolated practices should be instituted based on the prior medical history of the newly arrived animals. Those situations where isolation is recommended would have one or more of the following characteristics:

1. Recently collected (less than 30 days prior to arrival).
2. Recently exposed to a new arrival for which an adequate medical history is not available (less than 30 days prior to arrival).
3. Lack of a documented medical history.
4. Apparent medical problems at the time of arrival.
5. At the direction of the supervising veterinarian.

Standards for veterinary supervision, mixing of new arrivals with animals undergoing quarantine, keeper policies, sanitation, prophylactic measures including vaccination, identification, medical records and pathology are similar to those already described in the general section for mammals.

Following are recommendations and suggestions for appropriate medical procedures to be performed during or immediately prior to the quarantine period, by animal group:

**Cetaceans**

<table>
<thead>
<tr>
<th>Required</th>
<th>Recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CBC/sera chemistry panel</td>
<td>1. Direct and floatation 2.</td>
</tr>
<tr>
<td>Physical examination</td>
<td>fecal examination</td>
</tr>
<tr>
<td></td>
<td>2. Urinalysis</td>
</tr>
<tr>
<td></td>
<td>3. Blowhole and stool culture and cytology</td>
</tr>
<tr>
<td></td>
<td>4. Blood zinc levels</td>
</tr>
</tbody>
</table>

1995 JOINT CONFERENCE AAZV / WDA / AAWV 171
### Pinnipeds

1. CBC/sera chemistry panel
2. Physical examination

1. Direct and flotation fecal examination
2. Urinalysis
3. Morbilivirus titer
4. Leptospiral titer
5. Heartworm test (if appropriate)
6. Stool culture and cytology
7. Blood zinc levels

### Sirenians

1. CBC/serum chemistry panel
2. Physical examination

1. Direct and flotation fecal examination
2. Stool culture and cytology

### Carnivores (polar bear, sea otter)

1. Direct and floatation fecal examination
2. CBC/serum chemistry panel
3. Physical examination
4. Vaccination for canine distemper, feline panleukopenia, canine parvovirus, and rabies as deemed necessary by the attending veterinarian.

Additionally, others have made initial inquiries about regulations for invertebrate quarantine. It is anticipated that the above regulations will be changed and updated as new findings refine or knowledge of disease transmission and testing, and thus, appropriate quarantine protocols.
THE USE OF LONG TERM NEUROLEPTICS IN THE CONFINEMENT AND TRANSPORT OF WILD ANIMALS

Hymie Ebedes, BVSc
Department of Agriculture and Conservation, Private Bag X 180, Pretoria 0001, South Africa

Jacobus P Raath, BVSc
Kruger National Park, Private Bag X 402, Skukuza 1350, South Africa

The manipulation of wildlife, whether by capture, confinement or translocation, should be conducted in a professional manner, as humanely as possible and with minimum discomfort to the animal. This can be accomplished by combining the use of correct equipment, proven methods and trained personnel with the aid of tranquilizers. The need for longer acting tranquilizers has arisen and the use of longer acting human tranquilizers such as haloperidol (Serenace, Searle), zuclopenthixol acetate (Clopopoxin-acuphase, Lundbeck), pherphenazine enanthate (Trilafon, Scherag) and pipothiazine palmitate (Piportil depot, Maybaker) has been implemented and evaluated.

The most important findings with the use of these tranquilizers are as follows: There is a lag-phase between intramuscular injection and onset of tranquillisation at 15 min for haloperidol, one hour for zuclopenthixol and 72 hours for pherphenazine and pipothiazine. This implies that the tranquilizers have to be used with short acting tranquilizers or be stacked to maintain tranquility. The average duration of effect at the doserates recommended in Table 1 is 16 hours for haloperidol, 3 days for zuclopenthixol, 7 days for pherphenazine and up to 28 days for pipothiazine. The duration of effect is dose dependant and varies from one species to another and even between individuals within a species. Extrapyramidal symptoms can be seen at higher doserates and can be treated with repeated injections of 5 - 20 mg biperiden (Akineton, Knoll) or 5 - 10 mg diazepam (Valium, Roche). Dexetimide (Tremblex, Janssen), with its longer duration of action should also be experimented with. Suppression of the appetite is a possible side effect that should be considered. Smaller species need higher doserates than larger ones, as do younger animals within a species. They are not recommended for use in very young and very old animals. Because they are in an oil base, these long acting tranquilizers must never be administered intravenously.

Long acting tranquilizers have reduced mortality rates considerably during animal manipulations and their use should be further investigated.
<table>
<thead>
<tr>
<th>SPECIES</th>
<th>INTERMEDIATE ACTING TRANQUILIZERS</th>
<th>LONG ACTING TRANQUILIZERS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Haloperidol</td>
<td>Acuphase</td>
</tr>
<tr>
<td>Impala</td>
<td>AM=20</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td></td>
<td>AF=15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SA=10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L=5</td>
<td></td>
</tr>
<tr>
<td>Springbok</td>
<td>AM=20</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td></td>
<td>AM=15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A=10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S=7,5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L=5</td>
<td></td>
</tr>
<tr>
<td>Black Wildebeest</td>
<td>AM=20</td>
<td>1mg/kg</td>
</tr>
<tr>
<td></td>
<td>A=15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S/C=10</td>
<td></td>
</tr>
<tr>
<td>Blue wildebeest</td>
<td>AM+F=20</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td></td>
<td>S=10</td>
<td></td>
</tr>
<tr>
<td>Blesbok and Bontebok</td>
<td>AM=15</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td></td>
<td>A=10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S=7,5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L=5</td>
<td></td>
</tr>
<tr>
<td>Eland</td>
<td>AM+F=20</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td></td>
<td>S=10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gemsbok</td>
<td>Don’t exceed 20 mg in adults</td>
<td>1 mg/kg??</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Hartebeest</td>
<td>AM=30</td>
<td>AM+F=100</td>
</tr>
<tr>
<td></td>
<td>A=20</td>
<td>1mg/kg??</td>
</tr>
<tr>
<td></td>
<td>S=10</td>
<td></td>
</tr>
<tr>
<td>Lichtenstein Hartebeest</td>
<td>AM=40</td>
<td>1mg/kg</td>
</tr>
<tr>
<td></td>
<td>A=30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S=20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C=10</td>
<td></td>
</tr>
<tr>
<td>Tsessebe</td>
<td>AM=30</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td></td>
<td>A=20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S=10</td>
<td></td>
</tr>
<tr>
<td>Kudu</td>
<td>AM+F=20</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td></td>
<td>S=10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPECIES</td>
<td>INTERMEDIATE ACTING TRANQUILIZERS</td>
<td>LONG ACTING TRANQUILIZERS</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td></td>
<td>Haloperidol</td>
<td>Acuphase</td>
</tr>
<tr>
<td></td>
<td>AM=15</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>Nyala</td>
<td>A=10</td>
<td>??</td>
</tr>
<tr>
<td>Roan</td>
<td>AM+F=30</td>
<td>AM=300</td>
</tr>
<tr>
<td></td>
<td>S=20</td>
<td>A=170-250</td>
</tr>
<tr>
<td></td>
<td>C=10</td>
<td>S=100-150</td>
</tr>
<tr>
<td>Sable</td>
<td>AM+F=20</td>
<td>AM=300</td>
</tr>
<tr>
<td></td>
<td>S=15</td>
<td>A=100-200</td>
</tr>
<tr>
<td>Waterbuck</td>
<td>AM=20</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td></td>
<td>A=15</td>
<td>up to 600mg</td>
</tr>
<tr>
<td>Cape Buffalo</td>
<td>Not Used</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>up to 600mg</td>
</tr>
<tr>
<td>Giraffe</td>
<td>AM=30</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td></td>
<td>A=20</td>
<td>??</td>
</tr>
<tr>
<td>Grey rhebok Mountain redbuck</td>
<td>AM=20</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td></td>
<td>A=15</td>
<td>??</td>
</tr>
<tr>
<td></td>
<td>S=10</td>
<td>??</td>
</tr>
<tr>
<td></td>
<td>L=5</td>
<td>??</td>
</tr>
<tr>
<td>Klipspringer</td>
<td>AM+F=5</td>
<td></td>
</tr>
<tr>
<td>Steenbok</td>
<td>AM+F=8 ??</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>Grey Duiker</td>
<td>AM=15</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td></td>
<td>A=10</td>
<td>??</td>
</tr>
<tr>
<td>Suni</td>
<td>AM+F=5</td>
<td>10 - 20</td>
</tr>
<tr>
<td>Burchells and mountain zebra</td>
<td>AM+F=</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td></td>
<td>20 - 40</td>
<td>??</td>
</tr>
<tr>
<td>Black rhino</td>
<td>Not used</td>
<td>AM+F=300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S=100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>J=50</td>
</tr>
<tr>
<td>White rhino</td>
<td>Not used</td>
<td>AM+F=300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A=250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S=100-200</td>
</tr>
<tr>
<td>Elephants</td>
<td>See graph</td>
<td></td>
</tr>
</tbody>
</table>

1995 JOINT CONFERENCE AAZV / WDA / AAWV 175
<table>
<thead>
<tr>
<th>SPECIES</th>
<th>INTERMEDIATE ACTING TRANQUILIZERS</th>
<th>LONG ACTING TRANQUILIZERS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Haloperidol</td>
<td>Acuphase</td>
</tr>
<tr>
<td>Leopard</td>
<td>Not used</td>
<td>AM=70</td>
</tr>
<tr>
<td></td>
<td>A=50</td>
<td></td>
</tr>
<tr>
<td>Lion</td>
<td>Not used</td>
<td>1 mg/kg</td>
</tr>
</tbody>
</table>

AM=Adult male; A=Adult female; S=Sub-Adult; J=Juvenile; C=Calf; L=Lamb
XPS = Extra Pyramidal Symptoms

Figure 1.
THE "IDEAL" ANIMAL SHIPMENT

Darin Collins, DVM*
Woodland Park Zoological Garden, 5500 Phinney Avenue North, Seattle, Washington 98103, USA

Introduction

The ideal animal shipment is best described as the well-coordinated transfer of an animal between a departure and destination site without risk of injury, damage to health or inhumane treatment to the animal(s). This involves considerable communication and shared responsibilities among the veterinarians, keepers and curators of both institutions before, during and after the shipment.

A curator at the shipping institution will generally initiate an animal transaction and thus begin the process of an animal shipment. The curator will commonly take the primary role of making shipping arrangements, the veterinarian will assist with the proper documentation as it pertains to state, federal and international law and the keeper will oversee the animal and shipping container preparations.

The objectives to be covered here are the basic elements common to successful animal shipments with the "ideal" situation being emphasized. The working assumptions of this paper will be that the animal shipment is permitted by state, federal and international laws and regulations; that tranquilization, immobilization or other prophylactic medications are not necessary; and that quarantine considerations both pre and post-shipment have been thoughtfully considered and discussed between the shipping and receiving institutions. Realizing that shipments commonly occur with groups of animals, this discussion will refer to one animal being moved from one captive situation to another.

Advance Arrangements and Reservations

A decision will be made by the shipper as to the mode of transportation for the shipment. The shipper will decide upon an air carrier or mode of ground transportation. The typical factors of cost, species in question, distance of transport and carrier service to the area of destination commonly determine the mode of transportation. The ideal shipment will be the quickest means of transport with the fewest number of stops or layovers to minimize any unnecessary handling and climatic changes for the animal. Air shipments will understandably require appropriate ground transportation to the air carrier as well as to the point of destination once the air shipment arrives.

The shipper will need to finalize the route and decide upon any special care requirements of the animal while in transit. Special feeding or specific temperatures restrictions will need to be arranged with the air carrier. The transportation of animals over state or international boundaries will require the proper documentation and permits for the species being transported. Any customs or veterinary clearances will need to be scheduled in advance.
Advance arrangements with the Center for Disease Control will be required to inspect an importation of non-human primates upon arrival and to the point of destination. The USDA will also need to be contacted to seal and unseal shipment containers of animals being shipped between Permanent Post Entry Quarantine institutions.

The shipper will lastly confirm that the consignee is aware of the shipping details and has made arrangements to take delivery of the consignment upon arrival. The shipper should indicate how the receiving institution can acknowledge receipt of the shipment upon arrival of the animal.

Preshipment Testing and Medical History

Mandatory preshipment disease testing will be specific as determined by state, federal or international regulations. The USDA has a toll free telephone number (1-800-545-8737) with recorded information on testing requirements and permits. Veterinarians at the receiving institution will also consider the species in question and evaluate the needs for elective preshipment testing in accordance with their own preventive medicine protocols. Coordination between the dates of receipt of test results and the animal's shipping date can be critical, such as with tuberculin testing.

The ideal animal for shipment is free of any disease which would restrict the animal's ability to travel. Preshipment testing can identify disease or parasite conditions that warrant treatment prior to shipment. Preshipment test results can prevent the animal in question from being considered further for shipment. The shipping and receiving veterinarians should have the opportunity to discuss any preshipment testing results and how those results might effect the animal being shipped and/or the collection at the receiving institution.

The animal's full medical history would ideally be forwarded well in advance of the shipment to the receiving institution's veterinarian. Sending the animal's MedARKS disc is the most efficient means of sharing an animal's full medical history between institutions. Communication between veterinarians to discuss case histories with animals that have chronic illness or ongoing therapies should occur. Copies of radiographs, video footage of ultrasound procedures and other photographic documentation of the animal should be shared well in advance of the animal's shipment. Elective procedures such as permanent identification, sex determination and photographs should be scheduled accordingly by the shipping institution.

Documentation

Proper documentation and permits must accompany the animal shipment. The type of documentation required varies greatly depending upon the carrier, species being transported and the destination. Acceptable preshipment test results will result in permits dependent upon the animal's health status being obtained. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the IATA Live Animals Regulations govern the movements of animals via the airlines and require proper import and
export permits. The air waybill is the contract with the airline and will need to be completed before the carrier will accept live animal cargo. Air carriers also require a Shipper's Certificate that serves as an affidavit that all the information on the air waybill is correct, that the animal is in good health and that the shipment is not in violation of international law. It also identifies the species being shipped and indicates that the specific IATA container requirements have been met.

The veterinarian at the shipping institution will need to prepare and sign a health certificate. Health Certificates for international shipments originating from the US will require the completion of USDA Veterinary Services Form 17-140. The VS Form 17-140 is only valid with the USDA Veterinary Seal and the signature of the USDA Area Veterinarian in Charge. Proof of required preshipment testing must also accompany the health certificate. The animal being shipped should be in good health, be free of communicable disease and be in good condition to travel. Animals that are near term pregnant, in estrous or have antlers in velvet are examples of animals that are not in an ideal condition to be shipped. The health certificate must be accompanied by the USDA VS Form 18-20 for all domestic shipments which serves as a means to verify that the animal arrived in the condition stated on the health certificate.

The ideal shipment has all the required documentation with the correct number of signed and dated copies as required. A duplicate set of documents may be attached to the outside of the animal's container. The drivers of ground transports will need to be able to produce the documents required by state and federal inspectors at statelines.

**Primary Enclosure**

The shipper's responsibilities include the selection of the appropriate container or trailer for the specific needs of both the animal and the carrier. Air carriers will likely specify that the animal containers conform with the IATA Live Animals Regulations. The IATA Container Requirements are specific for the animal species concerned and are clear as to the acceptable materials and design principles. The shipper or the authorized agent is responsible for the compliance with international, carrier and International Air Transport Association regulations. The IATA regulations are accepted by CITES as the accepted guidelines in the transportation of animals by air. These regulations have been accepted as the minimum standard by most airlines for domestic as well as international flights. The IATA manual is available through the Publications Assistant, International Air Transport Association, 2000 Peel Street, Montreal, Quebec, Canada H3A 2R4.

It is essential that the shipping container be well constructed and allow for adequate ventilation. Dimensions of the container may be critical as the size of the compartment door on some airlines will determine if an animal shipment will be accepted. The dimensions as outlined by IATA will also be adhered to if any question arises as to the suitability of a container. The container must keep the animal safely inside throughout the entire shipment. Factors to be considered are the animal's ventilation requirements, the need for a leak-proof bottom with an acceptable absorbent bedding, food and water
containers and handles or spacer bars on the outside of the crate to allow for lifting. Any food or water must be able to be given via access ports readily identified on the animal's crate. The food and bedding that is provided must also be in accordance with the import regulations of the country of import and of the countries encountered during transit. Side, floor and ceiling panels equipped with special padding can be helpful for species that are prone to limb, head or horn trauma or injury to themselves at critical weight pressure points. The container should be clean and if the container is being reused, it must have been thoroughly disinfected.

Ground shipments require the same attention to container detail as air shipments. Fewer regulations govern container requirements with noteworthy exceptions. The CDC regulations for the importation of non-human primates requires container and vehicular specifications. For example, the personnel transporting the shipment must have separate ventilation from the animals.

Trucks or trailers for larger species where crates are impractical are typically used for transport. Trailers should be inspected for cleanliness and the appearance of being well maintained. The trailer tires should be checked for wear and proper inflation. Wheel bearings and brake linings should also be regularly inspected for proper maintenance. Trailers should have no projections to the inside of the animal area and have enough headroom to allow the animal to stand in a natural position. The flooring must be non-slip and have proper bedding. The inspection doors or windows must allow for the animal to be visually inspected and allow access for feeding and watering en route without risk of escape or potential injury to the animal. A means of illumination of the vehicle interior is important. The trailer should be inspected for the adequacy of ventilation. The loading ramp or steps should not have gaps or places where an animal's feet could get caught and side rails to prevent an animal from falling. The ramp gradient should be such that the animal loads without slipping. There should be a barrier or doors to prevent animals from falling out when the rear door is lowered.

Ideally the trailer would arrive well in advance of the shipping date to allow sufficient time for the animal to acclimate to the trailer. This also allows time for the keeper staff to plan an appropriate strategy for loading the animal without difficulty. The driver should be experienced with hauling animals and realize that the animal's level of comfort will depend on the driver's ability to navigate turns and change speed gradually. The trailer must become a transportable stall when the animal becomes acclimated to the trailer. Transportation time should include scheduling adequate rest periods to allow the animal time to eat with the trailer stopped. The trailer would be of sufficient size to allow the animal freedom of movement during these periods of rest. The optimal ventilation and temperature should be able to be regulated.

Specific Animal Needs

The specific needs of the individual animal during shipment and after can best be determined by input from the animal keeper caring for the animal being shipped. The
quarantine and unit keepers at the receiving institution will want to anticipate the animal's needs prior to the animal's arrival. The American Association of Zookeepers has available the Animal Data Transfer Form that allows institutions a format for exchanging information about diet, previous reproductive and medical history, enclosures, cleaning and disinfecting procedures and any personal comments about the animal. The ADTF is available free of charge upon request from Bernie Feldman, Burnet Park Zoo, 500 Burnet Park Drive, Syracuse, NY 13204. Specific animal needs during a shipment might include feeding or watering during a shipment. These arrangements must be made with the carrier and confirmed in writing at the time of departure. An attendant may be required as stipulated in the Marine Mammal Protection Act to accompany the shipment.

The ideal situation would be for the receiving institution to send the new keeper to the shipping institution prior to the animal's shipment. Having the new keeper on site to learn the animal's routine, its normal behavior pattern and to become familiar with the diet being fed would be optimal. Shipping a supply of the animal's present diet can be helpful in the transition as in the case of medicated or specially formulated diets. If the animal will be consuming a new diet at the receiving institution the new diet should be introduced prior to the animal's shipment. Perhaps the shipping institution could videotape the animal's routine care being performed and send the videotape ahead of the animal. More typical and still ideal is for the animal's present keeper to travel with the animal to the new institution to ease the stresses associated with moving to a new location.

**Handling Procedures by Shipper and Carrier**

The actual crating or loading of an animal for shipment will occur at variable times prior to leaving the shipping institution. The total time contained should ideally be minimized. Consideration should be given to conditioning an animal to the confines of a container before the actual day of shipping.

The air waybill will need to be presented prior to the air carrier accepting the live animal cargo and found to be correct. Proof of advance reservations with all the carriers participating in the carriage of the animal will need to be produced. Flight numbers for which reservations are held for the entire routing will need to be indicated. All relevant permits, including CITES where necessary, licences and certificates required for export, import or transport will need to be attached to the air waybill and the container.

The labelling and marking of the outside of the container of air shipments is the responsibility of the shipper. Labels that indicate "This Way Up" are required on all four sides where possible and one "Live Animals" label is required. The container requires a label to indicate name, address and telephone number of the consignee and the scientific and common name of the animal. All labels must conform to IATA specifications of size, color and text. Any animals that can inflict venomous bites or stings shall have their crates labelled as "Poisonous".
The ideal handling of a shipment has all the required labelling as determined by law. There is no question as to the intended destination of the shipment. Any special needs or instructions are clearly labelled and the animal is provided for as stipulated. The air carrier or transporting personnel handle the cargo without incident and the animal is transported humanely and without injury. The animal would ideally enter the shipping container and remain calm once contained without the use of drugs.

**Destination and Delivery**

The final phase of an animal shipment is the arrival of the animal at its intended destination. Advanced arrangements must be made for the consignee to receive the animal upon arrival. All those responsible for accepting the animal shipment must be fully aware of the details of the animal's scheduled arrival. Animals should be off-loaded first. Carriers will typically use lateral ramps for the off and on-loading of animals to minimize the chance of an animal falling within the confines of the container.

Individuals arriving to accept animal shipments should anticipate the needs of the animal. This might include weather concerns, ventilation and any need for the direct observation of the animal during transportation to the receiving institution.

The receiving institution will ideally accept the animal into an appropriate quarantine situation. The shipment ends when the animal is out of the confines of the shipping container. Animals at this time should have ready access to food, water and comfortable surroundings to begin the acclimation to their new surroundings.

A representative from the receiving institution should notify the shipping institution within 24 hours to report on the animal's shipment. Any unusual findings such as dead or injured animals, damaged crates or animals that did not arrive as scheduled should be reported to the shipping institution and the carrier. Communication from the receiving veterinarian should be communicated to the shipping veterinarian as it pertains to the animal's condition upon arrival if it is not consistent with information provided in previous communications or as stated upon the animal's health certificate. Information about the animal after its quarantine exam should also be communicated to the shipping veterinarian if it is not consistent with the animal's medical history.

**Conclusion**

The ideal animal shipment remains an "ideal". Every shipment will differ considerably in various details. The basic objectives of animal transportation remain constant, that an animal be transported without harm and be in compliance with all applicable laws. The challenge to attain the "ideal" comes from within ourselves to adhere to the basic objectives and to become innovative and consider any means to keep the animal's welfare first and foremost in the planning process of an animal shipment.
LITERATURE CITED

LATEST DEVELOPMENTS IN TRANSLOCATION TECHNIQUES OF THE ARABIAN ORYX, Oryx leucoryx

Marc Ancrenaz, DVM*, Stéphane Ostrowski, DVM, Alain Delhomme, Technical Breeder
National Wildlife Research Center, P.O. Box 1086, Taif, Saudi Arabia

Arnaud Greth, DVM
3 rue Larochelle, 75014 Paris, France

Introduction

An intensive captive breeding of Arabian oryx (Oryx leucoryx) was established at the National Wildlife Research Center (NWRC), Taif, Saudi Arabia, in 1986, for the propagation in captivity and reintroduction into the wild of this medium-size antelope.

Because of their nervous disposition and their aggressiveness, members of the Hippotraginae family are highly susceptible to stress and capture myopathy syndrome. They are therefore difficult to restraint, to immobilize and to transport.

The oryx bred at the NWRC for reintroduction purposes are mother-reared in large enclosures (25 ha). They have almost no contact with human beings, and are handled only rarely. By the time they are translocated, the animals are very wild, nervous, afraid of man and highly sensitive to stress. A high mortality rate (between 9% and 66%), related to capture myopathy, occurred during the first translocations of Arabian oryx from the NWRC to Mahazat as-Sayd, a fenced protected area situated 200 kms from Taif by road.

New techniques based on the boma training and on the use of long-acting tranquilizers were developed to undertake Arabian oryx translocations over longer distances and longer periods of time (up to 9 hours).

Previous Translocations of Arabian Oryx

Description of the different methods of transportation

Between 1991 and 1993, 40 (15.25) oryx, aged between 9 and 16 months, were translocated from the NWRC to Mahazat as-Sayd protected area. The animals were transported by road. The different transportations lasted 2-2.5 hrs and were always carried out in the early morning during winter, when the conditions were cool (5 to 15 °C).

On the day of the translocation, all the oryx were handled, blindfolded, blood sampled, weighed, fitted with ear-tags or radio-collars, and injected i.m. with 2 mg selenium, 10 ml of a vitamin cocktail, 240 mg methylprednisolone and 20 mg/kg body weight oxytetracycline. All were released in a 25 hectare enclosure when they arrived to the reserve.

The translocations were carried out according to three different procedures. The first steps of two procedures were identical; the animals were held with their mother in large
enclosures (15-25 ha) until they were translocated. Twenty-seven oryx were anesthetized with a combination of etorphine (0.05 mg/kg) and xylazine (0.20 mg/kg), administered i.m. via a dart gun (Gut 50, Telinject, Romerberg, Germany).

After handling, 6 (3.3) of the 27 oryx had anesthesia reversed with diprenorphine (0.1 mg/kg) and atipamezole (0.04 mg/kg). They were injected with haloperidol (10 mg i.m. and 10 mg i.v.) before being placed in individual crates and moved to Mahazat. In the second procedure, twenty-one (8.13) oryx were directly loaded into a car after recumbency and then directly driven to the reserve under anesthesia. They were injected with the reversal agents after they arrived at the reserve.

In the third procedure, 13 (4.9) calves 6-month old were separated from their mothers and placed together in a 4 ha enclosure. One month prior to the translocation, the group was enclosed in a corridor (80 m long, 6 m wide). The walls were fitted with plastic sheeting. On the day they were translocated, groups of 4 or 5 individuals were moved into a trailer (2.5 long x 1.8 wide x 2.2 m height) and driven to Mahazat. During the journey, the animals were continuously monitored by video-channel.

**Results**

Various mortality rates were encountered in the three procedures (Table 1). Different syndromes of capture myopathy were recorded during or shortly after the transportations: two animals died of a peracute form', two died of an ataxic myoglobinuric syndrome', and two died of a necrolysis of the gastrocnemius muscle. In the third procedure, the animals remained very quiet and frequently recumbent during the journey. However, lameness of a hindquarter, hypercontraction of the gastrocnemius muscle and malposition of the hoof were recorded in four individuals three hours after they were released. Two of them, were injected i.v. with 400 mEq sodium bicarbonate/ 100 kg body weight and recovered. Two of them did not receive any treatment and finally died after two and five weeks respectively. Post-mortem examination showed a necrolysis of the gastrocnemius muscle with loss of substance. Histological findings were mainly located in the muscle tissue: necrotic lesions, focal mineralization, sarcolemnal proliferation and fibrosis.

No capture myopathy symptome occurred when the oryx were moved under anesthesia, but two of them died of septicemia within ten days following the translocation. *Pasteurella haemolytica* and *Streptococcus bovis* were isolated after bacterial culture.

**Recent Translocation of Arabian Oryx Involving "Boma Training" Technique and Long-acting Tranquillization**

In 1995, Arabian oryx were translocated to Uruq Bani Ma’arid, an unfenced protected area located in the edge of the Empty Quarter, 1200 kms from Taif by road. In view of the preceding results, a new procedure was developed in order to airlift the animals.
Constitution of the groups to be translocated

Oryx calves were removed from their mothers when 6 months old and placed in bachelor herds, held in 0.2 ha enclosures. They were to be translocated when between 10 and 24 months of age. Three months prior to the translocation, groups of four animals of similar age and size were placed in a pre-transportation enclosure. A social hierarchy was promptly established within each group. When fights were too frequent during this period of adaptation, the most aggressive animals were removed and placed in another group. After the social relations were eventually determined, the composition of each group remained unchanged until the animals were released.

Description of the facilities

- Pre-transportation enclosure: each group of 4 oryx was placed in a pre-transportation enclosure. This enclosure was divided in two different parts:
  - Indoor pen: 15 square meters (5 m x 3 m) with wooden walls and shade cloth cover roof. Food and water were provided ad libitum in this part.
  - Outdoor pen: 60 square meters (6 m x 10 m), fenced with chain-link mesh. A sliding gate separated the two pens.

- Off-loading ramp and passageways: the outdoor pens were connected with a off-loading ramp 2.5 m wide and 3 m long. This ramp formed a funnel directed to a corridor 1 m wide, 8 m long. The passageway conducted to the loading platform and was used to move the oryx from the outdoor pen to the mass crates. The walls of the passageway were wire-mesh fences fitted with tarpaulin.

- Mass crates: the mass crates used for transportation were made in wood and the floor was covered with woven rubber mats. The size of the crate was 2.5 m long x 2 m wide x 2 m height; it was large enough to hold one group of 5 oryx. The ventilation was provided with 3 lines of circular openings running the length of the crate along the upper half of the four side walls. The crates were in close connection with the loading platform when placed on the carrier truck.

Boma training

During the first six weeks of the training, each group was enclosed every two days in the indoor pen for increasing periods of time, ranging from 2 to 24 hours. Progressively, the oryx became accustomed to being moved into the passageway and into the crate by the keepers who utilized pieces of canvas to gently push the animals. The oryx were closed into the mass crates and the horns of the more aggressive animals were fitted with plastic pipes. Twice a week, each group was driven around the NWRC for durations of 30 min to 2 hours. Each group was released in the pre-transportation pen after the driving. One week prior to the translocation, each group was closed in the mass crate over night every other day.
Use of long-acting tranquilizers

The biological effects of perphenazine enanthate (Trilifan, Shering-Plough, Levallois, France) in Arabian oryx were studied in previous experiments carried out at the NWRC with 15 individuals. When administered i.m., the effective dosage of perphenazine enanthate averaged 3 to 3.5 mg/kg body weight. The first signs of tranquilization were recorded 24 hours following the i.m. injection. The animals were quiet, they remained standing and the flee distance decreased. The best state of tranquilization was achieved between 3 and 4 days following the injection and lasted for approximately 24-36 hours. However, the animals were still able to run away when approached by keepers. The effects of the tranquilization with perphenazine had totally disappeared one week after injection. Food intake was unchanged during all the tranquilization period. Side-effects were recorded with higher dosages; akathisia, dyskynesia and parkinsonism. These side-effects spontaneously disappeared.

Three days prior to the translocation, all the oryx to be transported were handled, weighed and injected i.m. with 2.5 to 3 mg/kg body weight perphenazine. The horns were fitted with plastic pipes and cotton swabs were placed in the ears of all the animals. Then, they were released in the outdoor pen.

Translocation

Each group of tranquilized oryx was closed in the mass crate on the evening before the journey. In the early morning the animals were driven to the airport. The crates were loaded within a C 130 military plane. After a one hour flight, the plane landed 150 kms from the reserve. The crates were off-loaded from the aircraft, placed on trucks and transported by road to the reserve.

Release of the animals in the reserve

At their arrival, the oryx were placed in small pens. Water was provided ad libitum, whereas dry alfalfa and hay was given only the following day. After three days, the animals were handled and fitted with radio-transmitters; plastic pipes and cotton swabs were removed. The animals were released together in a 4 ha enclosure five days after the transportation.

Results

Seventeen (8.9) oryx were boma trained and prepared for the air transportation (Table 1). One female showed signs of gastrocnemius necrolysis during the training, after having been handled at the beginning of the training period. She was administered i.v. with bicarbonate but complete recovery took more than six months. She was not translocated to the reserve.

One male showed extra-pyramidal iatrogenic symptoms (dyskynesia) two days following the i.m. injection of trilifan. Ten mg diazepam (Valium, Roche, France) injected half i.v. and half i.m. immediately halted the symptoms.
Sixteen (8.8) young oryx were airlifted in two different shipments from the NWRC to Uruq Bani Ma'arid. The journeys lasted 9 and 7.5 hours respectively. During the transportation, the oryx were very quiet, without any fighting. No real signs of excitation, fear or stress were recorded during the flight.

The animals appeared tired (reluctant to walk, ears down, dehydrated) when they arrived to the reserve, but fully recovered within a few days. They did not display symptoms of capture myopathy, and no pathological problems were encountered during the acclimatisation period in the 4 ha enclosure. The 16 animals were successfully released into the wild three months after the transportation.

**Discussion and Conclusions**

The Arabian oryx bred at the NWRC and intended for release operations, are very sensitive to handling and physical restraint. Under the stress of translocation, they are prone to develop a muscular necrolysis, particularly affecting the gastrocnemius muscle. A treatment with i.v. infusions of 400 mEq sodium bicarbonate/100 kg body weight is prone to treat the affection, but the recovery process can take several months.

Boma training aims to tame down and to accustom the animals to the conditions they will face during translocation: movements between enclosures, pens and crates, confinement in a close space for a long period of time, sounds, sights, smells and disturbances associated with human presence and activities⁵. The best results are achieved when training is initiated at least four weeks prior to the translocation⁴. In order to achieve the best results, it appears essential to not interrupt the boma training after it has been initiated, and not to handle the animals on the day they are translocated.

The use of long-acting neuroleptic aims to reduce anxiety and motor activity for a prolonged period. The most noticeable effects in wild ungulates are a modification of the animal's attitude towards its surroundings (indifference to captivity, decreased aggression), a loss of fear of humans and a general relaxing effect, so the animals are better able to cope with captive conditions⁴. Nevertheless, food consumption remains unchanged during the period of tranquilization. Compared to the other species of wild ungulates in which perphenazine has been utilised⁵, the Arabian oryx needed a relatively high dosage to achieve a good state of tranquilization. It seems essential to study the specific actions, the effective dosages, the side-effects of long-acting tranquilizers for each species before transportation.

The combination of boma training and long-acting tranquilization gave very good results when Arabian oryx were translocated over long distances. The animals were relatively insensitive to stressful situations and did not develop any pathological problems following a 9 hour road-air-road transportation.
LITERATURE CITED


Table 1: Results of transportation of Arabian oryx according to the different procedures.

<table>
<thead>
<tr>
<th>Boma training</th>
<th>Transportation technique</th>
<th>Sample size</th>
<th>Mortality (number of deaths within two weeks following the transportation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedure 1</td>
<td>No 1- Anesthesia with etorphine / xylazine 2- Injection of reversal agents 3- Tranquillization with haloperidol 4- Individual crate 5- Two hours by road</td>
<td>6</td>
<td>66% 2 animals died of peracute capture myopathy 2 animals died of ataxic myoglobinuric syndrome</td>
</tr>
<tr>
<td>Procedure 2</td>
<td>No 1- Anesthesia with etorphine / xylazine 2- Two hours by road 3- Injection of reversal agents</td>
<td>21</td>
<td>9.5% 2 animals died of septicemia</td>
</tr>
<tr>
<td>Procedure 3</td>
<td>No 1- Small groups placed in a trailer 2- Two hours by road</td>
<td>13</td>
<td>15% 2 animals died of a necrolysis of the gastrocemius muscle</td>
</tr>
<tr>
<td>Procedure 4</td>
<td>Yes 1- Tranquillization with perphenazine 2- Mass crate 3- Nine hours by road/air/road</td>
<td>18</td>
<td>0%</td>
</tr>
</tbody>
</table>
CHLAMYDIOSIS

Branson W. Ritchie, DVM, PHD*, Frank D. Niagro, PHD
Psittacine Disease Research Group, University of Georgia, College of Veterinary Medicine, Athens, USA

Thomas N. Tully, DVM
LSU School of Veterinary Medicine, Baton Rouge, LA, USA

Kenneth S. Latimer, DVM, PHD, Denise Pesti MS, Raymond Campagnoli MS, Kelly Van Vreede, BS, Phil D. Lukert, DVM, PhD
Psittacine Disease Research Group, University of Georgia, College of Veterinary Medicine, Athens, USA

Chlamydia psittaci, the etiologic agent of psittacosis, is a highly infectious, obligate, intracellular parasite that can induce disease in most species of free-ranging and domestic birds as well as in humans. Chlamydiosis should be considered in any bird experiencing clinical signs of respiratory or digestive disease including dyspnea, anorexia, depression, weight loss, diarrhea, rhinitis, conjunctivitis and green or yellow discolored urates indicative of liver disease.

This organism can be transmitted through direct or indirect contact with contaminated feces or respiratory secretions. Infected birds may shed chlamydia for up to a week before developing clinical signs of disease. This organism may remain infectious in dried feces for several months, thus fomites can be involved in transmission. The reported incubation period varies from a week to 42 days depending on the species of bird and the circumstances of a bird’s exposure to the organism (natural vs experimental). Epizootics are particularly common in crowded conditions where numerous birds are being congregated, and incidence rates in imported psittacine birds have been reported to be as high as 30% in some populations.

Clinical pathology is of limited value for diagnosing chlamydiosis because the laboratory changes vary among different species, as well as between individuals within the same species. Currently, a combination of serologic tests and attempts to isolate or detect the organism in feces is the best way to diagnose chlamydiosis. Test that detect the organism are best in birds that are clinically ill and likely to be shedding chlamydia. Serology is best for determining if birds have previously been infected or to detect active infections. Serology is of limited value in acute disease. In some birds, chlamydiosis may be diagnosed by staining nasal or conjunctival cells with Giemsa or Macchiavello’s stains.

While chlamydiosis may be difficult to diagnose, infections respond readily to treatment with tetracyclines. Doxycycline is currently the drug of choice. This drug is available in the United States in preparations designed for oral or intravenous administration. A preparation of doxycycline (Vibrovenos) that can be administered intramuscularly also is available in Europe and Canada. A single injection of Vibrovenos will maintain effective concentrations of the antibiotic in the blood for 5 to 7 days. Clinicians who wish to use this drug are encouraged to obtain the necessary permits from the USDA.
A DNA probe has been developed to detect the nucleic acid from chlamydia in feces, swabs of the liver or spleen and bone marrow. Ongoing research is will attempt to determine if Chlamydia psittaci causes latent infections, and if so, the cellular site where the organism may be found. If a site for persistence of infection can be defined and an easily obtainable sample can be used for testing (ie., blood), then it may possible to use DNA probe analysis to establish chlamydia-free flocks of psittacine birds.
A chronic disease characterized by symmetric feather dystrophy and loss, development of beak deformities and eventual death, was first described in various species of Australian cockatoos in the early 1970s. Subsequently named psittacine beak and feather disease (PBFD), the syndrome has been diagnosed in many other psittacine species. It is possible that the disease had been noticed as early as 1887 by Australian explorers that described characteristic feather changes in free-ranging red-rumped parrots (Psephotus) in South Australia.

The first clinically detectable sign of PBFD is the appearance of necrotic, abnormally formed feathers. Feathers are normally arranged in distinct tracts called pyterylae. Both the normal pattern of molt and the appearance of dystrophic feathers in birds with PBFD occur separately in each specific feather tract. The type of feathers that are initially involved and the distribution of feather loss depends on the stage of molt when clinical signs of disease appear. In young birds (less than 2 months old), all of the feather tracts may be affected during a 1-week period, whereas in older birds the disease is more prolonged with progressive feather changes during ensuing molts.

Psittacine beak and feather disease has been reported in Australia, North America, Europe and Asia. It is likely that PBFD virus originated in psittacine birds indigenous to Australia and that it has been introduced to other continents through the world-wide movement of birds to meet demands of the companion bird markets. Continued intercontinental movement of birds could easily result in the introduction of PBFD virus into free-ranging populations of the world's more endangered psittacine species.

Historically, PBFD virus was thought to only affect Old World and South Pacific psittacine birds, with white and pink cockatoos being particularly susceptible. However, the disease has also been documented in several genera of black cockatoos and in New World psittacines including Amazon parrots, macaws and a species of Pionus. Histologic or clinically suggestive lesions of PBFD have now been described in over 40 species of psittacine birds, and new susceptible species are being observed every year. Until 1993, PBFD had only been documented in birds within the Psittaciforme order, but a similar virus has been recovered from free-ranging doves in Australia with feather abnormalities similar to those described in affected psittacines. The actual host range of the PBFD virus remains largely unknown.
Experimental infection studies suggest that the minimum incubation period for the appearance of dystrophic feathers is 21 to 25 days.\textsuperscript{8-10} Virus can be detected in the blood using DNA probes as soon as 2 days after natural exposure to the virus and long before an infected bird develops clinical signs of disease. Most adult birds infected with the PBFD virus will develop a transient viremia that can be detected using DNA probes. Most of these individuals subsequently seroconvert and remain clinically normal.

Both clinical experience and DNA probe testing of blood for the presence of PBFD virus nucleic acid suggest that the maximum disease incubation period can be years. Viral nucleic acid could be detected in the blood of one clinically normal Ducorp's cockatoo for 18 months before feather abnormalities developed. The bird was DNA probe test-positive during the 18 months before clinical signs of PBFD occurred.

One of the critical pieces of information that veterinarians should understand is how the PBFD virus can be transmitted. Armed with this information and the DNA probe test, one can implement testing and hygiene practices which will substantially decrease, if not eliminate, the possibility of a PBFD virus outbreak in the United States and Europe, where the virus does not infect free-ranging birds. In contrast, testing and hygiene may be less effective in controlling PBFD in Australia where infected free-ranging birds can easily introduce the virus to susceptible captive birds.

High concentrations of virus were demonstrated in feather dust collected from a room where birds with active cases of PBFD were being housed, implicating contaminated dust from any source (feather dander or dried, aerosolized excrement) as a major vehicle for the natural transmission of this virus.\textsuperscript{11} Given the ease with which feather dust can be dispersed both through natural air flow and through contact with clothing, nets, bird carriers, food dishes or insects, it is likely that contaminated feather dust is a major mode for transmission and environmental persistence of the PBFD virus. Within the sensitivity limits of the test, one can determine whether PBFD virus is present in a home or aviary by using viral-specific DNA probes to test environmental swabs for the presence of viral nucleic acid.

Psittacine beak and feather disease virus was demonstrated in the feces and crop washings from various species of psittacine birds diagnosed with PBFD.\textsuperscript{11,12} The recovery of PBFD virus in the feces and crops of diseased birds suggests that contaminated excretions and secretions from infected birds may be involved in disease transmission. It has been postulated that the demonstration of inclusion bodies containing PBFD virus in the cells lining the lumen of the palate, esophagus, crop, intestines and bursa could easily account for the virus shed oral secretions and feces.\textsuperscript{13}

Because viral nucleic acid can be demonstrated in the blood of infected birds, vertical transmission would be suspected. It has been determined that artificially incubated chicks from PBFD-infected hens will consistently develop PBFD, suggesting that vertical transmission of the virus does occur.
Psittacine beak and feather disease should be suspected in any bird with progressive feather loss involving malformed feathers. However, one cannot determine if a bird is infected with the PBFD virus strictly by examining the feathers. Visible feather changes grossly similar to those caused by PBFD virus can be induced by any factor that disrupts the blood supply to the developing feather, including avian polyomavirus, adenovirus, trauma, bacterial folliculitis, fungal folliculitis, septicemias, malnutrition, endocrine abnormalities and some drug reactions, particularly to penicillins and cephalosporins. Feather lesions identical to those caused by PBFD virus also can be produced by pinching developing feathers at or near the pulp cap. Conversely, birds with normal-appearing feathers can have the PBFD virus nucleic acid in their blood stream. The only effective method available for determining if a bird is infected with PBFD virus before feather lesions are present is DNA probe testing of blood.

In birds with feather abnormalities, PBFD virus infections can be confirmed by microscopic evaluation of affected feathers for the presence of characteristic intracytoplasmic inclusion bodies or by demonstrating viral nucleic acid in the blood using PBFD virus-specific DNA probes. Identifying basophilic intracytoplasmic inclusion bodies in birds with characteristic feather lesions is considered diagnostic. However, adenovirus and polyomavirus can cause intranuclear inclusion bodies that appear similar to those caused by the PBFD virus. Therefore, a confirmatory diagnosis of PBFD requires the use of viral-specific antibodies to demonstrate PBFD virus antigen or the use of DNA probes to detect PBFD virus nucleic acid. In a study comparing routine histopathology, immunoperoxidase staining and DNA probe detection of PBFD virus in tissues from suspect patients, DNA testing was found to be the most sensitive (97.7%) and specific (100%) diagnostic test. Routine microscopic evaluation of hematoxylin and eosin-stained tissues was found to be the least sensitive (72.4%) and specific (93.8%).

DNA Probe Testing

Use of viral-specific DNA probes is the most sensitive and specific test for detecting PBFD virus. In validating the DNA probe test designed to detect the PBFD virus in circulating white blood cells, it was found that the test correctly identified 377 of 378 known positive samples, resulting in a test sensitivity of 99.7%. One hundred known negative samples were correctly identified for a specificity of 100%. These probes can be used on biopsy samples of suspect feathers to confirm an infection or on a blood sample to demonstrate viral nucleic acid in circulating white blood cells of infected birds before clinical changes in the feathers are apparent. This test is available in the United States and in several European countries.
Figure 1: Diagnostic flow chart for PBFD virus.

**Feathers Normal**

Test blood for PBFD virus using DNA probes (Avian Research Associates, Milford, OH)

- A positive test in a bird with no feather abnormalities indicates that the bird has been exposed to PBFD virus and that viral nucleic acid is present in the blood. This bird must be retested in 90 days. If the bird is still positive when retested at 90 days, it indicates that the bird is either subclinically infected or that the bird is being repeatedly exposed to the virus. Subclinically infected birds can develop feather lesions at some future date. If the bird is negative when retested, it indicates that the bird was transiently infected and that the bird’s immune system was able to clear the virus from the blood. Birds with normal feathers that have cleared an infection should be considered resistant to PBFD. Most birds that are exposed to the PBFD virus will have virus present in their blood for a brief period.

- A negative test indicates that PBFD virus nucleic acid was not detected in the blood.

**Bird Has Abnormally Developing Feathers**

Test blood for PBFD virus using DNA probes

- A positive DNA probe test in a bird with abnormally developing feathers suggests that the bird has an active PBFD virus infection.

Management of a positive bird:

If a bird from a breeding aviary with feather abnormalities is found to be positive, remove the bird from the area as quickly as possible. Virus-infected birds with feather abnormalities shed large concentrations of virus in their feather dust which can be easily carried by the wind or on clothes, skin or hair to other birds. All areas, supplies, and equipment that could be contaminated with feather dust from the infected bird should be repeatedly cleaned and disinfected. One can determine whether cleaning efforts following an outbreak have been sufficient by DNA probe testing of air ducts, carpets, enclosures or any dusty area.

If a companion bird with feather abnormalities is found to be positive, the bird must never be exposed, directly or indirectly, to other birds outside of the household. Infected companion birds can live a long life when provided a stress-free environment and supportive medical care; however, anyone who maintains a PBFD-positive bird must be aware that the virus can be transported to other locations on clothing or in
hair. Be courteous of other birds and do not expose them to this virus by entering aviaries, pet shops or bird shows.

- If negative: A feather biopsy (including the feather follicle) should be submitted for microscopic examination. In some cases, birds infected with the PBFD virus may have a negative blood test if the concentration of virus in the blood stream is high or if the bird's white blood cell count is extremely low. These birds usually have severe feather lesions and can be diagnosed by microscopic examination of affected feathers. It should be noted that some PBFD-infected psittacines of South American descent have spontaneously recovered from the disease.

For DNA probe detection of active (feather lesions present) or subclinical infections, the recommended sample to submit is whole anticoagulated blood (0.2 to 1.0 ml in heparin, 20 units per ml of blood). In birds that have feather abnormalities, whole anticoagulated blood should be submitted for DNA probe testing and biopsy samples of diseased feathers should be placed in 10% formalin and held for further diagnostic testing, if necessary.

A positive DNA probe test in a bird that has feather abnormalities suggests that the bird has an active PBFD virus infection. A positive blood test in a bird that does not have feather abnormalities may indicate that the bird is latently infected or that it recently has been exposed to the PBFD virus and is viremic. A bird that tests positive and has no feather abnormalities should be retested in 90 days. If the bird is still positive, then it should be considered to be latently infected or is continuously being exposed to the virus. A negative test 90 days later indicates that the viral nucleic acid is no longer detectable in the blood and that the bird has eliminated the virus. Virus contamination of a sample from a toe-nail clip can cause a positive test in a bird that does not have the PBFD virus in its blood.

A negative DNA probe test for PBFD virus indicates that viral nucleic acid was not detected in the submitted sample. Some birds that have PBFD virus infections may be DNA probe-negative. These birds generally have severe infections, with a majority of their feathers being abnormal. These birds may be negative because the high concentration of nucleic acid in the sample interferes with the test reagents, or the white blood cell count may be decreased because of concomitant infections.18

It is common for birds that have recently been exposed to the PBFD virus to be DNA probe positive, even though the birds have normal-appearing feathers. The majority of these birds will be transiently infected and will eliminate the virus. These birds only develop disease if their immune system is unable to clear the infection. In 10,000 blood samples tested over a 1-year period, DNA probes indicated that approximately 5% of the birds were positive for PBFD virus. Many of these birds were clinically normal and were either subclinically infected or were transiently positive and subsequently were negative when retested 90 days later.18

The environmental stability of the PBFD virus is unknown. It would be prudent to consider its stability similar to that described for chicken anemia virus (CAV), which is similar in

1995 JOINT CONFERENCE AAZV / WDA / AAWV 197
ultrastructure and DNA composition to the PBFD virus. Chicken anemia virus has been found to be environmentally stable and remarkably resistant to inactivation. In liver tissue, CAV remained infectious when treated with amphoteric soap (10%), orthodichlorobenzene (10%), iodine (1%), sodium hypochlorite (bleach, 1%), methyl alcohol, ethyl alcohol, chloroform and heating to 80°C for 1 hour, even when boiled for 5 minutes. Dried material containing CAV remained infectious after treatment with ethylene oxide for 2 hours. Fumigation with formaldehyde for 24 hours only partially inactivated CAV. A 10% solution of sodium hypochlorite (bleach) or iodine was necessary to inactivate the virus in liver tissue. Cell culture-derived CAV was susceptible to iodine (1%), sodium hypochlorite (bleach), beta-propiolactone (0.4%), glutaraldehyde (1%) and heating to 80°C for 1 hour, but was resistant to phenol (5%), sodium azide (0.1%), thimerosal (0.1%) and urea.19

Until a safe subunit or cell-culture derived vaccine to prevent PBFD virus infections is available, DNA probe tests in conjunction with sound hygiene are the best ways to prevent PBFD. This disease can virtually be eliminated from captive birds in the United States and Europe by making certain that only DNA probe-negative birds without feather abnormalities are added to an established collection or aviary. In an effort to reduce the number of new cases of PBFD, it is advisable that all birds of a susceptible species be tested to determine whether they are latently infected with the PBFD virus.

LITERATURE CITED

7. Pass DA. Natural infection of wild doves (Streptopelia senegalensis) with the virus of psittacine beak and feather disease. Xth World Vet Poult Assoc Congress. Sydney, Australia: 1993: 165.
Introduction

Budgerigar fledgling disease (BFD), caused by an avian polyomavirus, was first reported in the United States and Canada in 1981.² Subsequently, polyomavirus infections have been described worldwide in various nonbudgerigar psittacine birds, finches and gallinaceous birds. While the viruses that infect these differing species appear to be morphologically and antigenically similar, the clinical presentation, distribution of lesions and epizootiology of infections vary dramatically in differing species.³⁻⁸

Peracute death with no premonitory signs is the most common clinical finding in young, nonbudgerigar psittacine birds affected by avian polyomavirus. Acute infections are characterized by death following a 12-to 48-hour period of clinical changes that may include depression, anorexia, weight loss, delayed crop emptying, regurgitation, diarrhea, dehydration, subcutaneous hemorrhage, dyspnea and polyuria.³⁻⁶,⁸⁻¹⁰

Neonates with polyomavirus infections may bleed profusely, or for a prolonged period, from intramuscular injection sites or from follicles where feathers have been removed. Subcutaneous hemorrhage over the crop and across the cranium is common.¹¹ While a propensity to bleed abnormally is suggestive of a polyomavirus infection, this clinical finding is not diagnostic for polyomavirus. Any disease involving vasculitis, clotting disorders or damage to the liver can cause similar hemorrhaging.

All species of psittacine birds or finches should be considered susceptible to the virus. It has been suggested that polyomavirus-induced disease occurs most commonly in young budgerigars, macaws, conures, eclectus parrots, lovebirds, ring-necked parakeets and caiques.³⁻⁸,¹²,¹³ In Australia, polyomavirus infections are considered particularly common in lovebirds.¹³ In comparison to the species listed above, polyomavirus-induced disease is considered less common in young cockatoos, grey-cheeked parakeets, lories, African grey parrots, hawk-headed parrots and Amazon parrots.³⁻⁶,⁸,¹³ It should be cautioned, however, that reported variances in susceptibility may represent a skew in the population of exposed birds and not an actual difference in susceptibility.

Gallinaceous birds also appear to be susceptible to polyomavirus. A virus that morphologically resembles a polyomavirus was recovered from the intestinal contents of asymptomatic turkeys. The recovered virus did not cause a discernible disease in experimentally infected birds.¹⁴ A polyomavirus-like agent was identified in the feces of an
ostrich located in the southeastern United States. (Georgia Vet Diagnostic Laboratory) A polyomavirus with similarities to BFD virus was recovered from the drinking water and feces associated with a chicken layer replacement farm in Germany. It was not determined if the virus in this poultry house originated from chickens, or was a contaminant from another source, but, serologic studies suggest that chickens are exposed under natural condition. Polyomavirus-specific antibodies have been demonstrated in broiler chickens from central Europe and the United States (Ritchie, et al unpublished). During a polyomavirus epornitic in a mixed species aviary, virus-neutralizing antibodies were detected in 2 golden pheasants and a Lady Amhurst pheasant that had been naturally exposed to affected psittacine birds, while a potentially exposed bantam chicken and 2 toco toucans remained seronegative (Niagro, et al, unpublished data). Inclusion bodies suggestive of polyomavirus have been described from Australia in a Kakariki, a peaceful dove, a brown pigeon and a canary (Reece, personal communication).

Experimental infections

Both budgerigar and nonbudgerigar psittacine birds have been shown to be susceptible to experimental polyomavirus infections; however, the characteristic disease has only been induced in some budgerigars. Experimentally infected 3-to 10-day-old budgerigar neonates died 11 days after being given BFD virus intramuscularly. When 25-day-old budgerigars were exposed intranasally to virus collected from the skin of diseased birds, they developed microscopic lesions characteristic of a polyomavirus infection but remained clinically normal. Budgerigars of a similar age that were given the same virus preparation subcutaneously, also remained clinically normal and did not develop any gross or microscopic changes suggestive of a polyomavirus infection.

In one trial, young seronegative budgerigars seroconverted within 16 days after being placed in the same enclosure with seropositive birds. Seronegative budgerigars also seroconverted when they were placed in enclosures adjacent to those containing seropositive birds. These findings suggest that direct and indirect transmission resulting in subclinical infections can occur in budgerigars.

Avian polyomavirus isolated in cell culture from budgerigars and administered orally or intramuscularly to blue and gold macaw chicks induced infections, but the birds remained subclinical. When blue-crowned conure chicks, 40-to 50-days-old were exposed to avian polyomavirus by intramuscular inoculation, they seroconverted, shed virus intermittently for 7 to 14 days and remained clinically normal. (Ritchie, et al, unpublished data) Mature Amazon parrots, African grey parrots and cockatoos exposed to avian polyomavirus by the intramuscular or intravenous routes will seroconvert and shed virus intermittently in the feces. Birds infected by the intravenous route develop transient diarrhea 5 to 10 days after inoculation, then recover. (Ritchie, et al, unpublished data) Birds experimentally infected with liver homogenates containing avian polyomavirus have been found to respond in a similar fashion to birds that are experimentally infected with cell-culture derived virus (Ritchie, et al, unpublished data).
The effect of avian polyomavirus (BFDV) on experimentally infected chickens varies dramatically with the age of exposure. Chicken embryos infected at 10 days of age died 10 days later, and had gross and histologic lesions characteristic of the disease. In contrast, chicken embryos infected at 11 and 12 days of age remained normal, developed precipitating antibodies that could be detected 2 weeks after hatching, and did not develop gross or microscopic changes suggestive of an infection.

Two-week to four-month-old broiler and specific-pathogen-free (SPF) chickens injected with avian polyomavirus by the intramuscular or intravenous routes developed virus-neutralizing antibodies, suggesting that they had been infected. Some experimentally infected chickens developed transient diarrhea but otherwise remained clinically normal. None of the experimentally infected chickens developed gross or histologic changes suggestive of a polyomavirus infection (Ritchie, et al, in preparation).

**Age susceptibility**

Nonbudgerigar psittacine neonates are considered to be highly susceptible to polyomavirus infection and the diseases it can cause. Infections may occur in either parent-or hand-raised neonates, however, disease may be more common in hand-raised chicks. In nonbudgerigar psittacines, clinical signs are most common at the time of weaning; however, neonates from 14 to 150 days of age have been reported to be susceptible to naturally induced avian polyomavirus infections and disease. Reported mortality (death) rates vary from 31% to 41% of the at-risk young birds. However, clinical experience suggests that 80 to 100% of the young birds in some highly susceptible populations may die.

In most cases, older psittacine birds (greater than 1 month in budgerigars and greater than 5 months in nonbudgerigar psittacine birds) exposed to avian polyomavirus seroconvert and remain clinically normal. However, occasionally, adult psittacine birds may die acutely with lesions suggestive of a polyomavirus infection.

No one has determined why most adult birds exposed to polyomavirus seroconvert following infection by this virus, while some develop clinical abnormalities and die. Factors that govern the susceptibility of young and adult birds to avian polyomavirus-induced disease might include the route of virus exposure, the quantity of virus to which the bird is exposed, a pre-existing resistance to disease based on previous exposure to the virus, a selective immunosuppression in the affected birds or the occurrence of strains of polyomavirus with increased virulence for certain species.

**Incubation**

The incubation period of avian polyomavirus has not been confirmed in nonbudgerigar psittacine birds, because experimentally infected individuals do not develop the clinical signs of disease that are characteristic in naturally acquired infections. Based on clinical observation, the incubation period of polyomavirus in nonbudgerigar psittacine birds has been estimated to be as long as 14 days but may be as short as 2 days.
fledglings with naturally acquired infections show peak mortality rates between the 15th and 19th day of life, suggesting that the incubation in this species may be less than 15 days. Budgerigar neonates experimentally infected by intramuscular inoculation died 11 days after being exposed to the virus.\textsuperscript{16}

**Prevention**

Detecting nonbudgerigar psittacine birds that are subclinically infected with avian polyomavirus is difficult, at best. Neither normal appearance nor the presence of antibodies correlate in a simple manner with the likelihood that these birds will shed virus. Of 106 serum samples collected from a group of breeding nonbudgerigar psittacines, 33\% were positive (titer greater than 1:10), 20.7\% were suspect (titer of 1:10) and 46.3\% were negative (titer less than 1:10). Adults from one flock that were exposed to diseased birds seroconverted (developed antibody titers) and raised seronegative, normal young in two subsequent breeding seasons.\textsuperscript{3} In another aviary, antibody titers were detected in 10 of 15 (67\%) blood samples taken from birds ranging in age from 6 weeks old to adults.\textsuperscript{11} During a polyomavirus outbreak, 32 of 76 (42\%) of the birds in the aviary had virus-neutralizing antibody titers that ranged from 1:20 to 1:1280. All of the seropositive birds were clinically normal, yet 5 of the seronegative birds and 3 of the seropositive birds excreted polyomavirus in their feces.\textsuperscript{23}

Polyomavirus nucleic acid can be detected in cloacal swabs taken from nonbudgerigar psittacine birds during a flock outbreak.\textsuperscript{23,24} During one outbreak in a group of mixed Psittaciformes, 41 of over 200 birds of 35 different species were found to be shedding polyomavirus in their excrement during the peak of the epornitic. However, only 3 of these 41 birds were still shedding detectable quantities of virus when they were retested 60 days later, indicating that viral shedding is transient. In adult parrots naturally infected with avian polyomavirus, virus was detected in the excrement in 26\% of the birds when they were tested twice at a 4-to 6-month interval.\textsuperscript{19} Nucleic acid also has been detected in the excrement of experimentally infected psittacine chicks starting from 2 to 7 days following intramuscular inoculation.\textsuperscript{17} The recovery of viral DNA from the cloaca suggests that the virus could be shed from gastrointestinal, renal or reproductive tissues.\textsuperscript{17,23,24}

**Vaccination**

Because of the difficulty in identifying birds that are subclinically infected with avian polyomavirus, use of an effective vaccine would be the best way to prevent infections. During epornitics in mixed psittacine bird collections, infected survivors and asymptomatic birds exposed to them have been shown to develop polyomavirus -neutralizing antibodies.\textsuperscript{3,10,11} Seronegative young adult birds will seroconvert when housed adjacent to seropositive breeding adults, indicating that an antibody response does occur following natural exposure to the virus. The detection of virus-neutralizing antibodies in flocks of birds in which individuals are clinically normal suggests that many infections are subclinical.\textsuperscript{3,5,10,11,25} Collectively, these findings suggest that some exposed birds are able to mount an effective immune response. If a natural immunity to disease occurs, then it should be possible to
induce a similar protective immunologic response through vaccination. Experimental studies have indicated that vaccination does induce an immunologic response that is protective. In one study using blue and gold macaw chicks, an inactivated avian polyomavirus vaccine elicited polyomavirus-neutralizing antibodies in all the vaccinates. The induced immunologic response protected the vaccinated chicks from subsequent challenge with live virus. In other studies, an inactivated avian polyomavirus vaccine was shown to protect Amazon parrots, cockatoos, African grey parrots and chickens from infection (Ritchie, et al, in preparation).

Vaccination studies have indicated that several adjuvants including aluminum hydroxide, Acemannan, Equimmune and Permulum can be used safely in companion birds (Ritchie, et al, unpublished data). The safety and immunogenicity of avian polyomavirus vaccines, administered either intramuscularly or subcutaneously, have been evaluated in several flocks. In one study, a group of 233 mixed species Psittaciformes that ranged in age from 12 weeks old to greater than 5 years old were vaccinated. In another flock, a group of 169 adult, mixed species Psittaciformes were vaccinated. Vaccination stimulated a marked virus-neutralizing antibody response, particularly in birds that had been seronegative prior to vaccination. The results of these two trials are listed in Table 1.

Serious reactions have not been observed in any vaccinates, and the appetites and attitudes of all vaccinated birds have remained normal. Three types of reactions, yellowish discoloration of the skin, thickening of the skin or formation of a mass are expected at the site of subcutaneous vaccination. These reactions are a response to some protein in the vaccine (hopefully the polyomavirus proteins), and the changes should resolve without treatment 3 to 6 weeks post-vaccination. Similar reactions undoubtedly occur in many mammals vaccinated with products containing adjuvants, but the reactions are difficult to visualize because of the fur and thickness of the skin. In one field trial, some cockatoos and macaws experienced a heavy molt of up to 10 days duration that started 3 to 5 days after the second vaccination. It could not be determined if this molt occurred in response to vaccination, the stress associated with handling or climatic and other external factors. The molt was uneventful and appeared to have no adverse affect on the vaccinates.

The safety of a vaccine intended for widespread use has been established by performing field trials in flocks in which the virus-neutralizing antibody titers to avian polyomavirus were not determined prior to vaccination. From previous studies, it was expected that many of the birds in otherwise stable flocks would have pre-existing neutralizing antibodies. In one flock, 63% of the vaccinates were considered to have been previously exposed to avian polyomavirus because of the detection of virus-neutralizing antibodies prior to vaccination; in another flock, 26% of the vaccinates were seropositive. None of the vaccinates with pre-existing neutralizing antibodies developed an adverse reaction following vaccination. A similar vaccine was shown to be safe in naturally infected birds, even when the birds were vaccinated 5 times in a 49-day period.

It has been suggested that some of the histologic changes that occur in the kidneys of nonbudgerigarsittacine birds are caused by an immune-mediated process. However, these
observations were based on uncontrolled field cases and are not supported by experimental data. After some nonbudgerigar psittacine birds with pre-existing polyomavirus-neutralizing antibodies are vaccinated, they will develop an increase in antibody titer. Some vaccinates have been found to develop antibody titers that are extremely high (greater than 1:16,000) yet remain clinically normal, even when followed for over 2 years after vaccination. If neutralizing antibodies to polyomavirus were involved in the disease process, some of these experimentally vaccinated birds might be expected to be adversely affected, but in fact, they all remain clinically normal.26,27 Additionally, blue and gold macaw chicks, blue-crowned conure chicks, Amazon parrots and chickens that are experimentally infected with BFD virus will seroconvert and some of these individuals develop high neutralizing antibody titers (greater than 1:640).17 Experimentally infected blue and gold macaw chicks remained clinically normal 3 years after infection. Experimentally infected blue-crowned conure chicks and adult Amazon and African grey parrots remained clinically normal 18 months after infection (Ritchie, et al, in preparation).

Given the prevalence of polyomavirus infections in companion birds, as indicated by the detection of virus-neutralizing antibodies, it is noteworthy that an inactivated avian polyomavirus vaccine intended for widespread use does not cause adverse reactions in vaccinates. The guidelines provided by the manufacturer of this vaccine should be carefully followed. (Biomune, Lenexa, KS. 800-846-0230) Briefly, it is recommended that all of the birds in an aviary or home be vaccinated. This vaccination regime will help break the cycle of virus transmission by decreasing the number of new birds that are susceptible to infection. Adult breeding birds should be vaccinated twice with a 2 to 3 week interval between doses. It is best to vaccinate these birds several months before the onset of breeding. Regular boosters will be needed to maintain high levels of antibodies. Chicks should be vaccinated twice at a 2 week interval. It is important that young birds receive their last booster at least 2 weeks before they are shipped, or exposed to other birds. Companion birds are vaccinated twice with a 2 to 3 week interval between doses.

Aviculturists with large breeding facilities rarely handle or evaluate the overall health of their adults. The infrequent attention provided to these adults allows some problems such as liver disease, kidney disease, heart disease, cancers and proventricular dilatation syndrome to slowly progress in what appears to be clinically normal birds. Many of these hidden problems will be detected or exacerbated during the handling procedures necessary for vaccination. In the best managed aviaries in which adult birds are rarely handled, experience suggests that during the vaccination process pre-existing medical problems will be identified in from 2% to 4% of the adults.

As is the case with many other viral-induced diseases in companion animals, vaccination will play a pivotal role in reducing the incidence of avian polyomavirus infections. However, because no vaccine is 100% effective, vaccination should not be expected to compensate for the deleterious effects of poor management or hygiene. The techniques recommended for decreasing the occurrence of avian polyomavirus infections are listed in Table 2.
Given the extremely high prevalence of persistent polyomavirus infections, and the frequency with which budgerigars can shed the virus, one should never maintain young nonbudgerigar psittacines in the same airspace with budgerigars. The potential for intraspecies transmission of polyomavirus may be a particular problem for pet retailers that maintain both large and small psittacine birds.

**Controlling an outbreak**

For maximum security, birds vaccinated for avian polyomavirus should not be considered to be protected from infection until 2 weeks after they have received a booster. Thus, vaccination alone cannot be expected to control an outbreak. A DNA probe-based test (Avian Research Associates, Milford, OH. 513-248-4700) is extremely valuable for identifying birds that are shedding virus in their excrement during an outbreak. Birds that are shedding the virus can be separated from others in a nursery to prevent further transmission, while vaccinated birds are developing antibodies to the virus. By testing cloacal swabs of a bird at the time of death one can determine whether it is shedding virus, which in turn will help determine whether its environment may have been contaminated. If the environment is contaminated, then there is a potential for viral amplification in a susceptible population. If an infected bird dies soon after infection, it may not be shedding virus at the time of death, and thus the bird's environment may not be contaminated with virus. Birds that are clinically ill, are found to be shedding polyomavirus, or are in direct contact with birds that are clinically ill or shedding polyomavirus should be isolated (placed in a separate geographic location) from birds that are clinically normal and not shedding virus.

**ACKNOWLEDGEMENTS**

Major sustained contributions that have made this work possible have been provided by the Cowan Avian Health Foundation, the International Avian Research Foundation, Veterinary Medical Experiment Station, Richard and Luanne Porter, Terry Clyne, Knick Enterprises, Ted Lafeber, Kathleen Szabo, Avian Research Associates, Midwest Avian Research Exposition, International Aviculturist's Society, Gateway Parrot Club, Kentuckian Bird Society, Cream City Feathered Friends, Aviary and Cage Bird Society of S. Florida, Hookbill Hobbyists of Southern California, Greater Brandon Avian Society, and Zeigler Brothers Inc. Hundreds of aviculturists, bird clubs, and veterinarians have also made significant contributions. The authors thank Richard and Luanne Porter, Joel Murphy, Sue Sattler, Sue Topper, Diane and Micheal Perry, Jeanie and Scott Anderson, Bill Bennett, Britt Blanchard, Mary Ervin, Kathy Murphy, Kristen Johnson, Sherri and Aaron Jones, Will Pace, Don Sanders, Debbie Seaman, Pat Terry, Donna Travers, Bridgett Trulove, Marcus Valentine, Cynthia Webb and Diane Wolff for technical assistance in completing this project.

**LITERATURE CITED**


Table 1: Pertinent data from 2 avian polyomavirus vaccination field trials.

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of birds</td>
<td>233</td>
<td>169</td>
</tr>
<tr>
<td>Seronegative birds prior to vaccination</td>
<td>87</td>
<td>133</td>
</tr>
<tr>
<td>Seronegative birds that seroconverted</td>
<td>81 (93%)</td>
<td>126 (95%)</td>
</tr>
</tbody>
</table>
Table 2: Techniques for preventing or controlling polyomavirus outbreaks in the nonbudgerigar psittacine nursery.

Prevention

- Vaccinate susceptible adults and neonates.
- Never ship or accept an unvaccinated bird.
- Do not maintain budgerigars, cockatiels or lovebirds in the same airspace with other unvaccinated psittacine neonates.
- Clean and disinfect the nursery environment regularly.
- Ship only weaned birds.
- Use biosecure shipping containers to prevent virus exposure during transport.
- Maintain a closed aviary, and strictly limit visitations by non-aviary personnel.
- Never return a neonate to the nursery if it has been exposed to other birds.
- If new birds must be added to the flock, vaccinate and quarantine them for a minimum of 60 to 90 days.
- Never mix neonates from multiple sources in the same airspace.
- Use separate feeding instruments for each bird.
- Never use a feeding utensil and place it back in a common food container.

Control

- Isolate clinically affected birds.
- Carefully vaccinate exposed birds making sure that virus transmission is not facilitated by the handling procedure.
- Never place a clinically ill bird in the same airspace with birds in the nursery.
- Isolate birds in direct contact with clinically ill birds or those that are shedding.
- Completely clean and disinfect the nursery environment.
- Use the DNA probe to test the nursery environment for viral contamination.
MOLECULAR TECHNOLOGY AND AVIAN MALARIA IN THE BLACK-FOOTED PENGUIN

M. R. Cranfield, DVM*
Baltimore Zoo, Druid Hill Park, Baltimore, MD 21217, USA

T. K. Graczyk, Msc, PhD
Department of Molecular Microbiology and Immunology, School of Hygiene and Public Health, The Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD 21205, USA

T. F. McCutchan, PhD
Growth and Development Section, Laboratory of Parasitic Diseases, Building 4, Room B1-28, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, MD 20892-0425, USA

Introduction

Avian malaria (*Plasmodium relictum, P. elongatum*) infections are historically diagnosed by Giemsa-stained thin blood film and blood subinoculation method. Since parasitemia occurs late in the infection if at all and these time-consuming tests can produce false negatives, often there is no time for efficacious treatment and the answers to many of the fundamental questions about the pathogenesis of the parasite remain unanswered.

ELISA Test

At Baltimore, Graczyk et al. (1983) fund that anti-*P. relictum* and anti-*P. elongatum* immunoglobulins recognize the human malaria *P. falciparum* antigens R32tet32, PFR27 and crude red blood cell extract. The human ELISA therefore could be utilized to detect anti-*Plasmodium* antibodies in birds. Binding efficacy of anti-penguin IgG coupled to alkaline phosphatase was higher than other anti-bird species IgG. By examining the yolk of eggs, serial serum samples of neonates and serum samples of adults both captive and wild, several enlightening discoveries were made.

The *Plasmodium* antibodies were concentrated and passed through the yolk to offspring. Serum antibody levels in neonates were always higher than the females but without exposure decreased over time to almost 0 after three months. It is not known at this time whether these antibodies are protective. The ELISA test results have shown that all the naive penguins are infected with avian malaria within three weeks of exposure to the vector. However, in our experience only 40-60% of the birds become parasitemic and fewer are fatal cases. The antibody titers do not correlate with mortality or with parasitemia levels. The antibody levels are higher in the first two seasons and then level off to a steady state throughout the rest of the bird's life. It is known that penguins carry the parasite in the tissues for long periods of time if not a lifetime. Immunity is thought to be through premunition which would explain the continued antibody levels in a balance between the immune system and the parasite.
Because the parasite is carried for a lifetime, the positivity for malaria of wild penguins can be generated by a single exposure. When the blood of wild penguins was examined it was found that true antarctic species Adelie (*P. adeliae*) had 0% with antibody titers due to a lack of vectors. Birds that lived most of their times in cold climate but migrated through areas containing vectors had variable prevalence of antibody titers (see Table 1). Black-footed penguins from Republic of South Africa had 52% prevalence of antibodies compared to 100% in captive black-footed penguins. All yellow-eyed penguins (*Megadyptes antipodes*) from New Zealand were positive.

Table 1. The mean absorbance values (±SE) obtained at 405nm in the indirect ELISA for detection of immunoglobulins against *Plasmodium relictum* or *P. elongatum* in the penguin sera diluted 1/100 with phosphate-buffered saline.

<table>
<thead>
<tr>
<th>Penguin species</th>
<th>Sporozoite (R32tet32) <em>± SE</em></th>
<th>Gametocyte (PFR27) <em>± SE</em></th>
<th>Positive* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Spheniscus demersus</em> (n=44)</td>
<td>0.402 ± 0.034</td>
<td>0.313 ± 0.027</td>
<td>52</td>
</tr>
<tr>
<td><em>Pygoscelis papua</em> (n=12)</td>
<td>0.544 ± 0.053</td>
<td>0.391 ± 0.021</td>
<td>33</td>
</tr>
<tr>
<td><em>Aptenodytes patagonicus</em> (n=12)</td>
<td>0.488 ± 0.018</td>
<td>0.437 ± 0.021</td>
<td>58</td>
</tr>
<tr>
<td><em>Megadyptes antipodes</em> (n=5)</td>
<td>0.782 ± 0.022</td>
<td>0.661 ± 0.038</td>
<td>100</td>
</tr>
<tr>
<td><em>Spheniscus magellanicus</em> (n=7)</td>
<td>0.301 ± 0.031</td>
<td>0.241 ± 0.012</td>
<td>43</td>
</tr>
<tr>
<td><em>Eudyptula minor</em> (n=12)</td>
<td>0.402 ± 0.019</td>
<td>0.444 ± 0.021</td>
<td>92</td>
</tr>
</tbody>
</table>

* Above the cutoff level of 0.134

**Polymerase Chain Reaction Test (PCR)**

A PCR test developed at NIH for malaria utilizes genus-conserved primers, 566 and 570, 5'-GGATAACTACGGAAAAGCTGTAGC-3' and 5'CGACTTCTCCTTCTTTAAAGATAGG-3' respectively.

The application of this PCR diagnostic is unusual in that any *Plasmodium* target, regardless of species, is amplified due to the use of primers conserved among all members of the genus characterized to date (13 species). Subsequent species identification is based on the uniqueness of the *Plasmodium* small subunit rRNA hypervariable region that the "genus-conserved" primers flank and is detectable by direct sequence analysis or hybridization analysis of amplification products.
It is possible therefore to analyze a blood sample from a single bird and find multiple *Plasmodium* species or strains within one bird and their relative concentrations. The test is also capable of analyzing mosquitoes and historical blood smears. Eighteen thousand mosquitoes were collected from five sites throughout the Baltimore Zoo during the 1994 season.

Work completed to date shows that there are genetically distinct strains of morphologically identical *P. relictum*. Analysis of local *Culex* species mosquitoes in the same fashion revealed three phylogenetically distinct groups of avian *Plasmodium* within this vector in the area. One group contained sequences matching the sequence derived from the *P. relictum* model strain, providing a direct genetic link between the infected penguins and the mosquito vector, thus defining a complete transmission cycle.

The different species (*P. relictum* and *P. elongatum*) plus different strains of these wax and wane in their prevalence in the mosquito and penguin populations throughout the season. Several of the penguins, both symptomatic and asymptomatic, contained the same parasite as determined by DNA hybridization analysis. Therefore, infection in naïve animals of the same age and degree of exposure cannot fully explain the prognosis for the individual or the mortality in the population (unpublished). Further analysis of the collected data utilizing this exciting tool should answer several questions and allow for earlier diagnosis and treatment of malaria.

LITERATURE CITED

ATOXOPLASMOsis - An impEdiment to the bAli mynah (Leucopsar rothschilli) Species survival plan

Ellis C. Greiner, PhD
Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611, USA

Terry M. Norton, DVM, Dipl. ACZM
North Carolina Zoological Park, 4401 Zoo Parkway, Asheboro, NC 27203, USA

Kenneth S. Latimer, DVM, PhD
Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA

Robert Seibels
Riverbanks Zoological Park, PO Box 1060, Columbia, SC 29202, USA

Atoxoplasmosis has been implicated in the deaths of Bali mynahs being raised in the United states by captive propagation. During the past 3 years, fecal samples and buffy coat preparations from Bali mynahs from zoos and aviaries across the USA have been sent to the University of Florida for diagnosis of the etiological agent of this disease, namely Atoxoplasma sp. Individuals in 34 of the 36 facilities submitting samples had at least one positive Bali mynah. A minimum prevalence of 62% (152 positive / 245 examined) was determined based upon the presences of oocysts in the feces and / or zoites in the mononuclear phagocytes. Distribution of samples ranged from Rhode Island to Florida to California to Washington. Thus this coccidian is a common inhabitant of Bali mynahs in most facilities trying to produce young for the Bali mynah Species Survival Plan. The significance and means of control of this Atoxoplasma sp. are presently under study. Furthermore, Atoxoplasma has been seen in captive Bali mynahs in Indonesia.
NECROPSY FINDINGS IN 111 BLACK-BELLIED SEEDCRACKERS (*Pyrenestes ostrinus*) FROM THE RIVERBANKS ZOOLOGICAL PARK, 1989-1994

E.W. Howerth, DVM, PhD*, B.G. Harmon, DVM, PhD, K.S. Latimer, DVM, PhD, R.P. Campagnoli, MS, M.E. Dorminy, MS
Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA

T.M. Norton, DVM
Riverbanks Zoological Park, Columbia, SC 29209, USA. Current address: North Carolina Zoological Park, Asheboro, NC 27203, USA.

A breeding colony of black-bellied seedcrackers (*Pyrenestes ostrinus*) was established at the Riverbanks Zoological Park with two shipments of wild caught birds from the Congo, West Africa. The first shipment arrived in 1985 and consisted of 27 birds; the second shipment arrived in 1989 and consisted of 57 birds. Prior to 1990, extensive post mortem examinations were not performed, thus early losses were not well documented. Adult mortality became more frequent in 1991, and the colony was depopulated in 1994. The objective of this study was to correlate the necropsy findings in birds submitted from the colony to the Department of Pathology, College of Veterinary Medicine, University of Georgia, from 1989 through depopulation in 1994.

The necropsy records of 111 black-bellied seedcrackers were used for this study. Fifty-nine of these birds either died or had clinical signs that necessitated euthanasia; 52 of the birds were killed during depopulation of the colony. The original tissue sections were reexamined only if the original diagnosis was in doubt. In some cases, special stains, immunohistochemistry, *in situ* hybridization or electron microscopy were performed to clarify or substantiate a diagnosis. Results of cytology records were utilized when they were contributory to a diagnosis.

The most common diseases observed in this colony were: mycobacteriosis; atoxoplasmosis; amyloidosis; and intestinal coccidiosis (Table 1). Diseases and conditions seen less frequently were: polyomavirus infection (5.4 %); sepsis (5.4%); hepatic lipidosis (4.5%); visceral urate deposition (4.5%); soft tissue mineralization (3.6%); proventricular cryptosporidiosis (2.7%); candidiasis (1.8%); sarcocystosis (1.8%); and myodegeneration (9%).

Mycobacteriosis was the most common disease observed (Table 1); 22 of 34 (64.7%) of birds with mycobacteriosis were identified when the colony was depopulated. Concurrent diseases were observed in 85.3% of the birds with mycobacteriosis and included: amyloidosis; intestinal coccidiosis; atoxoplasmosis; soft tissue mineralization; hepatic lipidosis; proventricular cryptosporidiosis; sepsis; myodegeneration; polyomavirus infection; and oophoritis (Table 2). Birds of known age with mycobacteriosis ranged in age from 9 mo to 8 yrs (average age, 4.2 yrs).

Atoxoplasmosis was the second most commonly observed condition (Table 1). Seventeen of the 31 birds infected with *Atoxoplasma* sp. were identified during depopulation. Asexual
stages of *Atoxoplasma* sp. were identified in all 17 of these birds by cytologic examination of liver and spleen imprints; only one of these birds had identifiable asexual stages in formalin fixed tissues. In contrast, 10 of 11 birds that were diagnosed with atoxoplasmosis prior to depopulation had identifiable asexual stages of the organism in formalin fixed tissues. The age range of birds with *Atoxoplasma* infections was 9 mo to 7 yrs (average age, 3.3 yrs).

Amyloidosis was common (Table 1). Liver involvement occurred in 26 of the 28 cases (92.9%). Other organs were involved less frequently and included: spleen (6/28;21.4%); kidney (2/28;7.1%); lung (1/28;3.5%); proventriculus (1/28;3.5%); and intestine (1/28;3.5%). 85.7% of the birds with amyloidosis had concurrent inflammatory conditions (Table 2). Birds with amyloidosis ranged in age from 1 yrs to 8 yrs (average age, 4.6 yrs).

Intestinal coccidiosis was common both prior to and during depopulation (Table 1). Birds with intestinal coccidiosis ranged in age from 1 mo to 7 yrs. The species of coccidia was not determined in any of the cases. Only 11 of the 27 birds with intestinal coccidiosis (44.4%) had asexual stages of *Atoxoplasma* in visceral organs.

Acute polyomavirus infection was diagnosed by light microscopy in 6 of the birds; the first 2 cases were seen in 1989. These birds ranged in age from 2 mo to >5 yrs. One bird had concurrent mycobacteriosis; two birds had amyloidosis. Proventricular cryptosporidiosis was observed in 3 birds killed during depopulation. These birds ranged in age from 6 yrs to 10 yrs. Microscopically the organisms were associated with epithelial hyperplasia. Two birds had sarcocysts in skeletal muscle. Both birds were adult females; there was no evidence of active disease (sarcocystosis).

Mycobacteriosis, atoxoplasmosis, amyloidosis, and intestinal coccidiosis were the most common diseases identified in this colony, and many of the birds killed during depopulation had these diseases. *Atoxoplasmosis* infections in birds killed during depopulation were identified primarily by cytologic examination of tissue imprints, and asexual stages of the organism and evidence of active disease were rarely seen in tissue sections. This suggests that most of the infected birds killed during depopulation were carriers and not experiencing active disease (atoxoplasmosis). Amyloidosis usually involved the liver and was usually associated with a concurrent inflammatory condition suggesting that the amyloid was type AA. The species of intestinal coccidia was not determined; however, less than 50% of the cases had asexual stages of *Atoxoplasma* sp. in visceral organs suggesting that most of the cases of intestinal coccidiosis were due to a primary intestinal coccidia and not due to *Atoxoplasma* sp. Proventricular cryptosporidiosis, identified during depopulation only, probably was not a significant morbidity or mortality factor in this colony. Acute polyomavirus infection was not a significant mortality factor despite being present in the colony since 1989. Although there were sarcocysts in skeletal muscle of 2 birds, there was never any indication of acute sarcocystosis in the colony.
TABLE 1. MAJOR DISEASES IN 111 BLACK-BELLIED SEEDCRACKERS

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>DIED OR HAD CLINICAL SIGNS</th>
<th>KILLED DURING DEPOPULATION</th>
<th>TOTAL BIRDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYCOBACTERIOSIS</td>
<td>12 (10.8%)</td>
<td>22 (19.8%)</td>
<td>34 (30.6%)</td>
</tr>
<tr>
<td>ATOXOPLASMA INFECTION</td>
<td>14 (12.6%)</td>
<td>17 (15.3%)</td>
<td>31 (27.9%)</td>
</tr>
<tr>
<td>AMYLOIDOSIS</td>
<td>17 (15.3%)</td>
<td>11 (9.9%)</td>
<td>28 (25.2%)</td>
</tr>
<tr>
<td>INTESTINAL COCCIDIOSIS</td>
<td>19 (17.1%)</td>
<td>8 (7.2%)</td>
<td>27 (24.3%)</td>
</tr>
</tbody>
</table>

1 Number of birds with disease; (% with disease).
TABLE 2. CONCURRENT DISEASES IN BLACK-BELLIED SEEDCRACKERS WITH MYCOBACTERIOSIS AND AMYLOIDOSIS

<table>
<thead>
<tr>
<th>CONCURRENT DISEASE</th>
<th>MYCOBACTERIOSIS</th>
<th>AMYLOIDOSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUBERCULOSIS</td>
<td>NA</td>
<td>12/28 (42.9%)²</td>
</tr>
<tr>
<td>AMYLOIDOSIS</td>
<td>12/34 (35.9%)²</td>
<td>NA</td>
</tr>
<tr>
<td>INTESTINAL COCCIDIOSIS</td>
<td>11/34 (32.4%)</td>
<td>7/28 (25%)</td>
</tr>
<tr>
<td>ATOXOPLASMA INFECTION</td>
<td>6/34 (17.6%)</td>
<td>6/28 (21.4%)</td>
</tr>
<tr>
<td>MINERALIZATION</td>
<td>4/34 (11.8%)</td>
<td>3/28 (10.7%)</td>
</tr>
<tr>
<td>HEPATIC LIPIDOSIS</td>
<td>2/34 (5.9%)</td>
<td></td>
</tr>
<tr>
<td>PROVENTRICULAR CRYPTOSPORIDIOS</td>
<td>1/34 (2.9%)</td>
<td>1/28 (3.6%)</td>
</tr>
<tr>
<td>SEPSIS</td>
<td>1/34 (2.9%)</td>
<td>2/28 (7.1%)</td>
</tr>
<tr>
<td>MYODEGENERATION</td>
<td>1/34 (2.9%)</td>
<td></td>
</tr>
<tr>
<td>OOPHORITIS</td>
<td>1/34 (2.9%)</td>
<td></td>
</tr>
<tr>
<td>CANDIDIASIS</td>
<td>1/34 (2.9%)</td>
<td>1/28 (3.6%)</td>
</tr>
<tr>
<td>POLYOMAVIRUS</td>
<td>1/34 (2.9%)</td>
<td>2/28 (7.1%)</td>
</tr>
<tr>
<td>MISCELLANEOUS INFLAMMATION</td>
<td></td>
<td>4/28 (14.3%)</td>
</tr>
</tbody>
</table>

¹ NA=Not applicable
² Number of birds with concurrent disease/Number of birds with mycobacteriosis; (% with concurrent disease)
³ Number of birds with concurrent disease/Number of birds with amyloidosis; (% with concurrent disease)
POSSIBLE ALBENDAZOLE TOXICITY IN BIRDS

Ilse H. Stalis, DVM, Bruce A. Rideout, DVM, PhD
Department of Pathology, Zoological Society of San Diego, PO Box 551, San Diego, CA 92112-0551, USA

Jack L. Allen, DVM
Department of Veterinary Services, Wild Animal Park, 15500 San Pasqual Valley Rd., Escondido, CA 92027, USA

Meg Sutherland-Smith, DVM
Department of Veterinary Services, San Diego Zoo, San Diego, CA 92112-0551, USA

Albendazole is used occasionally at the San Diego Zoo and Wild Animal Park to treat parasitism in a variety of bird species. It has been useful for treating capillariasis as we have found it to be more effective than other anthelmintics against *Capillaria*. Because of several recent deaths in groups of healthy birds treated with albendazole, we suspect that albendazole may be toxic to some species of birds.

Species where albendazole toxicity is suspected include keas (*Nestor notabilis*), southern speckled pigeons (*Columba guinea phaenota*) and pink spotted fruit doves (*Ptilinopus perlatus perlatus*). In all species an 11.36% suspension of albendazole (Valbazen® SmithKline Beecham Animal Health, West Chester, PA 19380, USA) was used. Three keas were treated at 100mg/kg SID by gavage for 7 days. Twenty-four pigeons were treated at 100mg/kg once by gavage followed by 100mg/kg on the food for 3 days. Nine days later the birds were treated with albendazole at 50mg/kg by gavage once, followed by 50mg/kg on the food for 4 days. One pink spotted fruit dove was treated at 100 mg/kg by gavage for 3 days.

The 3 keas died 9 and 10 days and the fruit dove died 10 days after the start of treatment. Two of the speckled pigeons died 4 days after the start of treatment, 1 died 5 days after the start of treatment and 8 died 12-16 days after the start of treatment (these 8 had received the second gavage dose). At necropsy the keas and speckled pigeons had lost 6 to 24% of body weight, despite normal food intake. CBCs obtained on 2 speckled pigeons that died revealed leukopenia with a marked heteropenia. Characteristic lesions in affected birds included bone marrow depletion and systemic acute bacterial and/or fungal infections, usually with little inflammatory cell response. In other species albendazole toxicity causes bone marrow depression, leukopenia, and overwhelming infections. (V. Theodorides, personal communication)

Albendazole had not been used previously in speckled pigeons or pink spotted fruit doves. Other keas had been previously treated at 50mg/kg (probably on the food) with no adverse effect. Although the southern speckled pigeons were also treated with ivermectin at the same time as the first dose of albendazole, the other birds received no additional drugs.

Possible albendazole toxicity appears to be a species specific phenomenon or related to the dose. Albendazole has been used successfully in 5 red-eyed doves (*Streptopelia semitorquata*)
(50mg/kg once by gavage then SID on the food x 4 days) and 10 Philby's rock partridges 
(*Alectoris philbyi*) (50-100 mg/kg PO SID x 3-10 days).

Our results are not conclusive, but based on the histories and necropsy findings we suggest 
that albendazole may be toxic to some species of birds and should be used with caution. 
At this point we do not know if a lower dose of albendazole would be safe and effective.
LONG BONE FRACTURE MANAGEMENT IN SANDHILL CRANES: A CASE REPORT

Patrice N. Klein, MS, VMD, Dip. ACVÆ, Dorothy Thompson
Paxuxent Wildlife Research Center, National Biological Service, Laurel, Maryland 20708, USA

Introduction

Long bone fractures in Gruiformes are not imminently fatal, however medical management of these fractures is often unsuccessful due to complications and debilitation from long term restraint. This report describes the successful management and recovery of an endangered sandhill crane with a femoral fracture through a program of incremental physical therapy beginning two weeks post femoral fracture repair. A progressive regimen of assisted walking techniques, hydrotherapy, increasing exercise periods, and gradual reduction in support devices led to the achievement of independent locomotion and recovery to full musculoskeletal function for this bird.

Case Report

A 65 day old, juvenile Mississippi Sandhill Crane (Grus canadensis pulla) sustained a spiral fracture of the right mid-diaphyseal femur in a handling accident. A standard orthopedic procedure for internal fixation using a single intramedullary pin and full cerclage wire was performed to stabilize the fracture site. The bird recovered from surgical anesthesia and was placed in a suspended support sling. One week post-operatively, the bird escaped overnight from the support sling and sustained oblique fractures of the left distal ulna and radius. Surgical reduction of these fractures and internal fixation of the distal ulna was performed using an intramedullary pin and full cerclage wire. A figure-8 support bandage was placed to secure the left wing. Consequently, a remodeled, adjustable, suspended sling was constructed to provide stable reinforcement. The frame was constructed of 1 X 1 vinyl-coated wire (40in.X 24in.X 30in.) with reinforcing diagonal steel poles. The front end had a cut-out section to provide placement of a feed tray. The canvas sling was supported by metal dowels running lengthwise which could be adjusted to the appropriate height. Cloth ties woven through the canvas sling secured the bird in position.

Beginning two weeks post femoral fracture repair, and continuing for six weeks, a program of physical therapy was initiated to address the debilitation due to muscle atrophy resulting from protracted restriction. Initially, the bird presented with severe paresis of the skeletal muscles of both legs with uncoordinated movement and moderate digital flexor tendon contraction. Graduated periods of daily "sling walking" followed by incorporation of daily periods of hydrotherapy enabled the bird to progress to "harnessed walking" and gradual reduction in support devices. Consecutive radiographs documented substantial femoral fracture repair by four weeks and the femoral intramedullary pin was removed. The bird gained sufficient musculoskeletal strength at five weeks to sustain 10-12 hours per day of partially assisted walking exercise. The support sling was used only overnight until the bird was capable of standing up unassisted.
The following table summarizes the type and duration of therapy used in the management of skeletal muscle atrophy throughout the long bone fracture recovery period:

<table>
<thead>
<tr>
<th>week in recovery</th>
<th>physical therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3</td>
<td>Brief sling walking daily</td>
</tr>
<tr>
<td>3</td>
<td>Sling walking with hydrotherapy twice daily.</td>
</tr>
<tr>
<td>3-4</td>
<td>Sling walking, hydrotherapy, and brief assisted walks.</td>
</tr>
<tr>
<td>4-5</td>
<td>Harnessed walking with hydrotherapy twice daily; incremental walking periods to include assisted hock sitting.</td>
</tr>
<tr>
<td>5</td>
<td>12 hours per day of partially assisted harnessed walking with support sling overnight.</td>
</tr>
<tr>
<td>6</td>
<td>Discontinued use of support sling: overnight monitoring to assist bird.</td>
</tr>
<tr>
<td>7</td>
<td>Discontinued harness support: 24-hour monitored but unassisted locomotion.</td>
</tr>
<tr>
<td>8</td>
<td>Returned to assigned pen.</td>
</tr>
</tbody>
</table>

Discussion

In our experience, successful recoveries of cranes with femoral fractures are rare. Muscle atrophy, restraint intolerance with secondary trauma and food refusal, and stress related opportunistic infections complicate the recovery efforts. Partial success may be attributed to the use of environmental and sensory stimuli to support the psychological well-being of this bird. However, the use of an incremental physical therapy program is the pivotal component to the effective medical management of long bone fractures in gruiformes.

ACKNOWLEDGMENTS

The authors thank the PWRC caretakers, volunteer staff, and Dr. G. Olsen for their assistance.

LITERATURE CITED

MANAGEMENT OF SUBSTRATE IN AVIARIES TO REDUCE EXPOSURE TO FUNGUS AND MOLD

Bonnie L Raphael, DVM, Dipl. ACZM*, Christine Sheppard, PhD, Don Bruning, PhD, Eileen Maher, MS, and Patti Cooper

Department of Clinical Studies, Wildlife Health Sciences, Department of Ornithology, Wildlife Conservation Society, Bronx, NY 10640, USA

Introduction

Fungal infections in aviary birds are difficult to diagnose and treat once established. Factors which can influence or predispose birds to contracting fungal diseases such as aspergillosis are: high numbers of fungal/mold elements in the environment; poor air circulation; high humidity without air turnover; too dry an environment; improper substrate or bedding management; chronic stress; immunosuppression; age and species of birds.1-5

For many bird exhibits in zoos some sort of soft substrate is used on the floor or bottom of enclosures. In addition to offering a pleasing aesthetic experience for the zoo visitor, a soft substrate allows ground dwelling birds the opportunity to perform natural behaviors such as dust bathing, scratching for insects, and building nests and helps to prevent feet problems. Even in off-exhibit areas, particularly those set up for breeding, some sort of bedding may be used, especially with ground dwelling birds such as pheasants, ground pigeons, ducks and rails. Substances used for ground covering are usually chosen for their appearance, the ease with which they can be used and cost without much attention being paid to whether the substrate might be a significant biological contaminant.

Because aspergillosis is a significant cause of death in captive birds, a project was undertaken to document the relative levels of fungus and mold in: 1. enclosures with different kinds of cage furnishings, 2. different types of substrates prior to putting them into enclosures and, 3. enclosures undergoing different treatments.

Methods and materials

A method of quantifying the amount of fungus per unit of substrate is performed as follows: 10 grams of substrate sample is suspended in 90 grams of stock solution. The stock solution is sterile distilled water to which a surfactant, Triton X-100 (Sigma Co., St. Louis, Missouri 63178), has been added to make a 0.01% concentration. Six serial dilutions are made and 1cc of each dilution was plated out on a Sabouraud-dextrose agar (BBL, Becton Dickinson, Cockeysville, Maryland 21030) to which 40ug chloramphenicol/ml media has been added. The plates are incubated at 37°C for 24 hours and then all colonies on the plates are counted. Periodically, samples of colonies on several plates are examined microscopically to verify that the colonies are fungal. Subsequent to the 1st two trials, only 3 dilutions per sample are plated.
Samples were initially collected weekly from 3 sites in each cage. Over the course of 6 months, areas within several types of enclosures were identified as heavily contaminated and some changes, such as removing planter areas, were made. Additionally, some cages were treated with an antifungal agent, Mertect 340-F (MSD AGVET, Merck & Co., Raleigh, North Carolina, 27609 USA) then sampled weekly to determine if fungal growth was retarded. Subsequent to that, all of the old substrate (shredded bark) was removed from enclosures and replaced with either red cedar or dry hardwood shavings. Monitoring of cages continued and as a final treatment, spraying enclosures with Mertect was compared to periodic partial replacement of substrate.

Results

Results of culturing substages straight out of packaging or from storage piles were as follows (reported as colonies of fungus/gram substrate):

- Shredded bark/mulch (bulk/outside storage) >1,000,000
- Eucalyptus mulch (bag) >1,000,000
- Pine bark mulch (bag) 150,000
- Pine bark nuggets (bag) 37,000
- Hard wood shavings (bag) 18,800
- Aspen Lab grade shavings (bag) 3,400
- Red Cedar shavings (bag) 2,167

When planters were removed from enclosures, fungal counts dropped significantly. Enclosures which contained a variety of natural materials to be used for nesting had significantly higher fungal counts regardless of the substrate used. The red cedar fungal counts remained at lower levels longer than hard wood shavings. Spraying the substrates with Mertect at 3 week intervals helped suppress fungal levels on all substrates.

Mertect is a fungicidal containing thiabendazole intended for use on vegetables and fruits for human consumption. It has been used safely and successfully in poultry rearing facilities to reduce environmental fungus. At present it is being used at the WCS in a propagation building, and in selected exhibits. When used in the exhibits, it is applied after replacement substrate is added to worn areas, rather than spraying an entire enclosure at once.

Successful bedding management requires that substrate not get damp or wet. Cedar and pine are considered to be the best woods to use as substrate as they are somewhat fungistatic. However, any damp shavings are good media for fungal growth, especially when warm. When shavings have previously been wet and then dry out, fungus sporulates and can be easily aerosolized. Substrate that becomes damp should be removed from enclosures as soon as possible. Spraying substrate with a thiabendazole fungicidal agent is recommended in areas that cannot be kept dry, in areas where a highly contaminated substrate is used, or in areas where it isn’t feasible to completely replace substrate at 3-4 week intervals.
ACKNOWLEDGMENTS

The authors thank Sue Maher and the Department of Ornithology for assistance

LITERATURE CITED

A virus with characteristics of the family Paramyxoviridae has been reported as the causative agent in multiple epizootics of proliferative pneumonia of captive snakes in both the United States and Europe. In 1976 the first suspected paramyxovirus of reptilian origin was isolated from a fer-de-lance viper (*Bothrops moojeni*) that died during an epizootic at a serpentarium in Zurich, Switzerland in 1972. While most of the documented cases of ophidian paramyxovirus (OPMV) infection involved viperid snakes, isolates also have been obtained from colubrids, boids, and elapids. Still, viperidae seem to be more significantly affected than snakes in other families. Recent studies of three isolates within my laboratory have supported the categorization of these isolates into the subfamily Paramyxovirinae based upon morphologic appearance, physico- and biochemical properties, and cytopathologic effects. These isolates were found to be serologically unrelated to known avian and mammalian members of this group. An immunoperoxidase and fluorescent antibody staining technique have been developed, using a rabbit polyclonal anti-OPMV antibody, so that viral antigen can identified in tissue section. A hemagglutination-inhibition assay has been developed to determine exposure to OPMV by measuring OPMV-specific antibodies. Since January 1994, 218 samples from 22 zoological and private collections have been evaluated and 44 (20%) of the snakes were considered sero-positive. In several collections active infections were demonstrated by either increased titers in paired samples taken over a 1-month period and/or demonstration of supportive histopathology/immunohistochemistry and viral isolation. In a transmission study, 5 Aruba Island Rattlesnakes, *Crotalus unicolor*, were challenged with an OPMV isolate obtained from a dead Aruba Island Rattlesnake. Snakes were challenged intratracheally and euthanatized at post-challenge days 4, 8, and 15 days; two snakes died at days 19 and 22. An additional snake served as a control for normal pulmonary histopathology and ultrastructure. Inflammatory changes could be seen as early as post-challenge day 4, with a progression of changes over time. Viral antigen was identified in lung using immunoperoxidase staining and immunofluorescence and virus was re-isolated in cell culture, thus confirming Koch's postulates.
Green Turtle Fibropapillomatosis (GTFP), characterized by multiple benign fibroepithelial tumors, is a worldwide threat to populations of endangered green turtles (*Chelonia mydas*). Transmission experiments have demonstrated that the etiology of GTFP is a filterable infectious agent, most probably a virus and have ruled out the direct involvement of spirorchid trematode ova in pathogenesis. A herpesvirus has been identified in experimentally induced and spontaneous GTFP. Experiments to fulfill Koch's postulates for the herpesvirus identified in this study require a source of purified virus. While our ongoing attempts to culture the GTFP-associated herpesvirus *in vitro* have not yet been successful, extraction of virus directly from infectious GTFP material by isopycnic ultracentrifugation gradients has yielded a fraction containing particles consistent in morphology and density to herpesvirus and no evidence for other viruses. Preliminary transmission experiments with this fraction have been started and DNA extracted from this material is being characterized. Meanwhile indirect evidence in support of a herpesvirus etiology has been obtained by serology. Using monoclonal antibodies to detect turtle IgY and IgM antibodies against this GTFP-associated herpesvirus, paired pre- and 1-year post-experiment plasma samples from all transmission experiment turtles were tested. All 12 transmission positive (GTFP positive) turtles demonstrated herpesvirus sero-conversion. Control and transmission negative turtles did not sero-convert. Similarly, a serologic survey of free-ranging turtles with and without spontaneous GTFP also revealed a strong statistically significant association between herpesvirus sero-reactivity and fibropapillomatosis (100% of 20 GTFP affected, 10% of 20 GTFP-free turtles). Development of practical molecular and immunological diagnostic tests for subclinical GTFP also requires purified viral antigens or viral genes. Preliminary comparisons of mRNA expression between normal and tumor derived cell lines reveals several differences, one or more of which may be viral gene products. Thus substantial and rapid progress that has been made toward understanding GTFP and several lines of active experimentation are focusing on proving or disproving that herpesvirus is the GTFP agent. This body of work will eventually lead to the means to monitor, prevent, and/or control epizootics in free-ranging green turtles.
AN UPDATE ON OPHIDIAN CRYPTOSPORIDIOSIS

Michael R. Cranfield, DVM*
Baltimore Zoo, Druid Hill Park, Baltimore, MD 21217 and School of Medicine, Johns Hopkins University, Baltimore, MD 21205, USA

Thaddeus K. Graczyk, MSc, PhD
Department of Molecular Microbiology and Immunology, School of Hygiene and Public Health, The Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD 21205, USA

Introduction

The apicomplexan genus Cryptosporidium contains 6 species1 that have been identified in numerous hosts including mammals, birds, reptiles, and fishes.2 The first complete report on Cryptosporidium in snakes was provided by Brownstein et al at the Baltimore Zoo.3 The name Cryptosporidium serpentis was assigned to the reptile-derived isolates by Levine (1980)4 and since that time multiple studies have shown that extensive pathologic changes in the snake's gastric region can occur in association with cryptosporidiosis.1 Cryptosporidial infections in snakes can cause considerable morbidity and can limit longevity within an ophidian collection.5

Due to low sensitivity of routine diagnostic methods, various clinical manifestations of the disease and the lack of efficient pharmacological treatments, institution policies range from a "head in the sand" approach to euthanasia in an attempt to create Cryptosporidium-free breeding populations. Based on present knowledge of the disease both extremes are wrought with potential dangers. A better approach would be to screen fecals on a regular basis to know prevalence and to conduct post-mortems to know morbidity/mortality. With this data, educated discussions on a species by species and institution by institution basis could be attempted.

Life Cycle and Transmission

The life cycle of human, calf, and presumably snake isolates of Cryptosporidium differs somewhat from that of coccidia (Eimeria and Isospora spp.). Each intracellular stage of Cryptosporidium occurs within a parasitophorous vacuole confined to the microvillous region of the host cell whereas comparable stages of Eimeria or Isospora spp. occupy parasitophorous vacuoles usually deep (perinuclear) within host cells. Oocysts of Cryptosporidium sporulate while they are within the host cells and are infective when released in the feces, whereas oocysts Eimeria or Isospora spp. do not sporulate until they are passed from the host and exposed to oxygen at temperatures below 37°C.

Using infected mice, it was found that approximately 20% of the oocysts of Cryptosporidium within host enterocytes do not form a thick, two-layered, environmentally resistant oocyst wall; the four sporozoites in this autoinfective stage are surrounded only by a single-unit
membrane. Soon after the sporozoites are released from the host cell, the membrane that surrounds the four sporozoites ruptures and these invasive forms penetrate into the microvillus region of other enterocytes reinitiating the life cycle. Most (approximately 80%) of the oocysts of *Cryptosporidium* sp. are similar to those of *Eimeria* and *Isospora* spp. in that they develop thick, environmentally resistant oocyst walls and are passed in the feces.

The thick-walled oocysts infect a new host. However, the presence of autoinfective, thin-walled oocysts and merozoites that can "recycle" are believed to be responsible for the severe infections in hosts exposed to a small dose and life-threatening disease in immune-deficient persons who do not have repeated exposure to these environmentally resistant oocysts.

Transmission is by the fecal oral route either directly or on contaminated objects. Recent studies at the Baltimore Zoo revealed that dosing fecal negative snakes with 2,000 oocysts via stomach tube produced fecal shedding or infection in approximately 70% of the specimens. The prepatent period ranged from 12 to 28 weeks post-infection. Snakes in our situation shed $0.4 \times 10^4$ oocysts/ml to $1.4 \times 10^5$ oocysts/ml (mean of $5.0 \times 10^4$ oocysts/ml) which is low numbers when compared to $8.0 \times 10^9$ oocysts/ml in bovine and $7.4 \times 10^6$ oocysts/ml in cats.

*Cryptosporidium* is not species specific but doesn’t appear to cross between reptiles and birds or mammals. Due to the transient nature of infection in mice *C. muris* does not appear to be a highly potential source of infection. The best disinfectants to reduce transmission on inanimate objects are 5% ammonia and 10% formal saline.

**Clinical Syndromes**

1. *C. serpentis* often causes long-term asymptomatic shedders in snakes which differs from *C. muris* and *C. parvum* in mammals that induces symptoms and then clears in immunocompetent individuals. Snakes can carry and shed the organism for decades as shown by a Trans Pecos rat snake in Baltimore that shed oocytes irregularly for 20 years. We observe this syndrome in 98% of our positive snakes.

2. A wasting syndrome characterized by gastric swelling and regurgitation.

The second syndrome is rare in most institutions but is felt to be more prevalent in some snake species. Others feel that stress and poor status of the immune system caused by concurrent disease, such as a virus or poor management, may cause the difference between the syndromes seen in one individual compared to another. A third opinion of which Baltimore agrees feels that perhaps different strains of morphologically similar *C. serpentis* are responsible for these different syndromes.
Treatment

The following drugs have been used with varying success:

1. Amprollium
2. SMZ
3. Paromomycin
4. Spiromycin
5. Halofuginone

At Baltimore, two studies were carried out with spiromycin and halofuginone. Halofuginone at either 1mg/kg every day or 0.5mg/kg every other day for three days caused sublethal histological toxic changes in the liver and kidneys and failed to clear the snakes of crypto. Spiromycin at 80mg/kg for three treatments every day caused a 60-100% reduction in fecal shedding and a clinical improvement in body condition but also failed to clear the organism as shown by stomach biopsy and later increase in fecal shedding.

The drug was given in beef baby food, and this and increases in environmental temperature may have aided the snake’s immune system to reduce the number of organisms shed. There have not been any controlled documented cases of clearing the organism with pharmacological treatment.

Diagnosis

One of the major drawbacks in studying and managing the disease is the lack of a definitive ante-mortem test to establish Cryptosporidium-free snakes to utilize as negative controls for scientific studies or the establishment of Cryptosporidium-free breeding populations.

The following ante-mortem tests are routinely used:

1. Fecal examination for the presence of the organism,
2. Stomach biopsy which is easily accomplished by entering the abdomen at the half mark between the head and the cloaca,
3. Examination of regurgitated prey for the presence of the organism,
4. Gastric lavage and examine for the presence of organisms,
5. A barium series showing gastric lumenal reduction or blockage in snakes with mid-body swelling and regurgitation.

A positive diagnosis is definitive, but a negative, even a repeated negative, is not definitive that the animal is Cryptosporidium-free. Rarely a negative stomach biopsy and post-mortems have been seen in shedding snakes. It is felt that the distribution of the organism is not uniform throughout the stomach mucosa and in light infection may be missed on histology.
In a high majority of the snakes for whose fecal shedding was monitored over years, it was found that they could be intermittent shedders with erratic periods of non-shedding. This means that positive non-shedding animals can be easily missed by fecal examination.

The accuracy of fecal, regurgitated prey mucus, and gastric lavage material is increased by the utilization of a modified acid fast stain. The *C. serpentis* organisms mean measurements are 6.3 x 5.5\(\mu\)m and stain red.

A recent study at Baltimore showed that the human commercial Meriflour™ monoclonal antibody test to identify cryptosporidia and giardia cross reacts with *C. serpentis*. It therefore can be utilized for direct immunofluorescence detection of *C. serpentis* in fecal or regurgitated material.

The sensitivity of the test is 16-fold greater than the modified acid fast test and therefore a very effective for screening collections. The test however is not species specific and therefore will not differentiate between reptilian organisms and mammalian organisms infecting prey species and just passing through the reptile’s digestive tract. The cost of the kit is $290 for 50 samples and requires a fluorescence microscope. ProSpect™, a newly developed cryptosporidia test developed for humans utilizes different antibodies. Early testing at Baltimore reveals that these antibodies do not appear to cross-react with *C. serpentis* antigens. Screening with both of these tests will increase the sensitivity and specificity.

**Pathology**

Consistent pathologic changes in snakes with cryptosporidiosis are limited to the stomach and vary in severity. Grossly, the lesion range from morphologically normal to an increase in the diameter of the stomach, with reduction in the gastric luminal diameter. The gastric mucosae of affected snakes may be edematous, with mucosal thickening and exaggeration of the normal longitudinal rugae, to which copious mucus is adhered. Mucosal petechiae, brush hemorrhages and focal necrosis are common.

In the normal snake stomach, the mucosal surface has tall columnar epithelium with microvilli that stain in the apical region with alcian blue, indicating the presence of acid mucopolysaccharides. Identical cells line the gastric pits. The gastric glands are well demarcated by mucous neck cells that stain uniformly with periodic acid-Schiff stain (PAS), which indicates neutral mucopolysaccharides. The remaining gastric glands are composed of cells with eosinophilic granular cytoplasm. These cells, termed granular cells, are combined chief and parietal cells.

Mildest pathologic changes in *Cryptosporidium* spp.-affected snakes consist of hyperplasia of mucous neck cells with abundant neutral mucopolysaccharides filling the gastric pits and glands and adhering to surface epithelium. Focal atrophy of granular cells with replacement by mucous neck cells is appreciated. In the columnar surface and pit epithelium, which stains uniformly for acid mucopolysaccharides in the normal snake, special stains indicate
alternating areas of acid and neutral mucopolysaccharides. There is edema of the lamina propria and submucosa; infiltration of plasma cells and lymphocytes with scattered heterophils is seen.

Many spherical to ovoid organisms are adherent to microvillar borders of surface, pit, and glandular epithelium. These are best seen in toluidine-blue-stained sections of plastic embedded tissues. Schizonts, 2.5 to 3.0 micrometers in diameter, have four to eight filiform merozoites and prominent narrow zones of attachment to microvillar surfaces. Trophozoites are 1.6 to 2.0 micrometers in diameter and have uniformly stained cytoplasm and a prominent nucleus. Macrogametes resemble trophozoites but have many metachromatic round to oval bodies within the cytoplasm.

Developmental stages of the parasite cannot be readily distinguished in paraffin-embedded tissues. Most organisms have foamy pink cytoplasm with distinct basophilic nuclei when stained with hematoxylin-eosin; cytoplasm stains light blue with azure-eosin. Occasional intracytoplasmic structures stain intensely with PAS. The organisms are Gram-negative.

Moderate changes attributable to reptilian cryptosporidiosis consist of complete replacement of granular cells by cuboidal to columnar epithelial cells with staining characteristics of mucous neck cells, as well as by surface-type epithelia. The mucous neck cells tend to occur in nests around pits that have formed in the hyperplastic epithelium. There is less intracellular mucopolysaccharide in the surface type of cell than in normally-staining mucous cells. There is much extracellular mucopolysaccharide. Cysts lined with low columnar to cuboidal cells frequently occur in the hyperplastic mucosa. Fibroplasia and collagenization of the lamina propria and submucosa usually are associated with an intense inflammatory response characterized by plasma cells, lymphocytes, and heterophils, as well as with edema. Cuboidal metaplasia of luminal epithelium is usually apparent at this stage.

In the most severe changes which occur in snakes, there is superimposition of mucosal necrosis on epithelial hyperplasia. Mucosal necrosis is often multifocal and contains many Gram-negative bacteria. When necrosis is confined to the mucosa, the inflammatory response is almost entirely of epithelioid cells. There is extension of this necrotic process into the submucosa of the stomach which induces an intense heterophilic response with abscess formation and edema. The protozoa are attached to the microvillar surface of gastric epithelium and are surrounded by a double membrane that is continuous with host plasma membrane.

Zoonosis

Unlike coccidiosis, cryptosporidiosis is not species specific and appears to be able to infect all animals within a taxonomic class. A recent study at Baltimore dosing neonatal mouse pups with C. serpentis did not result in infection when later examined histologically. This would suggest that C. serpentis is not of major zoonotic potential.
Conclusions

1. There is no definitive negative test.
2. Screening appears to be best accomplished utilizing a number of diagnostic tests with Meriflour™ and ProSpec™ yielding the most productive results.
3. There is no effective treatment to clear the organism but good supportive care and spiromycin may improve the animal’s condition and temporarily stop shedding.
4. Zoonotic potential is probably low but personal hygiene should be immaculate around snakes and infants, geriatric and immunosuppressed people should not handle reptiles.
5. Hopefully a serological test will be developed to aid in defining negative animals and therefore the formation of crypto-free breeding colonies.

LITERATURE CITED

PARADOXICAL PATHOLOGIC CHANGES IN VITAMIN D DEFICIENT GREEN IGUANAS (Iguana iguana)

Laura K. Richman, DVM*, Richard J. Montali, DVM
Department of Pathology, National Zoological Park, Smithsonian Institution, Washington, DC 20008, USA

Mary E. Allen, PhD, Olav T. Oftedal, PhD
Department of Zoological Research, National Zoological Park, Smithsonian Institution, Washington, DC 20008, USA

Metabolic bone disease is one of the most common disease seen in the green iguana (Iguana iguana). The nutritional causes include hypovitaminosis D, a poor source dietary calcium, improper calcium:phosphorous ratio and protein/energy deficiency. Nonnutritional causes include renal or liver disease, or primary hyperparathyroidism. The clinical signs and pathologic changes seen with metabolic bone disease are well established and include lethargy, inappetence, osteomalacia and replacement of bone with fibrocellular connective tissue.

Green iguanas have been shown to require exposure to ultraviolet light in the range if 285-315 nm (UV-B) for conversion of provitamin D3 to the active form of vitamin D3 (1,25 dihydroxycholecalciferol). In a previous study group of up to 20 green iguanas, substitution of a UV-B light source with a dietary form of vitamin D3 (2000 IU/kg diet) for one year was found to be an inadequate method of supplementation, with serum 25-OH-D3 concentrations often in the low range of <10 ng/ml.

In the present study group, 12 green iguanas housed in various facilities at the National Zoological Park were provided with a nutritionally complete iguana diet and had free access to UV-B emitting artificial lights. Occasionally, iguanas were presented for necropsy with a clinical history of lethargy anorexia and falling from perches, with a presumptive diagnosis of trauma from the fall. Postmortem examination revealed widespread soft tissue mineralization, vascular and basement membrane mineralization, cardiac and skeletal muscle degeneration and necrosis and occasionally, mild fibrous osteodystrophy.

Although many of the pathologic findings were suggestive of vitamin D toxicity, circulating levels of 25-OH-D3 were very low (range 7 to 36 ng/ml; normal, healthy basking green iguanas housed outdoors have been shown to have circulating levels of 25-OH-D3 in excess of 400 ng/ml). Reasons for the low vitamin D3 levels in iguanas in this study include territorial competition between males for the UV light and preferential dwelling distant from the UV source. Chronic deposition of mineral in many tissues of the affected animals suggests hypercalcemia or altered serum calcium:phosphorous ratio; however, multiple determinations of serum calcium and phosphorus were most often within normal range. It is possible that severe renal disease can increase plasma phosphate levels due to decreased glomerular filtration rate; however in most iguanas necropsied, the renal lesions were acute to subacute and often attributed to terminal dehydration, leading to gouty tophi deposition.

The possibility of hypovitaminosis E was explored as a cause of the skeletal and cardiac muscle degeneration; however preliminary serum vitamin E levels were within range of normal iguanas (average alpha-tocopherol level = 4.48 ug/ml, n = 5). Elevated parathyroid
hormone (PTH) levels are suspected; however determination of PTH levels and activity has been difficult. Conventional methods for measuring PTH levels are not applicable to the iguana PTH protein. Alternative methods for determining PTH activity are under current investigation.

Possibilities for the paradoxical metastatic soft tissue calcification in the face of low vitamin D levels include 1) metabolic derangements which alter the calcium:phosphorus ratio resulting in a calcium-phosphorous product that is optimal for metastatic calcification. 2) chronically low circulating vitamin D levels with subsequent hypocalcemia may produce an exaggerated PTH response, leading to excessive mobilization of calcium from bone; conditions may be optimal for soft tissue calcification. 3) less likely causes may be related to hypovitaminosis E, genetic predisposition or exertional rhabdomyolysis with subsequent mineralization. All possibilities are currently under investigation.

LITERATURE CITED

NORMAL VARIATIONS IN SELECTED PLASMA BIOCHEMICALS OF REPTILES

Bonnie L. Raphael DVM, Dipl. ACZM*, Paul P. Calle VMD, Dipl. ACZM, Mark S. Stetter, DVM, Barbara Mangold, DVM, Robert A. Cook, VMD
Department of Clinical Studies, Wildlife Health Sciences, Wildlife Conservation Society, Bronx, NY 10460 USA

Introduction

Knowledge of normal serum or plasma biochemical values is essential for accurate assessment of health status in any species. There are numerous literature references for normal biochemical values for various reptiles. Most, however, lack critical details such as numbers of animals sampled, age, sex, time of year, free-ranging versus captive, feeding histories, and laboratory methods utilized. These omissions limit the usefulness of the data. Clinical reports often make references to normal biochemical parameters but few indicate the extremes that may occur. Some references for normal blood values are not in literature easily accessible to veterinary practitioners. As a result, factual information may be poorly available, while anecdotal information abounds.

Case Report

A greater than 20 yr old, 75 kg reticulated python, (Python reticulatus) which had been exposed to an atypical mycobacterium was being bled serially during prophylactic treatment with enrofloxacin (2.5 mg/kg IM q7d). After 6 months, treatment was discontinued, but periodic blood sampling continued. In October 1993 the plasma calcium (CA) was 78 mg/dl, phosphorus (P) 14.8 mg/dl and cholesterol (chol) 257 mg/dl. These were in contrast to previous normals of 13.0-15.9, 3.1-5.0 mg/dl and 186-309 mg/dl respectively. All other chemistry values were within normal limits. When rebled one month later, CA was 100 mg/dl, P=17.9, and chol=392. In mid December CA was 134.2, P=18, and chol=548. At that time the animal was visibly enlarged through the caudal 1/2 of her body. Abdominal ultrasonography revealed multiple follicles of varying sizes. The snake was subsequently bled at 2 week intervals: calcium levels fluctuated as did phosphous and cholesterol. When ultrasonography was repeated in April, no follicles could be visualized and the CA, P, chol had dropped to 32.4, 6.0 and 312. When last measured in March 1995, the values were 19.0, 5.7 and 289 respectively.

Discussion

A review and compilation of biochemical values from the reptile literature revealed some interesting and important information. The foci of the review were calcium, uric acid and urea nitrogen. Literature sources included both papers or articles on normal biochemical status, as well as those referring to clinical or physiologic syndromes which included references to normal values.

Means of normal calcium for all species of reptiles are reported to be to be 9.8-14 mg/dl.1-5,7,16 Ranges of 10-22 for reticulated pythons15 and 7.7-16.7 (x=13.3 1.7) for anacondas
(Eunectes murinus), 14-23.7 for boas (Constrictor constrictor) have been reported. A recent report suggested that persistent hypercalcemia and hyperphosphatemia may be normal in indigo snakes (Drymarchon corais), although no comparison between females and males were made. Reptiles in active folliculogenesis may have elevated calcium, and phosphorus levels compared to other times, with values of up to 360 mg/dl, 54.5 mg/dl for calcium and phosphorus being reported. In a clinical report, a monitor in follicular stasis was found to have a calcium of 85. In a review of medical records at the Wildlife Health Center it was found that it was possible to predict the sex of many adult reptiles by evaluating plasma calcium levels.

Uric acid (UA) levels have been reported to range from 0.28 - 29.1 mg/dl depending on pre or post prandial collection and health status. UA is a measure of renal function but not dehydration. Normally, during periods of increased renal demand such as after ingestion of a high protein meal, uric acid will rise. Post-prandial UA levels of up to 29.1 in boas and 18.3 for monitors have been recorded. High uric acid levels may indicate renal dysfunction and be a precursor to visceral gout, or may merely reflect feeding status. In either case, if an elevated uric acid is encountered the animal should be resampled after a fast.

Urea nitrogen (BUN) is not routinely evaluated in reptiles although it is a useful test for assessing hydration, disease, feeding, metabolic and pregnancy status. Normal values have been reported to range from 0-265mg/dl. Chelonia have higher BUN than lizards and snakes due to squamates lacking the full complement of hepatic urea-cycle enzymes. Well hydrated herbivorous chelonia usually have negligible BUN values, often below the level of detection of automated chemistry analyzers. In contrast, normally hydrated carnivorous turtles have BUN ranging up to 75 and it isn’t uncommon to find elevated BUN levels in tortoises just emerging from hibernation.

In summary, although there are numerous published reports of biochemical values for reptiles, not all contain enough information for valid conclusions to be drawn. However, there are some important trends and principles that persist across the species. If seemingly aberrant results are encountered in an individual or group of reptiles, it is worthwhile to repeat the sampling and interpret the results in consideration of normal variation within the sex or species.

LITERATURE CITED

DISEASES OF SOLOMON ISLAND LEAF FROGS (Ceratobatrachus guentheri) AT THE WOODLAND PARK ZOO: A RETROSPECTIVE STUDY OF SEVENTY FIVE NECROPSY CASES, 1990-1995

Michael M. Garner, DVM, DACVP*
Northwest ZooPath, 15326 Broadway Ave SE, Snohomish, WA 98290-7042, USA

Darin Collins, DVM and Janis Ott Joslin, DVM
Woodland park Zoo, 5500 Phinney Avenue North, Seattle, WA 98103-5897, USA

The Woodland Park Zoo first acquired Solomon Island leaf frogs in 1988 and has since established a highly successful breeding program for this species. The animals are housed on leaf litter in wooden of fiberglass enclosures with glass fronts. Temperature, humidity (misting), and an incandescent light cycle are adjusted to simulate the native environment. The frogs are fed primarily on crickets and also receive occasional mealworms, waxmoth larvae and juvenile mice. The purpose of this retrospective evaluation was to attempt to establish trends in disease occurring within this colony. Necropsies were performed by a total of 14 different prosectors, and histopathology was performed by a total of 8 different pathologists. One pathologist (MMG) reviewed necropsy records and slides on all cases. The results are listed in Table 1. In 15 frogs lesions were not found and the cause of death was not determined, but many of these animals were autolyzed or had incomplete postmortem examinations. In the remaining frogs, significant disease trends were necrotizing granulomatous steatitis, inflammatory and degenerative eye lesions, noninflammatory intestinal nematodiasis, chronic glomerulopathy, nutritional and infectious dermopathies, and degenerative hepatopathies. Some or all of these conditions occurred concurrently in some animals, especially cataracts, steatitis, nephropathy and degenerative hepatopathies. Steatitis was characterized by circumscribed zones of epithelioid macrophages and multinucleate giant cells surrounding foci of necrotic fat and hemorrhage in the fat bodies or bone marrow. Inflammatory eye lesions were attributed to trauma. Cataracts were present in young and old frogs and comprised of mild lenticular degeneration in the fibers of the superficial cortex. Intestinal nematodes were not speciated and the incidence of infestation was reduced dramatically when a parasite control program was initiated (ivermectin). Renal lesions included varying degrees of glomerulosclerosis, tubulointerstitial nephritis, and tubular necrosis with exuberant regeneration. Many of these renal lesions occurred concurrently and occasionally bacteria or calcium oxalate crystals were detected within inflamed or necrotic tubules. In the one case in which cultures were obtained, heavy growth of Aeromonas hydrophila was isolated from affected kidney. Mineralization of the basement membrane or dermal collagen fibers in affected skin was attributed to nutritional oversupplementation with vitamin D and calcium or concurrent renal disease. Although hepatic disease was common it was usually accompanied by more severe lesions in other tissues. The majority of hepatic changes were attributed to concurrent metabolic, toxic, or nutritional derangements. Investigation into the cause of these diseases is ongoing and includes chemical analysis of tissues for vitamin E and selenium levels, analysis of feed for mycotoxins and standardization of necropsy procedures to include microbiology of select tissues.
Table 1. Lesions in Solomon Island leaf frogs from Woodland Park Zoo, 1990 - 1995.

<table>
<thead>
<tr>
<th>SYSTEM</th>
<th># OF LESIONS</th>
<th>DESCRIPTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose</td>
<td>17/18 (94%)</td>
<td>Steatitis (17): severe (9), moderate (2), mild (6)</td>
</tr>
<tr>
<td>Eye</td>
<td>11/15 (73%)</td>
<td>Cataract (5), uveitis/keratitis (5), conjunctivitis (1)</td>
</tr>
<tr>
<td>G.I.</td>
<td>29/47 (60%)</td>
<td>Nematodes (23), rectal prolapse (2), mycobacteria (1), tongue abscess (1), carcinoma (1), gastric mucosal necrosis (1)</td>
</tr>
<tr>
<td>Kidney</td>
<td>23/46 (50%)</td>
<td>Chronic glomerulopathy (19), nematodes (2), granulomas (3), cysts (1), glomerular thrombi (1)</td>
</tr>
<tr>
<td>Integument</td>
<td>13/27 (48%)</td>
<td>Mineralization (5), Capiollaria (2), ulcers (5), necrotic dermopathy (1)</td>
</tr>
<tr>
<td>Liver</td>
<td>19/52 (36.5%)</td>
<td>Necrosis (7), vacular hepatopathy (3), lipidosis (5), atrophy (2), granuloma (1), ito hyperplasia (1)</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>10/38 (24%)</td>
<td>Scoliosis (2), &quot;bones&quot; (2), hip problems (1), rhabdomyolysis (2), digit loss (2), hyperostosis (1)</td>
</tr>
<tr>
<td>Reproductive</td>
<td>7/31 (23%)</td>
<td>Ovarian degeneration (4), hermaphrodite (2), orchitis (1), oophoritis (1)</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>10/46 (21%)</td>
<td>Inflammation (7), thrombosis (2), cardiac lesion (1)</td>
</tr>
<tr>
<td>Spleen</td>
<td>1/9 (11%)</td>
<td>Fibrinonecrotic splenitis (1)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1/16 (6%)</td>
<td>Atrophy</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>1/32 (3%)</td>
<td>Granulomas</td>
</tr>
<tr>
<td>Endocrine</td>
<td>0/56</td>
<td>Adrenal 0/46, thyroid 0/0, pancreas 0/16, pituitary 0/0</td>
</tr>
<tr>
<td>Brain</td>
<td>0/4</td>
<td></td>
</tr>
</tbody>
</table>
A 5-year-old female green iguana (*Iguana iguana*) was presented with clinical signs of anorexia and weakness of one month's duration. Physical examination revealed anemia, icterus, and a large mass was palpable within the cranial abdomen. Following euthanasia a necropsy was performed; the mass measured 9 x 9 x 4 cm and filled most of the abdomen, arose to the left of midline on a vascular stalk in the region of the left adrenal which was not present, and was polycystic with caseonecrotic cores. Multiple pale nodules were also present in the heart, liver, and both kidneys. Microscopically, the abdominal mass as well as the metastatic foci in the kidneys, liver, and heart consisted of infiltrative sheets of large epithelial cells with abundant vacuolated cytoplasm, round vesicular nuclei, and a low mitotic index, consistent with an interrenal carcinoma, homologous to a mammalian adrenal cortical carcinoma. Ultrastructurally, the neoplastic epithelial cells had round to irregular nuclei with densely clumped chromatin, and abundant cytoplasm which contained numerous degenerate mitochondria and scattered lipid droplets.

Primary endocrine neoplasms have been rarely reported in reptiles, and this is the first report of a malignant interrenal cell tumor in a reptile. The widespread metastasis of this endocrine tumor is also rare for reptilian endocrine tumors. Interrenal tumors should be considered in the differential diagnosis for intra-abdominal masses in iguanas and other reptiles.
ULTRASONOGRAPHIC SEXING OF HELODERMATID LIZARDS

Kevin Wright, DVM*
Philadelphia Zoological Garden, Department of Herpetology, 3400 W. Girard Avenue, Philadelphia, PA 19104-1106, USA

Charles Pugh, DVM, Dip. ACVR, Lynn Walker VMD
Veterinary Hospital of the University of Pennsylvania, Department of Radiology, 3850 Spruce Street, Philadelphia, PA 19104-6010, USA

Introduction

The two living members of the family Helodermatidae (i.e., beaded lizard Heloderma horridum and gila monster Heloderma suspectum) have no reliable external features for gender determination. Several techniques have been described for sexing helodermatids: laparoscopy, eversion of the hemipenes by manual eversion or via the injection of 20 ml of sterile saline, comparative measurements of ischial dimensions, probing, and comparative cranium width.

Improper pairing of helodermatids (e.g., placing two males in the same cage under the assumption that it is a true male: female pair) will result in a failure to reproduce. The studbook for helodermatids was recently approved by the AZA Wildlife Conservation and Management Committee, definitive gender identification will become increasingly important for the captive management to preserve genetic diversity. Combat is a ritualized agonistic behavior between males that determines reproductive success. In captivity the combat arena is often too small to allow the loser to escape, so the loser can suffer serious to mortal wounds. Improper pairing can result in unexpected combat. A noninvasive definitive method of gender identification would minimize the occurrence of these unexpected/unwanted interactions.

Of the sexing techniques described for helodermatids in the literature, only laparoscopy was a definitive means of gender identification for both sexes as it was the sole method that allows direct observation of the gonads. Laparoscopy requires general anesthesia and invasive surgery. A 7-14 day pre-operative fast is needed to decrease the diameter of the colon which may obscure the gonads. In overweight lizards the gonads may be difficult to impossible to discern amid the visceral fat.

Eversion of the hemipenes allows definitive identification of male helodermatids. Some males will evert the hemipenes when provoked, while manipulation of the post-cloacal bulge may be successful in evertting a hemipenis in other males. Failure to evert a hemipenis is not definitive for a female helodermatid as poor technique may fail to externalize the hemipenis of a male. In the prehensile-tailed skink Corucia zebrata a surgical plane of inhalant anesthesia is necessary in order to manually evert the hemipenes of some males.
Presumably due to the strength of the hemipenal retractor muscles, large lizards require general anesthesia for the saline injection technique to be effective, although it was reported as effective in awake helodermatids. The hemipenal tissue is everted via the injection of a 20 ml saline bolus intramuscularly immediately caudal to the tip of the hemipenal sulcus. Failure to evert the hemipenes can result from improper placement of the injection. A further disadvantage to this technique is that the everted hemipenis can be damaged during the injection or while the organ is detumescing.

It is a common belief among helodermatid keepers that the cranium width of male helodermatids is proportionately greater than that of females. There have been no published studies that critically examine cranium width as a consistent (i.e., statistically significant) sexually dimorphic character. Obtaining this measurement in an awake lizard an be difficult as many individuals will attempt to bite anything that approaches their head. Probing was reportedly unreliable in beaded lizards, and in the author's experience (Wright) has not proven reliable in the gila monster.

The published report describing the radiographic sexing technique acknowledges that it is unreliable in distinguishing the sexes of animals of different snout-vent lengths. A further criticism of the paper is that only 7 known sex skeletal specimens were used to establish the criteria, and the 10 live animals that were assigned sexes by this technique were not reported as being sexed by a definitive technique. Radiographs require a minimum of two manual restraint procedures to position the lizard, or exposing the handler to x-rays, or general anesthesia. Radiographs can detect shelled ova if present in a female helodermatid.

One study described the ultrasonographic anatomy of the savannah monitor lizard *Varanus exanthematicus* but did not describe gonads. A review paper mentioned the possibility of detecting developing ova in lizards by ultrasonographic examination, but gave no details. An ultrasonographic examination might detect ovarian follicles, follicles undergoing vitellinogenesis, and oviductal eggs due to their cystic structure. Mature females of many lizard species have ovarian follicles throughout the year, so if the follicles have a detectable ultrasonographic presence this will be a definitive trait for a female.

**Materials and methods**

The animal inventory available for this study was 2.2.3 beaded lizards and 0.1.4 gila monsters. Sexes were known as follows: 0.1 beaded lizard (300340) -- 2 shelled eggs noted on a radiograph in 1993; 1.0 beaded lizard (300867) -- hemipenes were observed during copulation with 300340; 1.1 beaded lizard (300342, 300341) -- proven breeders; 0.1 gila monster (300954) had produced eggs at previous institution. At least three gila monsters were assumed to be male based on combat behavior and copulatory attempts noted in previous breeding seasons. Only eight of the available animals were used in the study due to time constraints.
Two ultrasonographic machines were used for the procedures, an Ultramark\textsuperscript{R9} with HDI\textsuperscript{R} Technology upgrade with a 10 MHz linear array transducer probe (Advanced Technology Laboratories, Inc., POB 3003, Bothell, WA 98041-3003), and an Aloka 500 with a curvilinear 5.0 MHz transducer probe (Corometrics, Inc., Wallingford, CT). The transducers were coupled to the helodermatids' ventral scutes using acoustic gel. The lizards were manually restrained in dorsal recumbency. The heart was located for frame of reference, and the abdomen scanned in a routine manner to identify as many soft tissue structures as possible. Two separate examinations were made, one on 10 December 1994 and one on 11 March 1995. On 10 Dec, four mature beaded lizards and 4 mature gila monsters were examined. 1.1 beaded lizards were of known sex as was 0.1 gila monster. Results are reported in Table 1. On 11 Mar 0.2 mature beaded lizards and 0.2 mature gila monsters were re-examined. Results are reported in Table 2.

Results

Follicles 5 mm in diameter were readily detectable using either ultrasonographic machine although the 10 MHz probe on the Ultramark\textsuperscript{R9} provided the best resolution. A 13 yr old gila monster born at the Philadelphia Zoo had been listed as a male based on its cranium width but was suspected of being a female based on its behavior. This animal had detectable ovarian follicles. One gila monster ovulated between examinations.

Discussion

This noninvasive technique for sexing helodermatid lizards can be quickly performed while the lizard is manually restrained. Abdominal fat pads did not interfere with the abdominal scan as was noted in a previous study using varanid lizards. Ultrasound examination allows direct assessment of ovarian activity. In healthy intact mature female helodermatids this procedure is a definitive method of gender assessment, and has none of the drawbacks of laparoscopy.

Regular ultrasound examinations can benefit the management of breeding groups of helodermatids by assuring that the group is of an appropriate sex ratio and may help time the introductions for breeding.

Unfortunately the testicles were not readily distinguished via ultrasound so at this point the technique assigns the male gender by the absence of ovarian follicles on the assumption that ovarian follicles are present throughout the year in mature female helodermatids. Continued studies will be conducted assessing ovarian follicular activity throughout the year as well as the onset of ovarian activity in juvenile helodermatids. The entire helodermatid inventory will be assessed.
LITERATURE CITED


Table 1. Results of 10 Dec 1994 ultrasonographic examination of some Philadelphia Zoo helodermatids.

| Beaded lizard | 300340 multiple 5 mm ovarian follicles |
| Gila monster  | 300344 vitellinogenic follicles |
|              | 300345 no cystic structures noted |
|              | 300346 vitellinogenic follicles |
|              | 300954 15 mm ovarian follicles |

Table 2. Results of 11 Mar 1995 ultrasonographic examination of some Philadelphia Zoo helodermatids.

| Beaded lizard | 300340 multiple 5 mm ovarian follicles |
| Gila monster  | 300346 vitellinogenic follicles |
|              | 300954 6+ oviductal ova |
CONSIDERATIONS IN THE EUTHANASIA OF REPTILES, AMPHIBIANS, AND FISH

Roy Burns, DVM  
Louisville Zoological Garden, PO Box 37250, Louisville, KY 40233, USA

Euthanasia techniques should result in rapid unconsciousness followed by cardiac or respiratory arrest and ultimate loss of brain function. Stress or anxiety experienced prior to unconsciousness should be minimized. Criteria to evaluate methods of euthanasia in animals are published.2

Euthanasia of reptiles, amphibians, and fish is performed for various reasons and in a wide variety of situations including laboratory, educational, clinical practice, and field settings. Methods of euthanasia appropriate for one setting may be impractical for another. Anatomy, physiology, and behavior should be considered in selecting appropriate methods of euthanasia for reptiles, amphibians, and fish.

Nociception in Reptiles, Amphibians, and Fish

The perception of pain can be difficult to measure. It is reasonable to assume that reptiles, amphibians, and fish experience pain, distress, or anxiety when exposed to noxious stimuli to a degree similar to that of mammals and birds. When considering euthanasia techniques, it is best to assume some degree of nociception exists.

The International Association for the Study of Pain defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage.22 Pain is a perception that depends on activation of a discrete set of receptors (nociceptors) by noxious (e.g., thermal, chemical, or mechanical) stimuli.20 Further processing in neural pathways (e.g., spinal chord, brain stem, thalamus, and cerebral cortex), enables noxious stimuli to be perceived as pain.20 The same basic pathways that process nociceptive information are present in all vertebrates.29 For pain to be experienced, the cerebral cortex and subcortical structures must be functional.2

The components of nociceptive pathways are present in reptiles. Reptiles have peripheral receptors including mechanoreceptors in skin and connective tissue, touch papillae, and joint capsule nerve endings.31 The thalamus and dorsal cortex of reptiles are involved in nociception.4

Amphibians also possess components of nociceptive pathways. There are pain and pressure receptors in the dermis.13 Amphibians have endogenous opioids that modulate central processing of noxious information.28

In fish, there is biochemical evidence for pain perception. Production of compounds related to adrenocorticotropic hormone, opiates, and presence of benzodiazepine receptor sites serve as biochemical evidence for pain perception in fish.15,26 Presence of a substance P-like
immunoreactivity, in the funicular nucleus of knifefish, *Apteronotus leptorhynchus*, is suggested to be associated with nociception.\(^{33}\)

Although in fish, specific pain receptors and the role of the central nervous system in pain perception have not been identified, sufficient anatomic and physiologic information supports the assumption that chronic as well as acute pain should be considered for procedures performed on fishes.\(^{7,32}\)

Reptiles, amphibians, and fish respond to noxious stimuli by flinching, muscle contractions, aversive movements away from the unpleasant stimulus, attempts to bite, and abnormal swimming behavior.\(^{25}\) For some species, recognition of response to chronic noxious stimuli requires an experienced observer. For example, color change, altered posture, and changes in water column utilization are fish responses to chronic painful stimuli that may be missed by an observer expecting responses typical to those of mammals.\(^{32}\)

**Unique Physiological Characteristics of Reptiles, Amphibians, and Fish**

Anoxia tolerance

Many reptiles and amphibians are capable of converting to anaerobic metabolism for prolonged periods.\(^{5}\) Turtles of the genus *Pseudemys* have survived up to 27 hours in an environment of 100% nitrogen.\(^{6,17,18}\) Green iguanas (*Iguana iguana*) can "breath hold" up to 4.5 hours.\(^{24}\) Amphibians also tolerate prolonged periods of anoxia\(^{31}\). Therefore, methods of euthanasia that induce unconsciousness by interruption of the blood supply to the head and anoxia, (e.g., decapitation, cervical dislocation, and exsanguination), are inappropriate for reptiles and amphibians when used alone, because rapid unconsciousness does not occur.\(^{12,25}\)

Freeze tolerance

In nature, the majority of reptiles and amphibians use behavioral tactics to elude exposure to freezing temperatures by choosing subterranean or aquatic hibernation sites.\(^{30}\) Some species of reptiles, amphibians, and fish have developed freeze tolerance (formation of ice crystals in extracellular spaces). The microenvironment in which freezing occurs significantly affects survival.\(^{31}\)

Freeze tolerance has been documented in four species of frog (wood frog, *Rana sylvatica*, grey tree frog, *Hyla versicolor*, spring peeper, *Hyla crucifer*, chorus frog, *Pseudacris triseriata*), a Siberian salamander, *Hynobius keyserlingi*, and hatchlings of the painted turtle, *Chrysemys picta*.\(^{31}\) These species inhabit cold climates. Adaptations described for freeze tolerance in these species include the presence of proteins that regulate growth of ice crystals in extracellular fluid, production of cryoprotectants from large glycogen stores accumulated before hibernation, ability to tolerate dehydration of up to 60% of total body water, and tolerance of ischemia.\(^{11,31}\) Presence of some of these adaptations have a seasonal pattern.\(^{31}\)
In fish, glycopeptides lower the freezing point of extracellular fluid compartments, protect cells and their membranes from hypothermic damage, and adsorb to faces of ice crystals and inhibit crystal growth.

Although hypothermia will make reptiles and amphibians more torpid, there is no evidence that it raises the pain threshold. For all species without adaptations for freeze tolerance, freezing at normal household freezer temperatures is contraindicated as a means of euthanasia because formation of ice crystals on the skin and in the tissues is likely to cause tissue damage, pain or distress.

Euthanasia Methods

Sodium pentobarbital

Administration of sodium pentobarbital (60 - 100 mg/kg) intravenously or in the pleuroperitoneal cavity can be used for euthanasia in most reptiles, amphibians, and fish. Time to effect may be variable but usually an effect is noticed within a few minutes and death occurs within 30 minutes. In frogs and toads, the subcutaneous lymph spaces can serve as alternate sites of injection.

Tricaine methane sulfonate (MS-222) and benzocaine

Immersion in solutions of MS-222 can be used for anesthesia or euthanasia of amphibians and fish. In amphibians, a concentration reported to be used for euthanasia is 1 - 10 grams/liter. Concentrations used for euthanasia of fish are 300 - 400 mg/liter (M. K. Stoskopf, personal communication), 500 mg/liter, and 1 gram/liter for a few minutes. MS-222 is acidic in solution and an initial excitement phase may occur.

Additional routes of administration of MS-222 are injection into the pleuroperitoneal cavity and injection into the lymph spaces. MS-222 rapidly desensitizes the exposed serosal tissues, making intrapleuroperitoneal injection essentially painless, except for the initial needle passage.

Benzocaine acts similarly to MS-222 but is relatively insoluble in water. It must be dissolved in acetone prior to being placed in aqueous solution. Immersion in a 100 mg/liter solution induces anesthesia in amphibians.

Inhalant agents (halothane, isoflurane, methoxyflurane)

Using inhalants as a sole means of euthanasia in reptiles is less satisfactory than other methods because high concentrations and long exposure times are required to ensure death. Modifying a reptile’s enclosure into an induction chamber and using a cotton swab soaked with a volatile agent can provide a means of delivering these agents safely to venomous or aggressive individuals.
These agents can be mixed in water or vaporized and bubbled into water for anesthesia of fish and aquatic amphibians. Euthanasia of these animals requires overdose of these drugs over a long period of time.

Carbon dioxide

Carbon dioxide administration is accepted as a means of euthanasia in reptiles, amphibians, and fish. In laboratory rats, it is suggested that analgesia produced by exposure to 70% carbon dioxide for 30 seconds is due to a novel form of environmentally induced nociception mediated by a non-opiate hormonal substance rather than the effects of hypoxia, endogenous opioids, or motor impairment. Although unconsciousness can occur rather quickly, exposure times required for euthanasia are long.

Decapitation

Decapitation relies on neurogenic shock and loss of blood to the brain to cause painless death. For amphibians, reptiles, and fish decapitation with a guillotine or heavy shears is considered effective in some species that have appropriate anatomic features provided it is followed immediately by pithing.

Pithing

Pithing is generally considered an adjunct to other methods of euthanasia and is to be performed on animals already rendered unconscious. Because of variation in size and anatomy of the skull in reptiles, pithing can be difficult and requires skill and training to perform appropriately. Pithing is used for some species of fish, the site can be determined in relation to the eye (M. K. Stosskopf, personal communication). In some amphibians (e.g., frogs), the location of the foramen magnum may be readily identified by a slight depression in the skin on the midline just posterior to the eyes when the animal is restrained with the neck flexed.

Exsanguination

As mentioned previously, amphibians and reptiles are extremely tolerant of anoxia. Methods which end life by anoxia when used alone and in a conscious animal are not considered humane. Exsanguination is an acceptable method of killing unconscious amphibians and reptiles.

Cranial concussion and stunning

These methods involve striking the head of the animal with a hard implement or object (or vice versa). The blow should at least result in unconsciousness and ideally should result in complete destruction of the brain. If the brain is not completely destroyed by the blow, this method should be followed by killing via exsanguination or double pithing. Cranial concussion and stunning are most easily performed on smaller reptiles, amphibians, and fish.
The skulls of many reptiles have two well developed bony encasements and can require more than the expected force to destroy. For large reptiles (crocodilians, large lizards, large constricting snakes, and marine turtles), gunshot or captive bolt pistols may be used to destroy the brain. Recommended points of aim for concussion have been published. One recommended point of aim is the thin area of bone under the parietal eye of some species of reptiles.

Rapid freezing

Immersion of an entire animal in liquid nitrogen achieves rapid death of an animal that weighs less than 40 grams. However, the Report of the AVMA Panel on euthanasia classifies rapid freezing as an unacceptable means of euthanasia when used without prior anesthesia.

Formalin

The AVMA does not list immersion in formalin as an acceptable means of euthanasia. For field researchers, published guidelines for the use of fish suggest that any fish that does not die rapidly following immersion in formalin should be killed before preservation. This recommendation is not supported by data. Formalin has no anesthetic qualities and the duration of consciousness after immersion in formalin is not documented.

Confirmation of Death

Distinguishing between deep anesthesia and death in reptiles, amphibians, and fish can be difficult but is important to avoid recovery of consciousness. It is common for the heart to continue beating after these animals appear to be dead. Electrocardiography or opening the body cavity can be used to confirm cardiac arrest.

Table 1. AVMA Classification of methods of euthanasia by species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Acceptable</th>
<th>Conditionally Acceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphibians</td>
<td>Inhalant anesthetics, CO, C0₂, barbiturates, MS-222, double pithing, benzocaine</td>
<td>Pithing, gunshot, captive bolt, stunning &amp; decapitation, decapitation and pithing</td>
</tr>
<tr>
<td>Fish</td>
<td>MS-222, benzocaine, barbituates CO₂*</td>
<td>Stunning &amp; decapitation, decapitation</td>
</tr>
<tr>
<td>Reptiles</td>
<td>Barbiturates, inhalant anesthetics, CO₂</td>
<td>Gunshot, captive bolt, stunning and decapitation, decapitation and pithing</td>
</tr>
</tbody>
</table>

* Carbon dioxide is listed in the literature as being acceptable, but is not listed for fish in the AVMA report.
Table 2. AVMA classification of euthanasia methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Classification</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbiturates</td>
<td>Acceptable</td>
<td>Amphibians, reptiles, other small animals.</td>
</tr>
<tr>
<td>MS-222, benzocaine</td>
<td>Acceptable</td>
<td>Fish and amphibians. Effective but expensive.</td>
</tr>
<tr>
<td>Inhalant anesthetics</td>
<td>Acceptable</td>
<td>Amphibians, reptiles, other small animals, zoo animals.</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>Acceptable</td>
<td>Small laboratory animals, amphibians, zoo animals, fish*</td>
</tr>
<tr>
<td>Decapitation</td>
<td>Conditionally acceptable</td>
<td>Amphibians, fish, and reptiles.</td>
</tr>
<tr>
<td>Pithing</td>
<td>Conditionally acceptable</td>
<td>Poikilotherms. Effective but death not immediate unless double pithed.</td>
</tr>
<tr>
<td>Exsanguination</td>
<td>Unacceptable</td>
<td>Should be done only in sedated, stunned, or anesthetized animals.</td>
</tr>
<tr>
<td>Stunning</td>
<td>Unacceptable</td>
<td>Stunning may render an animal unconscious, but is not a method of euthanasia. If used, it must be followed by a method to ensure death.</td>
</tr>
<tr>
<td>Rapid freezing</td>
<td>Unacceptable</td>
<td>As a sole means of euthanasia is not considered humane. Animals should be anesthetized prior to freezing.</td>
</tr>
</tbody>
</table>

* Carbon dioxide is listed in the literature as being acceptable, but is not listed for fish in the AVMA report.

LITERATURE CITED

THE EVOLUTION OF ELEPHANT HUSBANDRY FROM FREE CONTACT TO PROTECTED CONTACT-A VETERINARIAN'S PERSPECTIVE

James E. Oosterhuis, DVM*
Zoological Society of San Diego, San Diego Wild Animal Park, 15500 San Pasqual Valley Road, Escondido, CA 92027-7017, USA

In North America there is an increasing movement towards protected contact management techniques for elephants kept in zoos. This means that elephants are managed with a protective barrier between the handler and the elephant and that all behaviors are rewarded by positive reinforcement.

This change has been brought about by an increased desire for employee safety and to avoid animal discipline.

Implementation requires facility modifications with the installation of an elephant restraint device being one of the biggest additions needed. The change also requires an extensive training program for both handlers and elephants.

The veterinarian has to learn to work around the protective barriers and to be aware that short painful procedures such as injections or foot abscess examinations will now take longer and require a lot of patience.

The most important concerns however, after human safety, must be for the elephants. In the short term their welfare, both mental and physical must be considered and in the long term their future offspring and their needs must be met.

250

1995 PROCEEDINGS JOINT CONFERENCE AAZV / WDA / AAWV
THE HOT ZONE: PRIMATE WELFARE AND INTERNATIONAL IMPORT REGULATIONS

Janis Ott Joslin, DVM*
Woodland Park Zoological Gardens, 5500 Phinney Avenue North, Seattle, WA 98103, USA

Introduction

Managing captive population of nonhuman primates means developing selective breeding programs in order to maximize genetic diversity. Inherent in this process is the need to bring in new animals from outside the United States in order to expand the founder representation in the captive population. Since nearly one-third of nonhuman primate species are considered rare, vulnerable or endangered according to the International Union for the Conservation of Nature and Natural Resources, it is unlikely that individuals will be imported directly from the wild but more likely will come from captive bred stock from Europe and Canada. It is most likely that these individuals have been born and raised in a zoological garden and have been maintained in a social setting most of their life.

Importation of these animals into the United States now requires that the receiving institution be approved for primate importation by the Center for Disease Control (CDC). Presently there are only a handful of "lucky" zoos in the U.S. that are so approved. Approved institutions must then follow "guidelines" that CDC is "recommending" which include assuring that animals in transit into the U.S. do not contaminate any air handlers, foodstuffs and other animals; once at the quarantine facility the animals are isolated in individual squeeze cages so as to minimize the risks of injuries to the animal handler when restraining the nonhuman primate for examinations. Anyone working around these animals must wear coveralls, preferably disposable coveralls, dust/mist respirator masks, boots, gloves, goggles and caps. Respirator suits are "recommended" for any individual who has a beard, otherwise facial covering is needed. The institution is required to write up an outline of Standard Operating Procedures (SOP) for unloading the animals from the plane upon arriving, for transporting the animal from the airport to the quarantine facilities for daily feeding and cleaning and for performing the three required tuberculin tests during the 33-day CDC quarantine period.

In August, 1992, CDC distributed a draft of technical standards for importation and quarantine of nonhuman primates for comments. These CDC recommendations were developed to minimize contact between the nonhuman primates and humans as a result of the "Ebola" virus scare of 1989. It is true that the old CDC primate importation standards were minimal to say the least and that there needed to be a reevaluation of the facilities approved for primate importation. There was also a need to develop new requirements for handling of newly imported nonhuman primates straight from the wild in light of what could have occurred with the Ebola-related filovirus. CDC, however, has elected to require institutions to follow the same "guidelines" for any nonhuman primate importation as though it were straight from the wild, regardless of the origin of the animal.
Case History

Thus, with a recent importation of an adult female gorilla (Gorilla gorilla gorilla) who had lived the last 20+ years in captivity in the Toronto Zoo in Toronto, Canada, staff at Woodland Park Zoo were faced with the responsibility of gowning up in disposable coveralls complete with goggles, masks, booties and respirators to greet this animal on a daily basis. The Toronto Zoo agreed to send along a keeper with this gorilla in order to make the move and transition to new caretakers easier. Imagine the gorilla’s surprise when it reached Seattle and was greeted by its former keeper and new caretakers who were now dressed in moon suits. Thus the only way for the animal to recognize its caretakers was by their voice, mannerisms and maybe a foggy glimpse of a person’s eyes. The gorilla responded accordingly to this transition period by going off feed, hair plucking, excessive picking of old scabs causing open sores and reverting back to her old habit of frequently regurgitating and reingesting (R and R). The R and R occurred most frequently when her moon-suited caretakers were in her view. The gorilla also refused to shift cages for cleaning for several days after its arrival, and periodically throughout the quarantine period whereas it had reliably shifted its cages back in Toronto.

The gorilla’s behavior obviously worried her keeper from Toronto and the staff at WPZ. In an effort to alleviate the animal’s anxiety, the gorilla was medicated with Haloperidol at a dose of 5mg twice a day through the course of the CDC quarantine; then the dose was gradually decreased to 1mg twice a day over a 2-month period of time during the animal’s acclimation period. This had a minimal effect but did improve her shifting reliably. Unfortunately, what could have been a calm and smooth transition for this gorilla turned into a nightmare that lasted over six weeks.

CDC requires that the TB tests be performed three times at 10 day intervals and thus, the CDC quarantine ends after the 72 hour negative reading of the third tuberculin test. If the animal is TB tested on the date of arrival, then the quarantine would end after 33 days. Due to this gorilla’s obvious signs of stress during her first days in quarantine, we elected to postpone her first quarantine tuberculin test until six days after her arrival thus necessitating extending the CDC moon suit requirements for these additional days.

During this quarantine period, a female gorilla in the zoo’s collection became ill, requiring the ill gorilla to be isolated in a cage which was going to be used by the new gorilla as an introductory cage. This thus postponed the new animal’s release date from quarantine by several weeks. The plan was to have the regular gorilla keepers come to the hospital and spend time socializing with the quarantined gorilla prior to the animal’s move to gorilla holding. Since the gorilla had been TB tested and tested for hepatitis at the Toronto Zoo a couple of weeks prior to shipping, we requested that CDC waive the moon suit requirement after 33 days of quarantine and that several weeks later the third TB test be performed the day she would be sedated for her move to gorilla holding. On that date, she would receive chest x-rays and reproductive examination by our medical consultants. Since CDC recommendations do not allow the animal to be moved from the quarantine room without prior approval for good cause, this seemed like a reasonable way to get these
additional procedures done prior to its transfer. This would also eliminate the need for one additional immobilization as it was apparent that the darting procedure was extremely stressful to this gorilla.

When CDC was contacted about this request, they responded that the CDC quarantine would not be over until the third TB test was read at 72 hours. So in an effort to end the animal's apparent stress, she was TB tested the third time 35 days after arriving at WPZ.

Four days later, the monkey suits came off and the gorilla was able to socialize with people again and get the much needed primate enrichment objects for her entertainment. Due to the strict disinfectant recommendations and the need for incineration of cage materials, we were prohibited for cost reasons from bedding her heavily and using a lot of treat logs and toys during her CDC quarantine.

Discussion

This case history serves to show the dilemma that arises for the zoo veterinarian between CDC recommendations and the United States Department of Agriculture (USDA) animal welfare regulations. According to USDA, the licensed veterinarian for the facility is responsible for directing the "plan for environmental enhancement adequate to promote the psychological well-being of nonhuman primates...the plan must address the social needs of nonhuman primates of species known to exist in social groups in nature... individually housed nonhuman primates must be able to see and hear nonhuman primates of their own or compatible species unless the attending veterinarian determines that it would endanger their health, safety or well-being...the physical environment in the primary enclosures must be enriched by providing means of expressing non-injurious species-typical activities.\textsuperscript{1} All of these directives are in contradiction to what happened with the gorilla in the above example.

One could reasonably argue that the risk of disease to humans was nil from this animal from Canada or any nonhuman primate with a known long term captive history from Canada or Europe. In addition, the requirements for treating these animals at a animal biosafety level 3, the precautions (gloves, respirator, goggles, coveralls, etc.) were excessive both for the institution and the animal during its quarantine. Under these circumstances, it should be the veterinarian's discretion as to the level of disease containment that is necessary during the quarantine period.

There should be a quarantine for newly arrived non-human primates from facilities in Canada and Europe or even from other zoos in the U.S. Indeed according to the Guidelines for Zoo and Aquarium Veterinary Medical Programs and Veterinary Hospitals prepared by the Veterinary Standards Committee of the American Association of Zoo Veterinarians in 1990,\textsuperscript{3} all new arrivals should be quarantined from the rest of the animal collection until the health of the new animals can be evaluated. The quarantine policy should be developed and administered by the zoo's veterinarian as part of the institution's preventive medicine program. The length of quarantine and quarantine procedures may

1995 JOINT CONFERENCE AAZV / WDA / AAWV 253
vary depending upon such things as the risk of disease potential and the source of the quarantined animal (wild vs. zoo captive born). The recommended quarantine period for nonhuman primates is 60 to 90 days. However, the guidelines clearly state that "any animal that is severely stressed by quarantine procedures may require an earlier release or modification of the facilities to avoid stress."

The zoo veterinarian must be allowed to make his or her own decisions as to the type of quarantine that is needed for the animal welfare of these nonhuman primates based on a realistic evaluation of perceived risk and a realistic evaluation of the individual animal's needs. Thus, if an animal needs physical contact with his previous keeper and needs environmental enrichment or needs a cagemate during its quarantine, this should be at the discretion of the facility's veterinarian, not CDC's regulatory agents. CDC's responsibility is to prevent disease transmission from nonhuman primates to humans and their need for Level 3 biosafety precautions from wild caught macaques may be justified. But when these precautions are applied to animals of minimal risk but at the detriment of these nonhuman primates, then the precautions seem unjustified and excessive.

This paper may appear to be more of an editorial than a scientific research article but presently these CDC regulations are only affecting about 50 registered importers and of these, only 8 are zoos. These importers are having to follow proposed technical standards written in August, 1992 since CDC has yet to publish their final proposal in the Notice of Proposed Rulemaking (NPRM) in the Federal Register for public comment. Representatives from the Association of Zoos and Aquariums (AZA) Animal Health Committee and from the AAZV Infectious Disease Committee have met with CDC and have made recommendations that nonhuman primates who were captive born or have been a long term captive animal have these standards waived if the originating institution can be "certified" as having an adequate veterinary program that would minimize the risk that nonhuman primates exported from these institutions. However, without firm proposed federal regulations in place, CDC is unlikely to change their stand on this issue.

This problem is presently affecting only a handful of institutions but as more and more zoos are called upon to import nonhuman primates for captive breeding programs, more zoos are going to need to become licensed importers. The veterinarians in these institutions will be caught up in the dilemma between what is best for CDC and what is best for the nonhuman primates in their care. It should be the zoo veterinarian's decision to opt for the latter.

LITERATURE CITED


254
SURGICAL REMOVAL OF AN INTRACRANIAL TUMOR IN A WESTERN LOWLAND GORILLA

Thomas P. Meehan, DVM,* Jaqueline M. Zdziarski, DVM, Michael B. Briggs, DVM
Chicago Zoological Society, Brookfield Zoo, 3300 Golf Rd., Brookfield, IL 60513, USA

Douglas B. Anderson, MD, Andrew S. Zelby, MD, Madeline Grigg-Damberger, MD, Chinnamma Thomas, MD
Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153, USA

Robert D. Murnane, DVM, PhD, Timothy M. Walsh, DVM
Zoological Pathology Program, University of Illinois College of Veterinary Medicine, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153, USA

A nine-year-old, male, western lowland gorilla (*Gorilla, g. gorilla*) presented in November of 1994 with a history of recurrent episodes of ataxia and weakness over the past ten months. In February of 1994 he had an episode that lasted 10 hours and in June, an episode that lasted 48 hours both followed by apparently normal behavior. During the June episode an examination revealed leukocytosis, and the animal was subsequently treated with antibiotics. By November, signs had progressed to generalized weakness and ataxia that waxed and waned over several weeks without full recovery. Physical examination was otherwise normal at this time, and serum chemistry profiles and complete blood counts were unremarkable.

The gorilla was immobilized and transported to Loyola University Medical Center (LUMC) for Magnetic Resonance Imaging (MRI) of the brain. The MRI revealed a 6 x 3 x 3 cm, right, intraventricular tumor with entrapment of the lateral ventricle and resulting hydrocephalus. Based on a good prognosis for slow growing, non-invasive, intraventricular tumors in humans, surgical removal was elected.

Several weeks later, the gorilla was transported to LUMC for surgery. The tumor was approached through a right occipital craniotomy with the bone flap entirely behind the occipital crest. Removal of the tumor was accomplished using a Zeiss operating microscope. Post-operative recovery was rapid with only minor, left-sided hemi-paresis.

Five days following surgery, severe weakness and ataxia returned. A cerebrospinal fluid analysis revealed moderately elevated pressure (by human normals), large numbers of neutrophils without toxic change, and no evidence of microorganisms. A ventriculoperitoneal shunt was installed for the possibly elevated intracranial pressure, and treatment with antibiotics and steroids was instituted for the ventriculitis. Dexamethasone was started at 10 mg every six hours PO for three days followed by an eight day tapering dose schedule. Chloramphenicol treatment of 1.25 grams every six hours IM or PO for ten days was also initiated. Ranitidine 150 mg PO every twelve hours was also given to prevent steroid induced gastric ulcers. Thereafter, recovery was uneventful. The gorilla was returned to the gorilla holding area 16 days post-operative and introduced to the gorilla group six weeks post-operative.
Histopathology, flow cytometry and immunohistochemistry were performed on portions of the mass obtained during surgery. Histologically, the mass consisted of a well-vascularized proliferation of a mostly monotonous population of small lymphocytes, with multiple fading follicles and vascular mural invasion. There also were occasional remnants of choroid plexus. The lymphocytes were polyclonal by flow cytometry and immunohistochemistry, although the majority of cells were CD3 positive by immunohistochemistry. Differential diagnoses for the tumor are an atypical, lymphoproliferative lesion, or a low-grade lymphoma arising from ectopic, follicular lymphoid hyperplasia.

Follow-up complete blood count, bone marrow cytology, and peripheral lymph node biopsy four months post-operative were normal. A follow-up MRI four months after surgery showed no evidence of remnants of tumor following surgery or tumor recurrence.
SPIROCHETE-INDUCED GASTRITIS IN BLACK AND WHITE COLOBUS MONKEYS
(Colobus guereza)

Jacqueline M. Zdziarski, DVM*
Chicago Zoological Society, Brookfield Zoo, 3300 South Golf Road, Brookfield, Illinois, 60513, USA

Sheila Davis, MS
University of Illinois Laboratories of Veterinary Diagnostic Medicine, Department of Diagnostic Microbiology, College of Veterinary Medicine, 2001 S. Lincoln Avenue, Urbana, Illinois, 61801, USA

Timothy F. Walsh, DVM and Robert D. Murnane, DVM, PhD
Zoological Pathology Program, University of Illinois College of Veterinary Medicine, Loyola University Medical Center, 2160 South First Avenue, Maywood, Illinois, 60513, USA

During February of 1994, a 7 mo, female, black and white colobus monkey (Colobus guereza) was found dead in a mixed species exhibit containing seven other colobus monkeys, mandrills (Papio sphinx), Sykes monkeys (Cercopithecus mitis albogularis), and sooty mangabeys (Cercocebus torquatus atys). The only significant finding on gross necropsy was marked gastric mucous secretion with moderate gastric dilatation. Histologically, hematoxylin and eosin (HE) stained sections revealed a moderate to severe, pyogranulomatous gastritis with extensive mucous secretion and massive, luminal to infiltrative bacterial proliferation. Warthin-Starry, Gram, and Giemsa stains of stomach revealed massive numbers of two distinct populations of silver staining, spiral bacteria. One of the two spiral bacteria was thick, long, and curved to gently coiling with a periodicity of approximately 4-7 µm. The other spiral bacterium was thin, shorter, and tightly spiraled with a periodicity of usually 1 µm or less. Both coiled bacteria extended deep into the glandular crypts and were often present within mucosal cells. Gram negative and Gram positive rods were also detected but considered insignificant. Aerobic and microaerophilic bacterial culture attempts were unsuccessful and acid fast stains were negative. In addition, transmission electron microscopy (TEM), performed on the gastric mucosa, showed at least two types of spiral organisms present both intra- and extracellularly: a long and large, 0.5-0.75 µm wide, loosely coiled bacterium, and a smaller, 0.2-0.4 µm wide, tightly coiled bacterium with periodicity of approximately 0.7-1.3 µm. Flagella-like structures were present on both organisms.

The remaining three male and four female colobus monkeys, aged 1.5-8 yr were evaluated for the presence of these spiral bacteria. The animals were anesthetized with ketamine HCl intramuscularly (15 mg/kg), intubated and maintained on isoflurane. A flexible fiberoptic, 8 mm gastroscope (Richard Wolf Medical, Vernon Hills, Illinois, 60018, USA) was inserted into the presaccus and saccus fermenting chamber. Four of seven animals had thick, white mucous covering large areas of the gastric mucosa, with one animal having little to no mucosa visible due to extensive adherence of the thick mucous. In four of seven monkeys, the gastric mucosa appeared normal, whereas two animals had thickened mucosa. The pars pylorica was not visualized. Ten gastric biopsies were taken from each animal for histopathology and culture; samples were also frozen at -70°C.
Histopathology of HE stained gastric biopsies revealed usually a mild to moderate, and occasionally moderate to severe infiltration of lymphocytes, plasma cells, macrophages, and in some areas, neutrophils in the lamina propria in all seven of the colobus monkeys. Mild to moderate crypt separation or loss was identified in six of seven animals. Warthin-Starry stain demonstrated silver positive, spiral bacteria on the surface, within mucous, occasionally in the crypts, and apparently within mucosal cells of all animals. Two morphologically distinct organisms were identified: one was a large, long, gently spiraled bacteria, and the second a smaller, tightly coiled organism. These bacteria morphologically matched those detected in the deceased juvenile.

Immediately after gastroscopy, tissues for culture were streaked onto plain chocolate agar plates and chocolate agar plates supplemented with vancomycin, polymyxin B and amphotericin B. Additionally, biopsies were placed in Leptospira spp. transport media, Treponema spp. liquid media, Helicobacter spp. liquid media, and Campylobacter spp. thioglycollate media. All samples were placed in GasPak anaerobic jars (Becton-Dickinson Microbiology Systems, Rutherford, New Jersey 070970, USA) containing a fresh catalyst and a microaerophilic gas pack for shipping. Approximately five hours later, the biopsies in the Campylobacter spp. thioglycollate media were streaked onto Columbia blood agar plates containing 5% sheep blood, and also streaked onto Campylobacter spp. blood agar plates which contained vancomycin, polymyxin B, amphotericin B and cephalothin®. These plates were placed in another anaerobic jar with a fresh gas pack. All samples were incubated at 37°C. All cultures were negative for Treponema spp. at 30 days and Leptospira spp. at 12 weeks. Numerous colony types were present in the Campylobacter and Helicobacter spp. media. All colonies were screened with Gram stains for any curved, spiral or coccoid organisms. Any bacteria that fit these criteria were subcultured onto fresh Columbia blood agar plates for propagation. Two distinct bacteria were identified. The predominant colony type, grossly small pinpoint colonies, was cultured from six of seven animals. The bacteria in these colonies are large, Gram negative, microaerophilic, oxidase and urease negative, gently curving to spiral organisms. A second, mucoid type colony was cultured from two of seven animals. These bacteria are smaller, Gram negative, microaerophilic, oxidase and urease negative, coccoid to curved organisms. On dark field microscopy, the first organism is gently curved to coiled, while the second is tightly coiled, and both organisms are highly motile in wet mounts. TEM of cultures of the two types of organisms were also performed. TEM of the predominant, pinpoint colonies revealed a larger, mostly 0.4-0.5 μm but occasionally up to 1 μm wide, and loosely coiled bacterium with a periodicity of approximately 2-3 μm. The second organism is smaller, mostly 0.3-0.4 μm and occasionally up to 0.6 μm wide, and much more tightly coiled. Periodicity of the smaller organism was not able to be accurately determined with TEM due to very tight coiling, but was <1 μm. Both organisms also had flagella-like structures externally, although they were more numerous on the smaller bacteria.

In summary, the death of the juvenile colobus was due to overwhelming infection of spirochetes of at least two types, and one or both of which were pathogenic. The remaining colobus all have low-grade gastritis associated with at least two types of spirochetes. The possibilities that the spirochetes are normal flora, opportunistic pathogens, or primary
pathogens in black and white colobus must be considered. Further, the spirochetes cultured from biopsies of live animals are not compatible by chemical tests with known, described spirochetes of mammals, although the smaller organism is morphologically similar to Helicobacter or Gastrospirillum spp., whereas the larger organism is unlike any previously described spirochete. Future characterization through scanning electron micrographs, and genetic and ribosomal analyses will aid in the identification of these organisms.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Steven A. Raverty and the John G. Hines Veterans Administration Hospital Histology Laboratory for technical assistance, and Alberta Schultheis for editing the abstract.
A 7 1/2 year old female western lowland gorilla (Gorilla g. gorilla) presented in September 1994 with a history of abortions in May 1993 and July 1994. The 1993 abortion occurred late in the first trimester and Listeria monocytogenes was isolated from the fetal tissues. The second abortion occurred at approximately mid gestation. Retrieved tissues were examined and found to be consistent with a normal placenta from a spontaneous abortus. There was no evidence of Hydatidiform Mole. One month following the second abortion the gorilla was anesthetized for musculo-skeletal radiographs and a reproductive tract examination. Prior to a planned invasive reproductive procedure, a urine HCG pregnancy test was conducted and was positive. Invasive procedures were not performed and abdominal ultrasonography demonstrated a small cystic adnexal structure. Serum Beta Subunit HCG (BHCG) levels were consistent with an early pregnancy.

Two months later the gorilla was depressed, partially anorexic and lethargic. The anesthetized animal had pale mucous membranes and a marked anemia. Ultrasonography revealed a large cystic structure in the left mid-caudal abdomen. Culdocentesis yielded frank nonclotting blood indicative of hemoperitoneum. An abdominal exploratory laparotomy demonstrated an 8 cm x 8 cm x 8 cm multiloculated hemorrhagic left ovarian mass. A left salpingo-oophorectomy was performed. Histological diagnosis of the abdominal mass was a choriocarcinoma.

A retrospective analysis of the serum BHCG levels (see table 1) determined that it would be a useful tumor marker. Chemotherapy was instituted using oral etoposide (VePesid®, Bristol Laboratories, Princeton, New Jersey 08543) at 100 mg/meter^2 and intramuscular methotrexate sodium (Lederle Parenterals Inc. Carolina, Puerto Rico 00630) at .4 mg/kg. The animal was immobilized at regular intervals during the course of treatment to monitor her progress. The gorilla’s attitude and condition improved and then remained stable for approximately six weeks following the initiation of chemotherapy. However, the BHCG continued to rise and over time, the anemia and weight loss were noted to become more
severe. Euthanasia was performed due to her failure to respond to chemotherapy and her failing physical condition. Necropsy revealed extensive metastasis of the tumor affecting multiple organ systems.

<table>
<thead>
<tr>
<th>DATE</th>
<th>Wt (kg)</th>
<th>Hct</th>
<th>BHCG (IU/L)</th>
<th>History</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/09/94</td>
<td>61.36</td>
<td>34</td>
<td>17,920</td>
<td>Initial exam-r/o pregnancy</td>
</tr>
<tr>
<td>11/10/94</td>
<td>60.90</td>
<td>22</td>
<td>1,098,075</td>
<td>Pre-op mass removal</td>
</tr>
<tr>
<td>11/28/94</td>
<td>62.95</td>
<td>24</td>
<td>400,000</td>
<td>Post-op, pretreatment</td>
</tr>
<tr>
<td>12/13/94</td>
<td>55.45</td>
<td>14</td>
<td>500,000</td>
<td>Post 1st round chemotherapy</td>
</tr>
<tr>
<td>12/30/94</td>
<td>57.72</td>
<td>21</td>
<td>770,000</td>
<td>Post 2nd round chemotherapy</td>
</tr>
<tr>
<td>1/11/95</td>
<td>55.45</td>
<td>17</td>
<td>2,370,000</td>
<td>Post 3rd round chemotherapy</td>
</tr>
<tr>
<td>1/30/95</td>
<td></td>
<td>13</td>
<td></td>
<td>Euthanasia</td>
</tr>
</tbody>
</table>

Table 1.: Progression of selected laboratory data on 6-yr-old female gorilla with choriocarcinoma.
ENTERIC Mycobacterium paratuberculosis IN A MANDRILL (Papio sphinx)

Timothy F. Walsh, DVM*, Robert D. Murnane, DVM, PhD
University of Illinois Zoological Pathology Program, Loyola University Medical Center Stritch School of Medicine, Rm. 3940, Bldg. 105, 2160 South First Avenue, Maywood, Illinois, 60153, USA

Robyn Barbiers, DVM
Lincoln Park Zoological Gardens, 2200 North Cannon Drive, Chicago, Illinois, 60614, USA

Michael Collins, DVM, PhD
University of Wisconsin, School of Veterinary Medicine, 2015 Linden Drive West, Madison, WI 53706-1102 USA

Mycobacterium paratuberculosis infection, is a common, global cause of chronic granulomatous enterocolitis in domestic and wild ruminants (Johne’s disease), with enormous economic impact. In addition to the usual ruminant host, the organism can infect a wide range of species, including monogastric animals and birds, however, most of these infections were experimental. Only one report of confirmed spontaneous M. paratuberculosis infection in a nonhuman primate species was found in the literature. This outbreak affected 29 of 38 individuals in a colony of stumptail macaques (Macaca arctoides). Additionally, the association of M. paratuberculosis in human patients with Crohn’s disease has sparked much interest and controversy regarding a possible etiologic relation. This report documents a case of M. paratuberculosis infection in a mandrill (Papio sphinx).

A 21 month old, 4.26 kg, female mandrill arrived at Lincoln Park Zoo and was placed in 30 day quarantine on July 22, 1993. The animal was negative for intradermal tuberculin test (0.1 Coopers Mammalian OT in the right, superior palpebra) and for enteric pathogens including Campylobacter sp. and Salmonella sp. Serology was reported as negative for herpes virus SA8, Herpes simiae (herpes B), measles, simian immunodeficiency virus, simian retroviruses 1, 2 and 5, simian T-cell leukemia virus-1 and encephalomyocarditis virus, while positive for cytomegalovirus. The animal was vaccinated for tetanus (toxoid 0.5 ml IM) and measles (0.5 ml IM), and was de-wormed with ivermectin (0.2 mg/kg SQ). Serum chemistries, CBC, and abdominal and thoracic radiographs were normal and the animal was released from quarantine August 24, 1993.

On September 20, 1993, the animal had watery diarrhea and a distended abdomen. Balantidium coli and Entamoeba coli were found by fecal exam and were considered normal flora. Three days later the animal was examined for recurring bloat and a traumatic wound to the right third digit. The animal was thin, weighing 4.2 Kg, but otherwise considered normal. ClavamoxR (15 mg/kg PO BID) was prescribed for the traumatic wound. On November 11, 1993 the animal was again bloated and weighed only 3 kg. An elevated alkaline phosphatase (851 IU/L) and CPK (431 IU/L) were the only significant aberrations in a CBC and serum chemistry panel. Recurrent diarrhea and continued weight loss prompted abdominal radiographs, fecal culture, and repeat blood chemistries on March 18, 1994. Radiographs revealed gas distended bowel loops and sand-like material with dense, metallic specks in the distal colon. Fecal cultures for Salmonella, Shigella, Campylobacter,
and *Yersinia* sp were negative. The only significant blood chemistry abnormality was elevated alkaline phosphatase (570 IU/L). Small metallic slivers were found in the stool on March 19, 1994, and believed to have come from stainless steel pads used to block pipes from pests. Three days later the animal weighed 2.54 kg, and gastrointestinal contrast series suggested a possible foreign body in the cecum and a colonic stricture. With dietary supplementation, weight increased to 3.17 kg by March 25, 1994. However, by April 4, 1994, weight had dropped to 3.07 kg, and a nasogastric tube was intermittently placed to supplement caloric intake. Fecal trypsin was reported as inadequate on April 5, 1994, and pancreatic enzymes were added to the rations. Blood lead levels were not detectable. Exploratory surgery on April 26, 1994 revealed prominent, enlarged, firm, mesenteric lymph nodes of normal color and consistency. A small amount of ingesta and large amounts of gas were present throughout the gastrointestinal tract. Full thickness biopsies of colon, cecum, small intestine, and biopsies of mesenteric lymph nodes were collected. The animal recovered from anesthesia but subsequently died April 29, 1994. At necropsy on April 30, 1994, the animal was in fair postmortem condition, emaciated, and had a severely distended abdomen. The stomach was markedly distended with ingesta, and the intestines were severely distended with large amounts of ingesta and gas. Lymph nodes throughout the body were prominent. Representative samples of all organs were fixed in 10% neutral buffered formalin, routinely processed for histopathology, sectioned at 3-6 μm, and stained with hematoxylin and eosin. Throughout the duodenum, jejunum and ileum, histopathology revealed severe villi blunting and fusion, and crypt loss. The lamina propria had moderate to extensive accumulations of large, granular, occasionally multinucleated histiocytes containing massive numbers of small, usually 1-2 μm long, acid-fast positive (Ziehl-Neelsen stain), rod bacteria. In numerous areas of intestine, the histiocytes extended through the muscularis mucosa into the submucosa. Scant to moderate numbers of lymphocytes and plasma cells were scattered throughout the lamina propria, and there were rare crypt abscesses. The cecum and colon also had mild to severe infiltration of similar, acid-fast bacteria-laden macrophages in the lamina propria, and mild to moderate accumulations of lymphocytes and plasma cells. Mucosal associated lymphatic nodules were common and occasionally contained macrophages with identical acid-fast organisms. Mesenteric, sublumbar, and ileal lymph nodes were mild to moderately depleted of lymphocytes, and edematous. The mesenteric and ileal lymph nodes also had small to moderately severe numbers of macrophages containing acid-fast bacteria, predominantly in the subcapsular and medullary sinuses and rarely within the follicular centers. Similar lesions were present in the antemortem surgical biopsies. Additionally, there was moderately severe, multifocal, myocardial fibrosis, mild to moderate, multifocal, pyogranulomatous interstitial pneumonia with no acid-fast bacteria, and lingual candidiasis. The histologic lesions associated with the intestines and mesenteric lymph nodes in this animal were similar to the spectrum of lesion reported in the colony of stump-tailed macaques. Samples of feces, ileum and colon were processed for isolation of *M. paratuberculosis*, using a radiometric culture medium as previously described. Tissues were homogenized with an equal volume of sterile saline in a Stomacher. Three grams of homogenized tissue
suspension or feces was mixed with 30 ml of 1.0% hexadecylcetylpyridinium chloride (HPC). After overnight decontamination and settling, the uppermost 10 ml of the suspension was aspirated into a syringe and filter concentrated using a 13 mm diameter, 3 µm pore diameter polycarbonate filter in a Swinex filter holder. The filter was placed into a BACTEC 12B vial (Becton-Dickinson, Sparks, MD) supplemented with 1.0 ml egg yolk suspension (Difco, Detroit, MI), mycobactin (1 µg/ml) (Allied Monitor, Fayette, Mo.), vancomycin (10 µg/ml), amphotericin B (20 µg/ml), and nalidixic acid (30 µg/ml). Growth was monitored weekly on a BACTEC 460 instrument. Growth of acid fast bacteria was detected after 4-6 weeks. The Mycobacterium sp. isolated in each vial was confirmed to be M. paratuberculosis using the commercial PCR-amplified DNA probe for IS900 (IDEXX Laboratories, Inc. Westbrook, ME) proven to be specific for the organism.

In summary, the prolonged disease course was caused by infection with M. paratuberculosis, and death was due to the combined effects of this infection and myocardial fibrosis, exacerbated by the stress of surgery and anesthesia.

Infection with M. paratuberculosis in ruminants predominantly occurs at a young age and has a protracted incubation, with symptoms often not apparent for many years. These circumstances probably occurred in this mandrill. Infection with M. paratuberculosis also poses a diagnostic dilemma in non-human primates, particularly if large numbers of acid fast organisms are not being shed in feces, or if mycobacterial agents are not considered as a differential diagnosis. Indeed, without current improved diagnostic techniques, past cases of M. paratuberculosis infection may have been overlooked, or misdiagnosed as M. avium infection. The protracted and insidious nature of this disease resulted in enclosure-mates and keepers being exposed to the organism. Unfortunately, there is not adequate information on the pathogenesis of this organism in primates to accurately assess the risks and significance for other members of the troop. Testing of exposed mandrills at this institution and the institution of origin are in process.

The authors thank Ms. Debra Teubert for her excellent technical assistance in performance of the microbiological cultures and Hines Veterans Administration Hospital Histology Laboratory for the preparation of histopathologic materials.

LITERATURE CITED

B VIRUS IN ZOO MACAQUES: CURRENT ISSUES

Richard J. Montali, DVM*
Division of Pathology, National Zoological Park, Smithsonian Institution, Washington, DC 20008, USA

Macaques (genus, Macaca), Asian primates in the family Cercopithecidae are divided into the following species groups: Fascicularis, include the crab-eating (cynomolgus monkey), Japanese, Taiwan and Rhesus macaques; Silenus-Sylvanus, include liontail macaques and the Celebes and Barbary "apes"; Sinica, include toque and bonnet macaques; and Arctoides, the stump-tail macaque. Macaques are an interesting group of primates that have been traditionally exhibited in zoo collections. Of the 16 or so species currently listed liontails are endangered, Celebes are near endangered and Barbary macaques are considered vulnerable.

Even though some macaque species have qualified for successful zoo exhibitry and conservation programs, they have an unenviable trait of carrying an alpha herpesvirus, herpes B, which is infectious for and potentially fatal in humans. The virus, first recovered from researcher Dr. B in 1933 has been since found to be enzootic among laboratory and wild macaques. In the macaque, B virus is mostly a latent disease (as much as herpes simplex is in humans) and only occasionally manifests as oral or genital ulcers. It has been uncommonly transmitted to non-macaque primates and rarely has caused fatal disease in the primary macaque host itself. Although the epizootiology of B virus is not fully established, studies in laboratory macaques have shown that B virus is spread to post-adolescent conspecifics mainly by intimate exposure within colonies, and that virus shedding-frequency at any given time is very low - often 2-3% even in colonies 100% seropositive for herpes B.

The less than 40 human cases of herpes B infections reported in humans since the index case in 1933, translates to fewer than 1.5 cases every other year, despite the many thousands of contacts known to occur between macaques and their caretakers. This indicates that transmission of herpes B from a macaque to a human is not a casual event. The virus is very labile, and human infection usually requires direct inoculation by a scratch or bite, or exposure of broken skin to secretions from a macaque shedding the virus. Other factors such as degree of susceptibility or resistance of the exposed individual may influence the infectivity of the virus and the severity of infection.

Notwithstanding the low hazard for infection, herpes B in humans can be life threatening, and strict prophylactic procedures have been developed to further reduce the risk to those handling macaques. Laboratory methods for diagnosing herpes B by the latest immuno and molecular techniques are available and new guidelines for the prevention and treatment of B virus infections in exposed persons have recently been published.

Results of some sporadic serosurveys of macaque species in several zoos have shown liontail, Japanese, Tibetan, Celebes and Barbary apes, and perhaps some other non macaque species
seropositive for herpes B or B-like viruses (Gledhill, L. unpublished SSP report; Hilliard, J., personal communication). It is likely that herpes B seropositivity of macaques has been pervasive in zoo and wildlife parks; yet, human clinical cases of infections with this virus have never been documented in these settings.

The awareness of a disease potentially fatal to humans has created concern and led some zoo managers to remove macaques from their collection; others have strongly opposed these sentiments and actions. The purpose of this presentation is to provide a balanced medical overview of the herpes B question from a veterinary perspective in order to ascertain the chances of zoo primate handlers actually acquiring the disease. In addition to the clean record established already for no human B virus at zoos, and from the vast amount of preventative knowledge gained with laboratory macaques, the possibilities of zoo workers contracting this disease in the future seem to be even less likely.

Zoos should be able to continue to maintain macaques safely with little stress to the keeper force if they adopt a plan that provides preventative methods and education to zoo employees. This should be carried out by a veterinary staff and medical consultants that are knowledgeable about B virus.

With reference to special management practices, all macaques in zoo collections should be considered potentially B virus carriers. Although serologic testing should be an option, trying to sort animals by casual screening of individuals is not an accurate determination of B virus status because seronegative animals may still be carrying the virus latently and seroconvert at a later time. Likewise, because of the nature of the herpesvirus, seropositive animals may revert to a negative status, yet still harbor the virus in a non-shedding state with the possibility of exacerbation. Therefore, dividing groups of zoo macaques by serologic status to the B virus is probably not a useful exercise at this time.

Prophylactic medical procedures for human exposures should be implemented which include special first-aid practices to eliminate any potential viral contamination of a wound, followed by diagnostic procedures to document viral presence and treatment strategies as outlined in the new guidelines. When done so, the hazard of human B virus in a zoo is probably less than that of rabies in an enzootic area (like the raccoon outbreak in Northeastern U.S), or even from a fatal injury by a megavertebrate or large felid.

In addition to these preventative methods, the development of specific-pathogen-free (SPF) colonies of several laboratory macaque species free from B virus has shown good results, and offers promise for eventually eliminating this virus in all captive macaque groups. It is likely that these methods could be applied particularly to the imperiled macaque species and should be perhaps considered by SSP and TAG veterinarians advising these groups as another option for resolving the B virus "dilemma".
LITERATURE CITED


Langers - B-like virus. J. Hilliard went more.
AN IDIOPATHIC PROLIFERATIVE DISEASE OF BONE IN TWO SUBSPECIES OF RUFFED LEMUR (Varecia variegata variegata and Varecia variegata rubra)

Martha Weber, DVM*, Nadine Lamberski, DVM
Riverbanks Zoological Park and Botanical Garden, PO BOX 1060, Columbia, South Carolina 29202, USA

Kirk Heriot, MD, PhD
Lexington Medical Center, West Columbia, South Carolina 29169, USA

A proliferative bone disease was diagnosed in one black and white ruffed lemur (Varecia variegata variegata) and one red ruffed lemur (V. v. rubra) as an incidental finding during annual physical exam and tuberculin skin testing. The lesions noted on physical exam consisted of bilaterally symmetric firm swellings along the diaphyses of the bones of the distal limbs. Radiographs showed periosteal proliferation causing irregular enlargement of bone that involved the radii, ulnae, tibiae, and fibulae of each animal. Complete blood counts were unremarkable. One animal had a serum alkaline phosphatase of 2250 IU/L while the alkaline phosphatase of the other was within normal limits. Electrophoretic fractionation of the alkaline phosphatase was not useful in determining the source of the enzyme. Serum levels of ionized calcium, vitamin D, and parathormone did not indicate excessive parathyroid activity. Nuclear scintigraphy revealed increased uptake of technetium-99m-methylene diphosphonate in the affected limbs and in each animal's skull. Histologic examination of biopsies from affected bones showed extensive remodeling of the bone with prominent and disorganized cement lines. This condition is distinctly different from the previously reported bone disease of black lemurs and is most similar to Paget's disease in humans.
TREATMENT OF BILATERAL NASAL POLYPOSIS AND CHRONIC REFRACORY INHALANT ALLERGIC RHINITIS IN A CHIMPANZEE (Pan troglodytes)

Genevieve A. Dumonceaux, DVM*, Lyndsay G. Phillips, DVM, Diplomate ACZM
Veterinary Medical Teaching Hospital, University of California, Davis, California 95616, USA

Nadine Lamberski, DVM
Riverbanks Zoological Park, Columbia, South Carolina 29202, USA

Donald Clutter, MD
Sacramento Ear, Nose and Throat Surgical and Medical Group Inc., 3810 J St., Sacramento, California 95816, USA

Stephen M. Nagy, Jr., MD
Midtown Allergy Clinic, 4801 J St., Sacramento, California 95819, USA

A 30 yr old female chimpanzee (Pan troglodytes) was evaluated for a 15 yr history of inhalant allergies. This animal had recurrent annual episodes of facial swelling, nasal discharge, epiphora OU, and open-mouth breathing the past three years. Initial symptomatic treatment with prednisolone, and later the antihistamine terfenadine (SeldaneR, Marion Merrell Dow, Inc., 9300 Ward Parkway, PO Box 8480, Kansas City, Missouri 64114-0480, USA) reduced the severity of the clinical signs. In the spring of 1994 the signs reoccurred (facial swelling, purulent nasal discharge bilaterally, open-mouth breathing, epiphora OU) with increased severity and persistence. Response to terfenadine was equivocal. Only temporary response was seen with the switch to a second antihistamine, loratidine (OaritinR, Schering Corporation, Galloping Hill Road, Kenilworth, New Jersey 07033, USA) 10 mg p.o. s.i.d. Initially, the purulent nasal discharge responded to cephalexin (KeflexR, Eli Lilly and Company, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana 46285, USA) 500 mg p.o. b.i.d. but recurred and became refractory to a second treatment regimen.

In the fall of 1994 anesthesia with ketamine hydrochloride (KetasetR, Fort Dodge Laboratories, Fort Dodge, Iowa 50501, USA) 600 mg i.m. and isoflurane (AerraneR, Anaquest, Inc., 110 Allen Road, Box 804, Liberty Corner, New Jersey 07938-0804, USA) was performed for diagnostic examination including an IgE FAST-plus (fluorescent allergosorbent test) (Immugenex, Inc. Reference Laboratories, Palo Alto, California 94303, USA) and rigid nasal endoscopy. Multiple large polypoid masses were found bilaterally completely obstructing nasal air flow. The polyps, purulent material and plant fibers resembling hay were removed from the nasal cavities. An area of erosion was identified in the nasal septum communicating between the two passages. Upon anesthetic recovery normal nasal breathing commenced and persisted.

Histopathology of the masses yielded a diagnosis of inflammatory polyps. One polyp contained moderate hyperplastic epithelial change, but there was no evidence of neoplasia. In 1987 a close correlation was found between the IgE FAST-plus test and intradermal
allergy testing. The current FAST-plus test results (based on human reference levels) indicated significant levels of antigen specific IgE to several species of Sacramento Valley trees and grasses including perennial ryegrass, cultivated oats, oak, English plaintain, sycamore, Russian thistle, and cottonwood. An oral desensitization protocol was instituted similar to investigational techniques in human immunotherapeutic regimens. The treatment mix was placed in fruit juice at increasing dosage concentrations over several weeks. The chimpanzee is currently on an every other day dose of the most concentrated mix.

Immunotherapy decreases production of specific IgE. The goal is eventual immune system desensitization to offending allergens. The IgE FAST-plus test (a human clinical laboratory test) was performed to avoid prolonged and repeated anesthetic episodes for intradermal testing and desensitization.

The development of the polyps is believed to be due to antigenic stimulation and chronic irritation. At the one month post-operative recheck, minor polyp removal was performed. These polyps may have been residual mucosal remnants remaining after the initial treatment.

Periodic reevaluation is planned to monitor the animal's response to therapy. To date, endoscopic surgery and immunotherapy have eliminated the clinical signs. It is anticipated that this effect will continue even during the height of the chimpanzee's allergy season.

LITERATURE CITED

5. Sjovall, P. Oral hypo sensitization in allergic contact dermitis, 1990, Seminars in Dermatology, 9(3)206-209.
PRELIMINARY INVESTIGATIONS IN THE USE OF ORALLY ADMINISTERED CARFENTANIL CITRATE AND DETOMIDINE TO IMMOBILIZE DOMESTIC GOATS (Capra hircus)

Jonathan Sleeman, MRCVS* and Edward Ramsay, DVM
The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, Knoxville, Tennessee 37901-1071, USA

Ten healthy female domestic goats (Capra hircus) from the Knoxville Zoological Gardens were used to investigate the effectiveness of orally administered carfentanil citrate and detomidine with an absorption enhancer to induce and maintain chemical immobilization. The animals were fasted for 12-24 hours prior to anesthesia. The goats were physically restrained and the drug administered directly into the buccal cavity using a 3 ml syringe (without a needle).

Inductions of anesthesia were monitored every minute. Once sternal recumbency was achieved, temperature, pulse, respiration rate, oxygen saturation and indirect blood pressure measurements were taken every five minutes for 45 minutes. An assessment of depth of anesthesia was also made every five minutes by noting presence of palpebral and withdrawal reflexes. Muscle relaxation was noted as either poor, moderate or good. Reversal of the anesthesia was achieved by administering naltrexone at a dose of 100 mg naltrexone:1 mg carfentanil delivered and intravenous yohimbine or atipamezole at doses of 0.375 mg/kg or 0.3 mg/kg, respectively. The stages of recovery were noted at one-minute intervals.

Table 1 summarizes the anesthetic inductions of the preliminary trials.

Table 1

<table>
<thead>
<tr>
<th>Dose</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 mg/kg</td>
<td>carF</td>
</tr>
<tr>
<td>60 mg/kg</td>
<td>detomidil</td>
</tr>
</tbody>
</table>

Equal volume of 0.5% Sapcin
### Table 1: Summary of Anesthetic Inductions of Preliminary Trials.

<table>
<thead>
<tr>
<th>Drug Combination</th>
<th>Duration of Prancing Gait/Excitement Phase (MIN)</th>
<th>Time To Sternal Recumbency (MIN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 µg/kg carfentanil</td>
<td>17 (Anesthesia reversed)</td>
<td>Never achieved</td>
</tr>
<tr>
<td>60 µg/kg carfentanil</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>60 µg/kg carfentanil + 6ml molasses</td>
<td>18 (Anesthesia reversed)</td>
<td>Never achieved</td>
</tr>
<tr>
<td>60 µg/kg carfentanil + oral bandage</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>60 µg/kg carfentanil + absorption enhancer BL-9</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>60 µg/kg carfentanil + absorption enhancer Saponin</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>60 µg/kg detomidine</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>120 µg/kg detomidine</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>60 µg/kg carfentanil + 60 µg/kg detomidine + BL-9</td>
<td>5</td>
<td>27</td>
</tr>
<tr>
<td>90 µg/kg carfentanil + 60 µg/kg detomidine + Saponin</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15</td>
</tr>
</tbody>
</table>
Anesthetic monitoring data on 21 free-ranging adult African elephants (*Loxodonta africana*) including pulse oximetry readings on 16 elephants, were collected in October 1992 (n=12) and May 1993 (n=9) as part of the Botswana Department of Wildlife and National Park's elephant habitat utilization research program. All 21 elephants were handled in northern Botswana (capture sites: 17° 50' - 19° 32' S; 23° 07' E). One partial dart failure was suspected of leading to a prolonged time to recumbency, and this animal was excluded from the data set. The remaining 20 animals are discussed here, with 16 of them providing data via the pulse oximeter. Mean capture drug doses +/- SD (n=20) were: 9.5 +/- 0.53 mg etorphine hydrochloride with 2000 +/- 0 IU hyaluronidase. All elephants were deemed safely immobilized in sternal or lateral recumbency between 4.9 and 14.3 minutes post-darting, with the mean time to recumbency being 8.7 +/- 2.4 minutes. Those going down in sternal recumbency were pushed into lateral recumbency. The anesthetic monitoring protocol consisted of rectal temperature monitoring; continuous monitoring of heart and respiratory rates; cardiothoracic auscultation; palpation of auricular pulse for quality and regularity; and continuous real-time pulse rate and percent oxygen saturation of hemoglobin (SpO2) monitoring. Actual duration of pulse oximetry monitoring ranged from 3 to 24 minutes, with the mean duration (n=16) being 8.1 minutes of pulse oximetry monitoring. Differences between minimum and maximum SpO2 values for any given elephant ranged from 1 to 6 percentage points. The lowest SpO2 value recorded for any individual elephant was 70%; this elephant ranged from 70-75% over the 6 minutes it was evaluated. The highest SpO2 reading was 96%; this elephant ranged from 92-96% over the 9 minutes it was evaluated. Fifteen out of 16 oximeter-monitored elephants had mean SpO2 values over 80%, with 11 having mean SpO2 values over 85%. Standard deviations of the SpO2 readings over time for the 16 individual elephants ranged from +/- 0.52% to +/- 2.4%. The mean SpO2 of the composite "average" elephant was 87.3 +/- 2.8%. The generally stable SpO2 and pulse trends coincided with other normal clinical observations. All 21 animals walked away from their immobilizations without apparent ill effects.

Table 1 includes drug dosage data for the 20 elephants, as well as basic physiological and anesthetic event parameters for all 20 field immobilization exercises, along with ranges and means +/- standard deviations taking all data into account. Table 2 provides SpO2 readings over time for all 16 elephants successfully monitored with a pulse oximeter. Figure 1 provides a mean SpO2 profile and a mean pulse rate profile, a composite representing the "average elephant," derived from the data in Table 2 as well as pulse rate data collected. All SpO2 and pulse readings reported represent means for any given minute, and all times are +/- 30 seconds due to rounding off to the nearest minute for ease of comparison between animals. Individual elephants may have had gaps in their SpO2/pulse records for...
a variety of reasons, including occasional sensor detachment, intermittent failure to obtain a reading, or lapses in manual recording of data from the pulse oximeter that lacked a built-in printer (Nellcor N-180). Thus, any one minute in Figure 1 represents the average of readings from 1 to 13 elephants. The pulse oximeter was attached as soon as an elephant was observed to be safely anesthetized in lateral recumbency. Eleven minutes represents the soonest point in time post-darting that it was possible to get to at least one animal and successfully start to get pulse oximeter readings (a number of other anesthetic monitoring and research activities were being carried out simultaneously). The pulse oximeter was detached immediately prior to administration of the diprenorphine. Thirty-four minutes represents the longest point in time post-darting that the pulse oximeter was still on at least one elephant.

While the elephant's oxyhemoglobin dissociation curve is shifted to the left compared to that of adult humans, pulse oximeters measure oxygen saturation directly and thus are not affected by the position of this curve. Spectrophotometric comparisons between elephant and human hemoglobin along with further work correlating SpO₂ readings with arterial blood gas values in African elephants are necessary to facilitate quantitative clinical interpretation of data obtained from elephant pulse oximetry. For now, real-time observation of SpO₂ trends can be considered a valuable adjunct to basic elephant anesthetic monitoring protocols in the field or captive setting.
Table 1. Basic anesthetic event parameters for twenty *Loxodonta africana* field immobilization exercises. E = etorphine hydrochloride; D = diprenorphine hydrochloride. All twenty elephant darts contained 2000 IU hyaluronidase mixed with the etorphine. All times are in minutes. f = female; m = male. SD = standard deviation. NA = not available.

<table>
<thead>
<tr>
<th>ID</th>
<th>#/sex</th>
<th>Shoulder height (m)</th>
<th>E Dosage (mg)</th>
<th>D Dosage (mg)</th>
<th>Time to first effect</th>
<th>Time to recumbency</th>
<th>Time to first arousal post-D</th>
<th>Time to standing post-D</th>
<th>Rectal temp. (°C)</th>
<th>Resp. rate (range / min)</th>
<th>Total elapsed time</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/f</td>
<td>2.4</td>
<td>8.5</td>
<td>20.4 / 10.8</td>
<td>4.5</td>
<td>1.27</td>
<td>2.0</td>
<td>35.9</td>
<td>4 - 8</td>
<td>27.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/f</td>
<td>2.64</td>
<td>8.5</td>
<td>20.4 / 10.8</td>
<td>9.5</td>
<td>4.0</td>
<td>5.32</td>
<td>36.5</td>
<td>8 - 12</td>
<td>36.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/m</td>
<td>2.53</td>
<td>8.5</td>
<td>20.4 / 10.8</td>
<td>2.03</td>
<td>1.8</td>
<td>2.33</td>
<td>35.8</td>
<td>8</td>
<td>24.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/f</td>
<td>2.65</td>
<td>8.5</td>
<td>20.4 / 10.8</td>
<td>6.0</td>
<td>1.57</td>
<td>3.7</td>
<td>36.2</td>
<td>8</td>
<td>26.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/f</td>
<td>2.55</td>
<td>9.8</td>
<td>24.0 / none</td>
<td>4.6</td>
<td>1.85</td>
<td>2.85</td>
<td>37.1</td>
<td>8</td>
<td>27.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/f</td>
<td>2.56</td>
<td>9.8</td>
<td>24.0 / none</td>
<td>3.65</td>
<td>3.05</td>
<td>3.97</td>
<td>35.6</td>
<td>8</td>
<td>28.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/f</td>
<td>2.62</td>
<td>9.8</td>
<td>24.0 / none</td>
<td>3.22</td>
<td>2.67</td>
<td>9.6</td>
<td>35.8</td>
<td>8 - 12</td>
<td>33.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/f</td>
<td>2.7</td>
<td>9.8</td>
<td>24.0 / none</td>
<td>4.57</td>
<td>2.4</td>
<td>2.63</td>
<td>37.0</td>
<td>4 - 8</td>
<td>29.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/f</td>
<td>2.6</td>
<td>9.8</td>
<td>24.0 / 12.0</td>
<td>2.58</td>
<td>1.72</td>
<td>8.45</td>
<td>35.6</td>
<td>4 - 8</td>
<td>27.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/f</td>
<td>2.75</td>
<td>9.8</td>
<td>24.0 / 12.0</td>
<td>5.32</td>
<td>2.47</td>
<td>4.45</td>
<td>35.8</td>
<td>4</td>
<td>27.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/m</td>
<td>2.66</td>
<td>9.8</td>
<td>24.0 / 12.0</td>
<td>2.37</td>
<td>2.78</td>
<td>2.93</td>
<td>36.3</td>
<td>8</td>
<td>21.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13/f</td>
<td>2.7</td>
<td>9.8</td>
<td>24.0 / 12.0</td>
<td>3.47</td>
<td>2.17</td>
<td>2.48</td>
<td>35.7</td>
<td>4 - 8</td>
<td>24.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14/f</td>
<td>2.47</td>
<td>9.8</td>
<td>24.0 / 12.0</td>
<td>2.33</td>
<td>1.92</td>
<td>2.1</td>
<td>37.8</td>
<td>8</td>
<td>31.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15/f</td>
<td>2.6</td>
<td>9.8</td>
<td>24.0 / 12.0</td>
<td>4.1</td>
<td>12.47</td>
<td>2.67</td>
<td>2.9</td>
<td>36.7</td>
<td>8 - 12</td>
<td>39.37</td>
<td></td>
</tr>
<tr>
<td>16/f</td>
<td>2.57</td>
<td>9.8</td>
<td>24.0 / 12.0</td>
<td>2.83</td>
<td>1.38</td>
<td>1.83</td>
<td>37.3</td>
<td>8 - 12</td>
<td>31.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17/f</td>
<td>NA</td>
<td>9.8</td>
<td>24.0 / 12.0</td>
<td>3.2</td>
<td>2.08</td>
<td>3.17</td>
<td>37.5</td>
<td>4 - 8</td>
<td>33.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18/f</td>
<td>2.45</td>
<td>9.8</td>
<td>24.0 / 12.0</td>
<td>3.87</td>
<td>1.72</td>
<td>2.12</td>
<td>37.6</td>
<td>6 - 8</td>
<td>27.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19/f</td>
<td>2.72</td>
<td>9.8</td>
<td>24.0 / 12.0</td>
<td>3.85</td>
<td>1.33</td>
<td>2.68</td>
<td>35.9</td>
<td>6</td>
<td>23.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20/f</td>
<td>2.87</td>
<td>9.8</td>
<td>24.0 / 12.0</td>
<td>2.65</td>
<td>2.42</td>
<td>2.93</td>
<td>36.1</td>
<td>4 - 8</td>
<td>32.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21/f</td>
<td>2.77</td>
<td>9.8</td>
<td>24.0 / 12.0</td>
<td>1.55</td>
<td>2.47</td>
<td>3.62</td>
<td>36.3</td>
<td>8</td>
<td>26.77</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| mean | 2.62 | 9.5 | 23.3 / 11.7 | 3.8 | 8.7 | 2.2 | 3.6 | 36.4 | NA | 29.6 |
| SD   | 0.12 | 0.53 | 1.5 / 0.54 | 1.8 | 2.4 | 0.67 | 2.1 | 0.72 | NA | 4.8 |
| (range) | 2.40 | (8.5 | (20.4 - 2.87 | (15.5 | (4.92 | (1.27 | (1.83 | (35.6 | (4 - 12 | (21.9 | (39.37 |

*aOnly includes elephants that received some IM diprenorphine.*
Table 2. Percent oxygen saturation of hemoglobin (SpO2) readings over time for all 16 elephants successfully monitored with a pulse oximeter, as described in the text. Minute 1 is the minute during which the elephant was darted. REC = the minute during which the elephant became recumbent. ST = the minute during which the elephant stood. SD = standard deviation. Duration is the total inclusive time in minutes between the first and the last SpO2 reading. Pulse oximeter was attached as soon as animal was observed to be safely anesthetized in lateral recumbency. Pulse oximeter was detached immediately prior to administration of anesthetic reversal agent.
Figure 1.

Mean (+/- SD) SpO₂ and pulse rate profiles, representing the "average elephant," derived from data on adult African elephants (n=16) anesthetized with etorphine/hyaluronidase as described in the text. Solid circles: SpO₂ (%) versus Time (minutes); empty squares: Pulse Rate (per minute) versus Time (minutes). Minute 1 was defined as starting when the dart first hit the elephant. Pulse oximeter was attached as soon as animal was observed to be safely anesthetized in lateral recumbency. Pulse oximeter was detached immediately prior to administration of anesthetic reversal agent.

<table>
<thead>
<tr>
<th>Summary</th>
<th>SpO₂ (%)</th>
<th>Pulse rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>minimum value:</td>
<td>82.8</td>
<td>43</td>
</tr>
<tr>
<td>maximum value:</td>
<td>93</td>
<td>54</td>
</tr>
<tr>
<td>mean value (+/- SD) for total anesthetic period:</td>
<td>87.3 (+/- 2.8)</td>
<td>48.2 (+/- 2.4)</td>
</tr>
</tbody>
</table>
IMMOBILIZATION AND MONITORING OF FREE-RANGING WILD DOGS (*Lycaon pictus*) USING A KETAMINE/XYLAZINE/ATROPINE COMBINATION, YOHIMBINE REVERSAL AND PULSE OXIMETRY

Steven A. Osofsky, DVM*
Botswana Dept. of Wildlife and National Parks, Wildlife Veterinary Unit, Box 131, Gaborone, Botswana; current affiliation: Fossil Rim Wildlife Center, P.O. Box 2189, Glen Rose, TX 76043, USA

John W. McNutt, MS
University of California, Division of Environmental Studies, Davis, CA 95616, USA

Karen J. Hirsch, DVM
Botswana Dept. of Wildlife and National Parks, Wildlife Veterinary Unit, Box 131, Gaborone, Botswana; current affiliation: Camp Bowie Animal Clinic, 5709 Lovell Avenue, Fort Worth, TX 76107, USA

Free-ranging adult wild dogs (*Lycaon pictus*) ranging in age from 2.5 to 10+ years, habituated as part of an ongoing behavioral ecology research program, were successfully immobilized in November 1993 using 35 to 50 mg ketamine, 60 mg xylazine and 1.25 mg atropine. Administration of 5 mg of yohimbine, half intravenously and half intramuscularly, provided effective reversal, the yohimbine having been administered as soon as 32 minutes 55 seconds after darting in one dog. The mean +/- SD ketamine:xylazine ratio was 0.73 +/- 0.12:1, considered relatively low for successful use on free-ranging carnivores. Average drug doses +/- SD (n=4), based on educated estimates of body mass, were 1.6 +/- 0.22 mg/kg ketamine; 2.2 +/- 0.21 mg/kg xylazine; 0.046 +/- 0.0041 mg/kg atropine and (n+5) 0.19 +/- 0.019 mg/kg yohimbine. All dogs were immobilized in sternal recumbency within 12 to 13 minutes of darting, with the average time to sternal immobility being between 10 and 11 minutes. All dogs exhibited complete skeletal muscle relaxation, and none exhibited any signs of arousal during physical examinations/biosampling. All dogs were standing within 10 minutes 14 seconds of yohimbine administration, with the mean time to standing being 6 minutes 38 seconds (=/- 3 minutes 23 seconds). Table 1 summarizes basic anesthetic event parameters.

Continuous real time monitoring of pulse rate and percent oxygen saturation of hemoglobin (SpO2) trends, in addition to standard anesthetic monitoring procedures, indicated no adverse physiological responses to this drug combination (Figure 1). All five dogs exhibited relatively stable SpO2 profiles for the duration of monitoring (Figure 1). Differences between minimum and maximum SpO2 values for any given dog ranged rom 6 to 11 percentage points. The average of the five dogs' mean SpO2 values was 89% +/- 4.9%. The overall stable trends coincided with other normal clinical observataions. Figure 1 summarizes basic physiological parameters, including pulse oximetry and pulse rate profiles compiled from data recorded by the pulse oximeter for the five field immobilization exercises. On all graphs "0 minutes" refers to the time the pulse oximeter obtained its first set of readings.
The times to recumbent immobility post-darting and times to standing post-yohimbine found in this study are deemed safe for the free-ranging setting. All dogs appeared normal and returned to their packs shortly after the procedures. The ability to have dogs up and alert within minutes of administration of an antagonist is of the utmost importance in a free-range situation in the northern Botswana study site (19°07.32'S; 23°E) with its high predator density and omnipresent surface water. While the sample size for this study is small (n=5), this inexpensive, reversible, nonnarcotic combination previously unreported in free-ranging wild dogs, meets criteria for maximizing anesthetic and post-anesthetic safety. Since completion of this pilot project, 15 more Botswana wild dogs have been immobilized without incident using this protocol.
Table 1. Basic anesthetic event parameters for five *Lycaon pictus* field immobilization exercises. K = ketamine hydrochloride; X = xylazine hydrochloride; A = atropine sulfate; Y = yohimbine hydrochloride.

<table>
<thead>
<tr>
<th>Dog ID number/ sex</th>
<th>Est. age (yrs)</th>
<th>Est. body mass (kg)</th>
<th>K Dosage (mg/kg)</th>
<th>X Dosage (mg/kg)</th>
<th>A Dosage (mg/kg)</th>
<th>Time to sternal recumbency (min:sec)</th>
<th>Y Dosage (mg/kg)</th>
<th>Time to first arousal post-Y (min:sec)</th>
<th>Time to standing post-Y (min:sec)</th>
<th>Total Elapsed time (darting to standing) (min:sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/f</td>
<td>6</td>
<td>27</td>
<td>1.9</td>
<td>2.2</td>
<td>0.046</td>
<td>9:11</td>
<td>0.19</td>
<td>5:05</td>
<td>7:35</td>
<td>41:52</td>
</tr>
<tr>
<td>2/m</td>
<td>6</td>
<td>31</td>
<td>1.6</td>
<td>1.9</td>
<td>0.040</td>
<td>12:12</td>
<td>0.16</td>
<td>2:20</td>
<td>4:23</td>
<td>37:18</td>
</tr>
<tr>
<td>3/m</td>
<td>2.5</td>
<td>27</td>
<td>1.5</td>
<td>2.2</td>
<td>0.046</td>
<td>9:11</td>
<td>0.19</td>
<td>7:44</td>
<td>9:00</td>
<td>45:16</td>
</tr>
<tr>
<td>4/m</td>
<td>9</td>
<td>24</td>
<td>c</td>
<td>c</td>
<td>c</td>
<td>c</td>
<td>0.21</td>
<td>1:34</td>
<td>10:14</td>
<td>c</td>
</tr>
<tr>
<td>5/m</td>
<td>≥10</td>
<td>25</td>
<td>1.4</td>
<td>2.4</td>
<td>0.050</td>
<td>10:30</td>
<td>0.20</td>
<td>2:00</td>
<td>2:00</td>
<td>36:00</td>
</tr>
<tr>
<td>mean</td>
<td>6.7</td>
<td>26.8</td>
<td>1.6</td>
<td>2.2</td>
<td>0.046</td>
<td>10:11</td>
<td>0.19</td>
<td>4:11</td>
<td>6:38</td>
<td>40:07</td>
</tr>
<tr>
<td>+/-SD</td>
<td>2.9</td>
<td>2.7</td>
<td>0.22</td>
<td>0.21</td>
<td>0.0041</td>
<td>NA</td>
<td>0.019</td>
<td>2:48</td>
<td>3:23</td>
<td>4:16</td>
</tr>
</tbody>
</table>

(range) (2.5 - 10^+)(24 - 31) (1.4 - 1.9)(1.9 - 2.4) (0.040 - 0.050) (9:11 - 12:12) (0.16 - 0.21) (1:34 - 7:44) (2:00 - 10:14) (36:00 - 45:16)

a, female; m, male.

b Thick brush sometimes obscured line of vision, so exact times are not always available.

c Data omitted for this dog, as a total of three darts were needed due to partial injections and/or bounces upon hitting.

NA = not applicable.
Figure 1.

A) Dog Number 1

Parameters obtained as soon as animal was handled

Rectal temperature (F): 101.9
heart rate (per minute): 36
respiratory rate (per minute): 16

Event summary^a

<table>
<thead>
<tr>
<th>SpO2 (%)</th>
<th>Pulse rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>min: 89</td>
<td>40</td>
</tr>
<tr>
<td>max: 98</td>
<td>120</td>
</tr>
<tr>
<td>mean: 93</td>
<td>107</td>
</tr>
</tbody>
</table>

^aPulse oximeter was attached 5 minutes after animal was observed to be anesthetized in sternal recumbency, and was disconnected at the time yohimbine was administered. An excellent plane of anesthesia was attained, with only mild ataxia observed after reversal due to residual ketamine. The dog appeared completely normal when the pack was checked 2 hours after reversal.
Figure 1. (continued)

B) Dog Number 2

Parameters obtained as soon as animal was handled

Rectal temperature (°F): 101.8
heart rate (per minute): 129
respiratory rate (per minute): 16

Event summary

<table>
<thead>
<tr>
<th>SpO₂ (%)</th>
<th>Pulse rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>min: 85</td>
<td>118</td>
</tr>
<tr>
<td>max: 96</td>
<td>133</td>
</tr>
<tr>
<td>mean: 94</td>
<td>126</td>
</tr>
</tbody>
</table>

solid circles: SpO₂ (%) versus Time (minutes)
empty squares: Pulse Rate (per minute) versus Time (minutes)

Pulse oximeter was attached 2 minutes after animal was observed to be anesthetized in sternal recumbency, and was disconnected at the time yohimbine was administered. An excellent plane of anesthesia was attained, with mild ataxia observed after reversal due to residual ketamine. The dog appeared completely normal when the pack was checked 1 hour after reversal.
Figure 1. (continued)

C) Dog Number 3

Parameters obtained as soon as animal was handled

Rectal temperature (° F): 102.7
heart rate (per minute): 92
respiratory rate (per minute): 20

Event summary

<table>
<thead>
<tr>
<th>SpO2 (%)</th>
<th>Pulse rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>min: 79</td>
<td>98</td>
</tr>
<tr>
<td>max: 88</td>
<td>118</td>
</tr>
<tr>
<td>mean: 84</td>
<td>109</td>
</tr>
</tbody>
</table>

Pulse oximeter was attached 11 minutes after animal was observed to be anesthetized in sternal recumbency, and was disconnected at the time yohimbine was administered. A normal sinus rhythm was noted on a lead II ECG tracing obtained during the procedure. Plane of anesthesia appeared light initially, but gradually deepened. Recovery took several minutes longer than for previous animals, but there was no evidence of residual ketamine after reversal. The dog appeared completely normal when the pack was checked within 10 minutes of reversal.
Figure 1. (continued)

D) Dog Number 4

Parameters obtained as soon as animal was handled

Rectal temperature (°F): 107.4
heart rate (per minute): 96
respiratory rate (per minute): 12

Event summary

<table>
<thead>
<tr>
<th>SpO2 (%)</th>
<th>Pulse rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>min: 81</td>
<td>96</td>
</tr>
<tr>
<td>max: 87</td>
<td>101</td>
</tr>
<tr>
<td>mean: 84</td>
<td>99</td>
</tr>
</tbody>
</table>

This dog's physiological parameters have been affected by a prolonged capture process related to the dart failures described in the text. Hyperthermia was successfully managed with a combination of intravenous isotonic fluids, topical application of cool water, as well as topical application of isopropyl alcohol.

Pulse oximeter was attached 2 minutes after animal was observed to be anesthetized in sternal recumbency, and was disconnected at the time yohimbine was administered. Appeared slightly ataxic and sedate after reversal. As this exercise was completed towards the end of the day, we were unable to find the pack again until the following day. The dog appeared completely normal at that time.
Figure 1. (continued)

E) Dog Number 5

Parameters obtained as soon as animal was handled

Rectal temperature (°F): 100.2
heart rate (per minute): 44
respiratory rate (per minute): 12

Event summary

<table>
<thead>
<tr>
<th>SpO2 (%)</th>
<th>Pulse rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>min:</td>
<td>85</td>
</tr>
<tr>
<td>max:</td>
<td>96</td>
</tr>
<tr>
<td>mean:</td>
<td>91</td>
</tr>
</tbody>
</table>

Pulse oximeter was attached 8 minutes after animal was observed to be anesthetized in sternal recumbency, and was disconnected at the time yohimbine was administered. An excellent plane of anesthesia was attained, with only mild ataxia after reversal due to residual ketamine. The dog walked into a river, and then walked back out normally and called to pack-mates. The dog appeared completely normal when the pack was checked one-half hour after reversal.
Figure 1. (continued)

F) Averages of parameters from all dogs (n = 5)

Parameters obtained as soon as animals were handled: result +/- SD (range)

Rectal temperature (°F): 102.8 +/- 2.7 (100.2 - 107.4)^
Heart rate (per minute): 79 +/- 39 (36 - 129)
Respiratory rate (per minute): 15 +/- 3.3 (12 - 20)

Summary of events

<table>
<thead>
<tr>
<th></th>
<th>SpO2 (%)</th>
<th>Pulse rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average min +/- SD (range):</td>
<td>84 +/- 3.9 (79 - 89)</td>
<td>85 +/- 30 (40 - 118)</td>
</tr>
<tr>
<td>Average max +/- SD (range):</td>
<td>93 +/- 5.1 (87 - 98)</td>
<td>113 +/- 15 (95 - 133)</td>
</tr>
<tr>
<td>Average mean +/- SD (range):</td>
<td>89 +/- 4.9 (84 - 94)</td>
<td>105 +/- 15 (84 - 126)</td>
</tr>
</tbody>
</table>

^
When the dog (number 4) that was hyperthermic due to a prolonged capture process related to dart failures is excluded, the rectal temperature data summary (n = 4) is: 101.7 +/- 1.0 (100.2 - 102.7).
ANESTHESIA OF FREE RANGING WILD DOGS (*Lycaon pictus*) WITH FENTANYL AND XYLAZINE

Johan Hattingh - In memoriam

Jacobus P Raath, BVSc *
Kruger National Park, Private Bag X 402 Skukuza 1350 South Africa

Caroline M Knox, BSc MSc PhD
Department of General Physiology, Univ. of Witwatersrand, Johannesburg 2000, South Africa

Denise Kernes, HD Med Tech (Clin Path)
Department of Haematology, MEDUNSA 0204 South Africa

Dewald F Keet, BVSc
Directorate of Animal Health, P O Box 12 Skukuza 1350 South Africa

Michael G L Mills, B.A.BSc DSc
Specialist Scientist, Private Bag X 402 Skukuza 1350 South Africa

The drug combinations published for the anesthesia of wild dogs such as ketamine and xylazine, medetomidine and ketamine, tiletamine and zolazepam have the disadvantage that one of the compounds is not fully reversible with current antagonists. When anaesthetizing wild dogs in free ranging conditions, it is of the utmost importance to use anaesthetics than are completely reversible. This reduces vulnerability to predators during long recovery phases, prevents the possibility of isolation should the pack move off while the anaesthetized animal is still recovering and prevents drug dependant behavioral changes possibly influencing the hierarchial position of the animal. The authors experimented with the use of fentanyl and xylazine as they are completely reversible with naloxone or naltrexone and yohimbine.

Over the timespan of three years, free-ranging wild dogs were immobilised in the Kruger National Park using combinations listed in Table 1 in order to overcome the disadvantages of other drugs mentioned above. This resulted in the decision to use the combination of 2 mg fentanyl and 25 mg xylazine as a standard dose for adult wild dogs, and lower doserates in subadults and pups. The fentanyl (Janssen) was reconstituted using Dimethylsulfoxide (DMSO) to a concentration of 5 mg/ml, while the xylazine (Bayer) was used at a concentration of 100 mg/ml. They were darted with a 2.5 ml Telinject dart and a 40 mm needle at a distance ranging from 10 to 40 meters using the Telinject GUT 50 rifle. Once recumbent the dart was removed and the dart wound treated with antibiotics. The dogs were blindfolded, their ears stuffed with cotton and they were unobtrusively removed from the sight of the pack.

A physical examination was conducted on the animals, they were weighed and placed in lateral recumbency. During the anesthesia, physiological parameters such as heart rate,
respiration rate, mean arterial pressure, electrocardiogram, haematology and chemistry, and bloodgas values were measured in some individuals and results are given in Table 2.

After completion of the procedure the animals were moved to within close range of the pack. The earplugs and the blindfold were removed and the antidotes administered. This consisted of Yohimbine at 0.125 mg/kg intravenously, followed by Naloxone of 3 - 5 vials of 1 ml each of the 0.04mg/ml concentration or Naltrexone at 40 times the dose of the fentanyl. Both injections were given into the vena saphena lateralis.

After a good intramuscular injection of the drug combination the animals were found to be ataxic at 1.5 - 3 minutes and recumbency at 5 to 7.5 minutes. However, if the animals were approached too soon after recumbency it was found that they would respond to touching by getting up and running off in an ataxic manner. Therefore the decision was made to leave the dogs until ten minutes post darting. The animals were found to be very placid and easily manageable. The darting combination resulted in good anesthesia even for surgical invasion. Vocalisation does occur spontaneously during the anesthesia.

Without any top-ups, animals would remain under anesthesia for a period of 45 minutes to one hour, at which point recovery would be very sudden and the dog would run off as if having been administered with the antidote. This necessitates careful monitoring of the animal while under anesthesia.

Top-ups have been given routinely at 45 min consisting of 0,5 mg fentanyl and 10 mg xylazine. This ensures deep anesthesia for at least another 45 minutes to one hour.

After intravenous administration of the antidote, the animal will recover in 45 to 90 seconds and the reversal will be complete. The dogs jump up and run off immediately and often keep running for a while. For this reason the authors moved well away and out of sight from the dogs after administration of the antidote. Most often the other members of the pack will run up to the recovered dog, reuniting it with the pack.

A total of 112 anesthesia have been conducted with this combination in all age classes with no mortalities. One subadult that accidentally received an adult dose had a short grand mal seizure approximately five minutes after going down, but recovered spontaneously and showed no obvious side effects after administration of the antidote. The dog is still alive today.
Table 1.

<table>
<thead>
<tr>
<th>Drug combinations</th>
<th>Number</th>
<th>Dose rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telezol</td>
<td>12</td>
<td>0.65 - 4 mg / kg</td>
</tr>
<tr>
<td>Ketamine and Xylazine</td>
<td>1</td>
<td>200 mg and 25 mg</td>
</tr>
<tr>
<td>Innovar Vet and Ketamine</td>
<td>10</td>
<td>2.5 ml and 50 mg</td>
</tr>
<tr>
<td>Fentanyl and Demosedan</td>
<td>3</td>
<td>2 mg and 1.5 mg</td>
</tr>
<tr>
<td>Fentanyl and Xylazine</td>
<td>112</td>
<td>2 mg and 25 mg</td>
</tr>
</tbody>
</table>

Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate</td>
<td>33</td>
</tr>
<tr>
<td>Respiration Rate</td>
<td>9</td>
</tr>
<tr>
<td>Mean Arterial Pressure</td>
<td>119 ± 26</td>
</tr>
<tr>
<td>pO₂</td>
<td>73.9</td>
</tr>
<tr>
<td>pCO₂</td>
<td>63.8</td>
</tr>
<tr>
<td>pH</td>
<td>7.23 ± 0.05</td>
</tr>
</tbody>
</table>
COMPARISON OF DETOMIDINE / CARFENTANIL / KETAMINE AND MEDETOMIDINE / KETAMINE ANESTHESIA IN GREVY'S ZEBRA

Lin Klein, VMD*
University of Pennsylvania, New Bolton Center, 382 West Street Road,
Kennett Square, Pennsylvania 19348, USA

Scott B. Citino, DVM
White Oak Conservation Center, 726 Owens Road, Yulee, Florida 32097, USA

The agent most commonly used for immobilization of zebra has been the potent opioid etorphine, either alone or combined with phenothiazine derivative or $a_2$ agonist tranquilizers. Etorphine is presently not available in the United States, necessitating the development of other methods of immobilization. Carfentanil citrate (C) (Wildlife Pharmaceuticals, Ft. Collins, CO 80524) has been used successfully to immobilize a wide variety of ungulates, but zebras are somewhat resistant to carfentanil, and immobilization may be unsatisfactory even when high doses are used. Medetomidine and ketamine provide rapid immobilization in many artiodactylids, but their effects in zebras have not been reported. Initial experience with a combination of C, detomidine (D) (SmithKline Beecham, West Chester PA 19380) and ketamine (K) suggested that this combination provided satisfactory immobilization in zebras and allowed a reduction in the dose of C. This study was undertaken to investigate the behavioral, cardiopulmonary and biochemical effects of immobilization with a DCK combination and its reversal with naltrexone (N) (Wildlife Pharmaceuticals, Ft. Collins, CO 80524), yohimbine (Y), and tolazoline (T) (Ciba-Geigy, Basle, Switzerland), and to compare the effects with those of medetomidine (M) (Wildlife Pharmaceuticals, Ft. Collins, CO 80524) and K, and the $a_2$ antagonist atipamezole (A) (Wildlife Pharmaceuticals, Ft. Collins, CO 80524) in zebras.

Animals

A total of seven zebras (Equus grevyi), 8 months to 21 years old and weighing 227-445 kg, maintained as a breeding herd at White Oak Conservation Center in Yulee, Florida were immobilized for routine hoof care, physical examination, vaccination, and dental prophylaxis. One stallion and five mares were given DCK, and 11 months later, the stallion, 4 of the 5 mares and an 8 month old male were given MK. The animals were brought from pasture into stockade-fenced paddocks (the stallion separated from mares) 6-7 days prior to immobilization. The studies were conducted in March 1994, and February/March 1995.

Drug Administration

Initial administration of the immobilizing drugs was in the neck or shoulder, by projectile (plastic) syringe (Telinject USA, Inc., Saugus CA 91350). Supplemental K was given by hand-injection, IM or IV. Drug dosages listed are based on actual weights, measured at the conclusion of each procedure.
For the DCK study, D 150 ± 12 mg/kg was given initially and was followed by C:9.8 ± 0.7 mg/kg with K:2.0 ± 0.2 mg/kg when moderate sedation was obvious. When the opioid effect became evident, the zebras were blindfolded and assisted into a lateral position in a shaded area.

For the MK study, 3 zebras were given M and K simultaneously, and 3 zebras were given M alone first, followed by K when moderate sedation occurred. Dosage schedule for M and K is shown in Table 1. When the MK animals became ataxic and were unable to maintain a standing posture, they were either assisted, as with DCK, or allowed to go down unrestrained, and were moved into the shade.

All zebras were left in lateral recumbency with the head and neck raised. They breathed ambient air except for one 15 minute period, during which O₂ 15 LPM was given by nasopharyngeal insufflation. At the conclusion of the clinical procedures, the zebras were weighed and the antagonists were given.

The DCK zebras received N 980 ± 67 mg/kg: 1/2 IV, 1/2 SQ, along with Y:52 ± 6.7 mg/kg and T:260 ± 30 mg/kg, both IV. MK zebras received (A) at a dose rate of 4(n = 1) or 5 times (n = 3) the dose of M with 1/4 given IV and 3/4 given SQ, or 5 times the dose of M with 1/2 IV, 1/2 SQ (n=2). When arousal occurred, the zebras were assisted to a standing posture and then released, except that 3 MK zebras were allowed to recover unassisted.

Sample Collection

Samples were drawn for CBC and clinical serum chemistry when the animals became recumbent and at the conclusion of the clinical procedures. Venous blood samples were drawn for lactate analysis (YSI 1500 Sport Lactate Analyzer: YSI Inc., Yellow Springs OH 45387) during the first 15 minutes of recumbency and at the time of blood gas sampling. Arterial samples were drawn for blood gas and pH analysis (StatPal II Blood Gas and pH Analyzer: PPG Sensors, La Jolla CA 92037) in the first 15-30 minutes of recumbency and at 15 minute intervals thereafter. Blood gas and pH values were corrected for rectal temperature. Mean arterial pressure (MAP) was measured continuously from an auricular artery and EKG was also monitored continuously (Propaq Model 106 Portable Patient Monitor: Protocol Systems Inc., Beaverton OR 97008). HR, RR and rectal temperature were recorded as soon as immobilization occurred and at 5-10 minute intervals. Data are presented as x ± SD, and results were analyzed by ANOVA and Student's t-test. Differences were assumed to be significant if p was < 0.05.

Results

Ambient temperature and humidity ranged from 21-29°C and 44-71% (DCK) and 23-30°C and 47-70% (MK). The zebras were apprehensive before drug administration but not excited. Activity was limited to walking or short periods of trotting or cantering across the paddocks. In the DCK zebras, first signs of sedation after D were noted at 5 minutes. Sedation with D was characterized by lowered head and ears, relaxation of facial, tongue, and limb muscles, occasional stumbling, and a tendency of the females to place themselves between herdmates. Spontaneous motor activity was decreased, but the animals could be
aroused, and they maintained avoidance behavior. CK was given 22.3 ± 4.4 (17-28) minutes after D. After receiving CK, individual mares were separated from the group. Mild hypermetric pacing and increased ataxia were noted. Two animals stumbled and fell, but immediately stood and walked again. Varying degrees of head pressing and rigidity occurred, after which the zebras could be "hand walked" and assisted to recumbency. Recumbency occurred 13.7 ± 5.4 (6-21) minutes after CK. Three zebras were given additional K 0.5 to 0.7 mg/kg IV to improve or maintain muscle relaxation. Time from immobilization to antagonist administration was 89.7 ± 13 (66-99) minutes in the DCK group. Zebras stood 3.7 ± 1.4 (2-6) minutes after antagonist administration. The zebras were assisted to stand, and generally did not respond to being held until they were able to stand and walk with adequate control of legs. Mild ataxia and sedation were usually present. No renarcotization was noted.

In the MK group, the earliest signs of sedation occurred 2-4 minutes after drug injection and were similar to D. The adult male had a complete penile prolapse during onset and he exhibited mild to intense rubbing of his lips on his legs or the fence. With time, ataxia and muscle relaxation were more pronounced after M than after D, and all zebras receiving MK, either simultaneously or in sequence, eventually became moderately to severely ataxic before going down. They lost control of their legs and either staggered for several minutes or fell one or more times before losing their desire to stand and the avoidance reaction to being handled. Two animals fell with their necks twisted, but rolled immediately, realigning their necks. Four animals required additional K to achieve complete immobilization and relaxation. Once immobilized, the zebras generally became more relaxed over time. Two zebras were given K to extend the period of additional (75-93) immobilization (Table 1). Time from immobilization to antagonist administration was 82.3 ± 8.6 min. One zebra was very light and moving when given A (4 x M) and stood immediately but remained moderately sedated and ataxic. The other 5 zebras stood 5.4 ± 2.3 (3-9) min. after A (5 x M), but the quality of recovery varied, depending on the proportion of the dose given IV. The 3 zebras given A 1/4 IV, 3/4 SQ had unsatisfactory recoveries and were significantly ataxic after standing. Two were assisted to stand and fell one or more times after being released from the handlers. One was left to recover alone. She rolled over, stood, fell down and rolled again before being able to stand and walk. The two zebras receiving A 1/2 IV and 1/2 IM, and left to recover alone had excellent recoveries, and were only slightly sedate after standing. Urination was copious in all MK zebras after standing. The stallion and one mare were seen to be rubbing their lips after recovery. No resedation was seen.

Measured Variables (Table 2)

Mean $P_{aCO_2}$ was significantly higher, and $P_{aO_2}$ (while breathing ambient air) and pH, were lower with DCK than with MK. The $P_{aCO_2}$ increased during $O_2$ administration but the increase was highly variable among animals 9.6 to 223 mmHg. Mean venous lactate was higher with MK than with DK but only one value (4.3 mM/L, MK) was above the anaerobic threshold. Mean respiratory rate was higher with MK than with DCK. Rectal temperature was higher with DCK 38.9 ± 0.6 °C) than with MK (38.4 ± 0.5). Hypertension and
hyperglycemia were present in both groups, and heart rates were not significantly different. No arrhythmias or abnormal waveforms were noted on the EKG.

Conclusion

DCK in the dosage and administration scheme tested provided satisfactory induction and good immobilization, and N, Y and T produced rapid antagonism in captive Grevy's zebras. The ability to restrain and examine the DCK zebras while they were still standing, and to "handwalk" them was considered a clinically useful attribute of the drug combination. Based on $Pa_{CO_2}$ values, respiratory drive was better maintained with MK than with DCK, and all zebras given DCK had $Pa_{CO_2}$ levels below 60 mmHg, compared to only one in the MK group. Hypertension was present in both groups and, therefore, could not be attributed solely to an opioid effect. Hyperglycemia was attributed to the effects of the $\alpha_2$ agonists.

In the dosages and administration schemes tested, MK did not produce satisfactory induction. Once immobilization occurred, it was judged to be good. A, given at 5 times the M dose, with 1/2 given IV and 1/2 given SQ, provided rapid, controlled, antagonism. When only 1/4 of the dose was given IV, recovery was unsatisfactory.

ACKNOWLEDGMENTS

The authors would like to thank Wildlife Pharmaceuticals for providing medetomidine, ketamine, and atipamazol for the studies. The authors would also like to thank Nancy Lung VMD, Lee Young DVM, Cyd Mayer, Lisa Kolbach, Kari Wildeboer, Karin Largerstrom, Vincent Seccareccia, Rebecca Owens, Laura Kasbohm DVM, Steve Shurter and the White Oak hoofstock staff for their invaluable assistance in conducting the studies.
Table 1. MKA Drug Administration to Zebras

<table>
<thead>
<tr>
<th>Animal #</th>
<th>Initial Doses</th>
<th>Minutes</th>
<th>Additional K mg/kg</th>
<th>Antagonist</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M mg/kg</td>
<td>K mg/kg</td>
<td>M to K</td>
<td>imm</td>
</tr>
<tr>
<td>1</td>
<td>93</td>
<td>1.6</td>
<td>simul</td>
<td>1.5 IV</td>
</tr>
<tr>
<td>2</td>
<td>114</td>
<td>2.3</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>96</td>
<td>1.9</td>
<td>16</td>
<td>0.5 IV</td>
</tr>
<tr>
<td>4</td>
<td>94</td>
<td>1.9</td>
<td>27</td>
<td>0.9 IV,IM</td>
</tr>
<tr>
<td>5</td>
<td>153</td>
<td>2.0</td>
<td>simul</td>
<td>0.6 IV</td>
</tr>
<tr>
<td>6</td>
<td>165</td>
<td>2.2</td>
<td>simul</td>
<td>-</td>
</tr>
</tbody>
</table>

simul = medetomidine and ketamine given simultaneously.
imm = required to achieve complete immobilization.
ext = given to extend period of immobilization.
Table 2. Measured Variables in Zebras Given DCK or MK: $x \pm SD$

<table>
<thead>
<tr>
<th>Variable</th>
<th>DCK</th>
<th>MK</th>
<th>n</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaCO₂ mmHg *</td>
<td>54.7±5.7</td>
<td>45.0±4.5</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>PaO₂ mmHg *+</td>
<td>61.7±2.0</td>
<td>76.9±1.8</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>pH *</td>
<td>7.34±0.04</td>
<td>7.44±0.02</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>Lactate mM/L *</td>
<td>1.76±0.68</td>
<td>2.26±0.78</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>RR bpm</td>
<td>25.2±8.3</td>
<td>30.7±8.8</td>
<td>44</td>
<td>41</td>
</tr>
<tr>
<td>HR bpm</td>
<td>49.9±6.4</td>
<td>48.5±5.5</td>
<td>48</td>
<td>41</td>
</tr>
<tr>
<td>MAP mmHg</td>
<td>191±13</td>
<td>184±14</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Glucose mg/dl</td>
<td>209±38.5</td>
<td>192±59</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Temp °C *</td>
<td>38.9±0.6</td>
<td>38.4±0.5</td>
<td>39</td>
<td>30</td>
</tr>
</tbody>
</table>

* $p < 0.05$ DCK vs MK
+ values from zebras breathing ambient air
n = number of measurements
Sixteen (10 female and 6 male) captive born dama gazelles (*Gazella dama*), weighing 48 ± 10 kg (mean ± SD) were used to evaluate the cardiopulmonary effects of IM carfentanil and to validate the use of pulse oximetry in immobilized gazelles. Carfentanil (18 ± 2 μg/kg) produced rapid induction (6 ± 3 min) and moderate muscle relaxation, a significant (p < 0.05) persistent decrease in heart rate (87 ± 12 beats/min) and bradypnea (11 ± 4 breaths/min). Carfentanil produced significant elevations in systolic, mean and diastolic arterial pressures which were present throughout the immobilization period. Arterial blood gas analysis, performed at 10, 20 and 30 minutes after induction, showed PCO₂ and PO₂ values within normal limits. A period of moderate hypoxemia (SaO₂ < 95%) was seen 10 minutes after induction. For the remainder of the immobilization period, arterial oxygen saturation (SaO₂) was considered to be adequate. Oximetric saturation (SpO₂) values were generally lower than SaO₂, but reliably demonstrated trends in arterial oxygen saturation. Periods of hypoxemia were in most cases indicated by the oximeter reading and confirmed by SaO₂ measurements. An increase in CPK values (88 ± 53 U/L to 109 ± 48 U/L) was seen at 30 minutes post immobilization. Naltrexone reversal (1.8 ± 0.3 mg/kg) given half intravenously and half subcutaneously was rapid and uneventful. No cases of renarcotization were recorded. Time to standing after administration of the antagonist was 2 ± 1 min.
Anorexia is a common and serious problem in rabbits, often occurring post-surgically. Such animals die rapidly without nutritional support; however, methods currently available for administrating enteral nutrition to rabbits are stressful \(^1\) and impractical. The purpose of this work was to adapt a feeding tube technique described for dogs and cats \(^2\) to the rabbit patient (*Oryctolagus cuniculus*).

Gastrostomy tubes were percutaneously placed in five healthy New Zealand White rabbits weighing 2.7 kg to 3.55 kg. The rabbits were fasted 12-18 hours before anesthesia. Elizabethan collars were used during this period to prevent coprophagia. Following premedication with acepromazine and ketamine, anesthesia was induced with isoflurane via face mask. The rabbits were then intubated and maintained on isoflurane. The gastrostomy tubes were prepared by cutting off the wide connecting end of 16 French de Pezzer catheters, and removing the small bulb end of the mushroom tip. Once anesthetized the rabbits were placed in right lateral recumbency, and the left paracostal area was prepared for tube placement. Strips of gauze were used to gently pull the rabbit's jaws apart during gastroscopy, and to cover the teeth to prevent damage to the bronchoscope. An incision was made caudoventral to the twelfth rib before passing the bronchoscope to identify the site of cannula placement prior to distending the stomach. The bronchoscope was lubricated with lidocaine hydrochloride gel to prevent hypersensitive pharyngeal reflex, then was inserted through the mouth and advanced into the stomach. The stomach was distended with air using an electrical aquarium pump connected to a hose inserted into the biopsy port of the scope. This distension brought the gastric wall into contact with the left abdominal wall and pushed the liver, spleen and cecum away from the gastrostomy site. The procedure following this point was identical to that used for dogs and cats \(^2\). A modification was made to prevent the suture from slipping out of the snare. The snare and suture were pulled just inside the bronchoscope channel and were brought out through the mouth inside the bronchoscope. The procedure required 15-45 minutes, depending on amount of stomach content present. The tube was fixed externally by passing a flange made from the wide connecting end of the catheter over the tube in close contact with the abdominal wall. Tape was placed around the tube adjacent to the flange to anchor it in position. The flange served to immobilize the tube and prevent inward movement. The tube was closed with a three way stopcock, or PRN adapter.

The rabbits were initially fed a liquid diet through the gastrostomy tube consisting of strawberry Ensure Complete Liquid Nutrition\(^\circledR\) and Heinz Junior Peas\(^\circledR\), which was then supplemented with increasing proportions of solid food per os. Attitude, temperature, heart rate, respiratory rate and body weight were monitored daily. Complete blood counts and
biochemical profiles were performed prior to the gastrostomy procedure and weekly thereafter. The tubes were scheduled to be removed 14 days after insertion. The rabbits were euthanized and necropsied either seven or 14 days after tube removal. Administration of the liquid diet and removal of the tube were easily accomplished. No significant changes were noted in the rabbit's vital signs and there was no evidence of pain or systemic infection. At postmortem, all animals were in good body condition with no lesions other than those associated with the gastrostomy site. In all rabbits, local fibrous adhesion had resulted in a tight, strong adhesion linking the stomach and abdominal wall with no gross evidence of peritonitis or gastric leakage. Although some problems with patient compliance were encountered, the percutaneous endoscopic gastrostomy (PEG) tube is a successful method for administration of enteral nutritional support in the rabbit.

LITERATURE CITED

PRELIMINARY STUDIES ON THE USE OF MILBEMYCIN OXIME FOR TREATMENT OF POLAR BEARS (Ursus maritimus) WITH CHRONIC Balisascaris transfuga INFECTION

Gail E. Hedberg, AHT, R. Avery Bennett, DVM, MS, Diplomate ACVS*, Freeland H. Dunker, DVM
San Francisco Zoological Gardens, San Francisco, CA 94132-1098, USA

Dr. Bennett's current address is University of Florida, College of Veterinary Medicine, Department of Small Animal Clinical Sciences, Gainesville, FL 32610-0126, USA

Introduction

Milbemycin oxime (Interceptor; CIBA-Geigy Corp., Greensboro, NC 27419) is available as a tablet to be administered once monthly for the prevention of heartworm disease and control of hookworm, roundworm, and whipworm infections in dogs. The minimum recommended dose is 0.5 mg/kg (0.23 mg/#). The tablets are available in four sizes (2.3 mg, 5.75 mg, 11.5 mg, and 23.0 mg).

Though studies document its safety and efficacy in dogs, milbemycin has not previously been evaluated for safety and anthelminthic activity in ursid species. Chronic ascariasis has been present in several species of Ursidae at the San Francisco Zoological Gardens since 1980. Various anthelmintics had failed to clear the infection. Between August, 1993 and the present a clinical trial has been conducted to evaluate the efficacy and safety of milbemycin oxime in polar bears (Urus maritimus). This was a cooperative study between the Veterinary Department of the San Francisco Zoological Gardens (SFZG) and the CIBA-Geigy Animal Health Division.

Materials and Methods

Three adult female polar bears were used in this study because of their history of chronic infection with Balisascaris transfuga. Quantitative fecal egg counts were performed by Dr. Susan Wade of the Diagnostic Laboratory at the New York State College of Veterinary Medicine (NYSCVM) prior to any treatment. All bears were initially treated at a dose of 1.0 mg/kg once monthly. The tablets were mixed with the bears’ diets. Fecal samples were evaluated either at SFZG or the NYSCVM 3-4 weeks following treatment when samples could be obtained. The treatment schedule was changed to 1.0 mg/kg every other month in February of 1995.

All bears were immobilized for routine health examinations in June, 1994. Serum samples were collected during these examinations which occurred 80-130 min post-treatment. An accurate weight was obtained on each bear during the immobilization allowing for accurate dosing.
Results

Treatment of all bears was easily accomplished. There was no rejection of the medication and the volume was easily consumed. No side effects from the medication were observed.

The results of the fecal examinations are presented in Table 1. All three bears had fecal egg counts of *Balisascaris transfuga* of greater than 2000 eggs/gm prior to the first treatment with milbemycin. Within 6 months negative fecals were obtained for all 3 bears. They have continued to be negative for the subsequent 6 months.

Serum levels of milbemycin and time of sample collection following treatment are presented in Table 2. All samples contained levels of milbemycin oxime greater than 0.05 ppm.

Discussion

A variety of anthelminthic medications were used to treat the chronic ascarid problem in the bear collection at the SFZG during the 13 years preceding this study. Though the bears are maintained on a cement substrate reinfection continued to be a problem. Additionally, it had been difficult to effectively medicate the bears because of the volume of liquid or paste required to treat animals their size. Often the treatment would decrease the worm burden but the chronic infection was never eliminated. The ease of administration makes it possible to treat bears with milbemycin on a regular basis with the goal of eliminating the parasite. The individual dose ranged from 8-10 of the 23 mg tablets. These were easily administered in the animals’ diet.

A dose of 1.0 mg/kg milbemycin oxime appears to be safe and efficacious in polar bears. The dose of 1.0 mg/kg used in this trial is higher than the recommended canine dose of 0.5 mg/kg. Preliminary studies with gallinaceous birds indicate that a dose of 2.0 mg/kg is required to obtain negative fecal results. CIBA has conducted trials in domestic cats and expects to begin marketing milbemycin for use in cats at a dose of 1.0 mg/kg. In general, when extrapolating doses of anthelminthics for use in bears, the domestic cat dose is recommended. CIBA has also determined that at 5 mg/kg (ten times the recommended dose) no adverse effects were observed in dogs. CIBA reports that 90% of the drug’s action is local, within the gastrointestinal tract and that only 10% is from systemic absorption. The serum levels achieved in these three bears were well above the accepted level of 0.2-0.5 ppm expected in canine patients receiving a dose of 0.5 mg/kg. These results support that a lower dose should be effective in polar bears.

As this study progresses we will be evaluating the efficacy of milbemycin at a dose of 0.5 mg/kg monthly and every other month to determine if these doses are effective at maintaining negative fecal results. We have also used 1.0 mg/kg in Kodiak bears (*Ursus arctos middendorffi*) with equally positive results (Table 3).
ACKNOWLEDGEMENTS

The authors thank CIBA-Geigy, Animal Health Division; Phyllis Hilling LVT, Diagnostic Laboratory, NYSCVM; Susan Wade, PhD, Department of Parasitology; Daniel Jackson and Alex Weiss, Zoo Keepers; and Lory Palmer, Administrative Assistant for their assistance with this study.

LITERATURE CITED

### Table 1: Fecal Results for Polar Bears Treated with Milbemycin.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>185009</td>
<td>2,228</td>
<td>Rx'd 1579</td>
<td>NEG</td>
<td>Rx'd</td>
<td>60</td>
<td>Rx'd</td>
<td>300</td>
<td>Rx'd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>185044</td>
<td>4,714</td>
<td>1375</td>
<td>2900</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18269</td>
<td>2,664</td>
<td>6000</td>
<td>59,220</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>185009</td>
<td>199</td>
<td>Rx'd</td>
<td>750</td>
<td>Rx'd</td>
<td>no sample</td>
<td>Rx'd</td>
<td>no sample</td>
<td>Rx'd</td>
<td>no sample</td>
<td>Rx'd</td>
</tr>
<tr>
<td>185044</td>
<td>no sample</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>no sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18269</td>
<td>positive*</td>
<td>NEG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>12/20/94</th>
<th>12/23/94</th>
<th>1/1/95</th>
<th>1/30/95</th>
<th>2/9/95</th>
<th>2/22/95</th>
<th>3/23/95</th>
<th>4/1/95</th>
</tr>
</thead>
<tbody>
<tr>
<td>185009</td>
<td>1</td>
<td>Rx'd</td>
<td>no sample</td>
<td>Rx'd</td>
<td>no sample</td>
<td>NEG</td>
<td>Rx'd</td>
<td></td>
</tr>
<tr>
<td>185044</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18269</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rx'd - all 3 animals treated

Sample values noted as egg/gm

*Samples evaluated at SFZG
Table 2: Serum Concentrations of Milbemycin Oxime in Polar Bears

<table>
<thead>
<tr>
<th>Date</th>
<th>6/7/94</th>
<th>6/16/94</th>
</tr>
</thead>
<tbody>
<tr>
<td>185009</td>
<td>0.068 ppm</td>
<td>0.211 ppm</td>
</tr>
<tr>
<td>1 hr 20 min*</td>
<td></td>
<td>2 hr 10 min*</td>
</tr>
<tr>
<td>185044</td>
<td>0.254 ppm</td>
<td></td>
</tr>
<tr>
<td>1 hr 30 min*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18269</td>
<td>0.487 ppm</td>
<td></td>
</tr>
<tr>
<td>4 hr*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Time of sample collection following treatment

Table 3: Serum Concentrations of Milbemycin Oxime in Kodiak Bears

<table>
<thead>
<tr>
<th>Date</th>
<th>2/24/94</th>
<th>2/16/94</th>
</tr>
</thead>
<tbody>
<tr>
<td>18003</td>
<td>0 ppm**</td>
<td>0.214 ppm</td>
</tr>
<tr>
<td>3 hr 30 min*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18002</td>
<td>0.487 ppm</td>
<td></td>
</tr>
<tr>
<td>4 hr*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18008</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Time of sample collection following treatment

**Control
THE EVALUATION OF A VACCINE AGAINST ENCEPHALOMYOCARDITIS INFECTION IN ELEPHANTS (Loxodonta africana) UNDER CONTROLLED CONDITIONS

Jacobus P Raath, BVSc
Kruger National Park, Private Bag X 402, Skukuza 1350, South Africa

Roy G Bengis, BVSc MSc PhD MRCVS
Directorate of Animal Health, P O Box 12, Skukuza 1350, South Africa

Introduction

The Kruger National Park of South Africa has an estimated elephant population of 7500. An outbreak of encephalomyocarditis in the elephant population was first noticed in October 1993, peaked in January 1994 with 32 mortalities and declined steadily until November 1994. Altogether 64 elephants died during this outbreak of which 53 (83%) were adult bulls.2 Although this did not pose a major threat to the elephant population as a whole, the loss of 3% of the bull population was of concern. The impact on smaller groups of privately owned elephants could become significant. The vaccination of large tuskers to prevent the loss of their genetic material could become an important management option, should the incidence of the disease increase.

A killed virus vaccine was produced at the Onderstepoort Foot and Mouth disease high security laboratory using the virus isolated from the Kruger Park elephants as seeding stock. The vaccine was tested in laboratory rats and domestic pigs ensuring adequate antibody response. The vaccine was then tested in elephant calves confined in pens at Skukuza, followed by a trial in free ranging adult elephants.
This paper reports only on the results obtained in the elephant calf vaccine study.

Materials and Methods

Eighteen African elephant calves, as part of the annual culling quota, were made available by the National Parks Board for this phase of the vaccine evaluation project. Of these 18 elephants only 12 were part of the challenge trial.

The elephants were all of similar size, approximately 6 - 8 years old and in good health. They consisted of 16 males and two females. The elephants were confined in groups of three, in 6 adjacent pens at the elephant holding facility in Skukuza. They were given a combination of teff and lucerne ad lib, received freshly cut browse twice daily, and were supplemented with a concentrate (Elephant cubes - Epol RSA) daily. The elephants had free access to water.

The elephants were anaesthetized using a combination of etorphine hydrochloride (Logos AgVet) and azaperone (Janssen). Two animals in each pen were marked with either a single or double ear nick, resulting in an identification system for each calf of the pen number.
followed by either a 0, 1 or 2, representing the number of ear nicks. Baseline blood samples were collected from the upper ear vein in sterile vacutainers prior to vaccination.

The first twelve animals were vaccinated by deep intramuscular injection of 5 ml of the Onderstepoort vaccine. All injections were done on the right hand side in the gluteal area. The anaesthetic was reversed using diprenorphine hydrochloride (Logos AgVet).

The animals were all bledd once a week for three weeks (n = 18) and a further sample was collected in week 4 from the group to be used in the challenge study (n=12).

Following the development of the titres, it was decided to challenge two groups of vaccinated and two groups of unvaccinated controls with virulent EMC virus. The animals were anaesthetized with only etorphine hydrochloride from this point in the study. Blood samples in vacutainers, baseline ECG's and faecal samples were collected. Body temperatures were taken using a Bailey digital thermometer (Scientific Associates) by deep intrarectal placement of the sensor. The challenge was done by oral route in pens 2 and 7, and by intramuscular injection in pens 5 and 6. The challenge doses were 2 ml intramuscularly and 10 ml per os of a solution that contained 10^{7.9} TCID per millilitre. One out of each group of three elephants in a pen was left unchallenged in an attempt to determine if horizontal transmission would occur.

Subsequent to the challenge, the elephants were anaesthetized every second day, starting with groups 2 and 6 on day one and groups 5 and 7 the following day. This continued for a period of 14 days, resulting in seven immobilisations in each group. All elephants were placed in left hand lateral recumbency, and blood and faecal samples were collected and temperatures and ECG's recorded. Special sterilisation of all equipment was done between animals to avoid mechanical transfer of the virus. The vaccination sites were inspected for any adverse reaction.

Results

The results of the vaccine trial in the calves are given in Table 1.
## Antibody Response to EMCV Vaccination in Elephants

<table>
<thead>
<tr>
<th>Animal Nr.</th>
<th>Vaccination Status</th>
<th>Pre-bleed</th>
<th>First week</th>
<th>Second week</th>
<th>Third week</th>
<th>Fourth week</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/0</td>
<td>vaccinated</td>
<td>&lt;1/16</td>
<td>1/1825</td>
<td>1/1825</td>
<td>1/1825</td>
<td>1/2300</td>
</tr>
<tr>
<td>2/1</td>
<td>vaccinated</td>
<td>&lt;1/16</td>
<td>1/362</td>
<td>1/362</td>
<td>1/1448</td>
<td>1/1448</td>
</tr>
<tr>
<td>2/2</td>
<td>vaccinated</td>
<td>&lt;1/16</td>
<td>1/287</td>
<td>1/724</td>
<td>1/456</td>
<td>1/724</td>
</tr>
<tr>
<td>3/0</td>
<td>vaccinated</td>
<td>&lt;1/16</td>
<td>1/72</td>
<td>1/362</td>
<td>1/180</td>
<td>N/D</td>
</tr>
<tr>
<td>3/1</td>
<td>vaccinated</td>
<td>&lt;1/16</td>
<td>1/456</td>
<td>1/724</td>
<td>1/2300</td>
<td>N/D</td>
</tr>
<tr>
<td>3/2</td>
<td>vaccinated</td>
<td>&lt;1/16</td>
<td>1/144</td>
<td>1/1149</td>
<td>1/1448</td>
<td>N/D</td>
</tr>
<tr>
<td>4/0</td>
<td>vaccinated</td>
<td>&lt;1/16</td>
<td>1/362</td>
<td>1/1448</td>
<td>1/1149</td>
<td>N/D</td>
</tr>
<tr>
<td>4/1</td>
<td>vaccinated</td>
<td>&lt;1/16</td>
<td>1/180</td>
<td>1/1448</td>
<td>1/2300</td>
<td>N/D</td>
</tr>
<tr>
<td>4/2</td>
<td>vaccinated</td>
<td>&lt;1/16</td>
<td>1/2300</td>
<td>1/2300</td>
<td>1/1825</td>
<td>N/D</td>
</tr>
<tr>
<td>5/0</td>
<td>vaccinated</td>
<td>&lt;1/16</td>
<td>1/228</td>
<td>1/724</td>
<td>1/1148</td>
<td>1/1874</td>
</tr>
<tr>
<td>5/1</td>
<td>vaccinated</td>
<td>&lt;1/16</td>
<td>1/362</td>
<td>1/575</td>
<td>1/228</td>
<td>1/590</td>
</tr>
<tr>
<td>5/2</td>
<td>vaccinated</td>
<td>&lt;1/16</td>
<td>1/912</td>
<td>1/912</td>
<td>1/1448</td>
<td>1/1636</td>
</tr>
<tr>
<td>6/0</td>
<td>unvaccinated</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
</tr>
<tr>
<td>6/1</td>
<td>unvaccinated</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
</tr>
<tr>
<td>6/2</td>
<td>unvaccinated</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
</tr>
<tr>
<td>7/0</td>
<td>unvaccinated</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
</tr>
<tr>
<td>7/1</td>
<td>unvaccinated</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
</tr>
<tr>
<td>7/2</td>
<td>unvaccinated</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
<td>&lt;1/16</td>
</tr>
</tbody>
</table>
The results of the challenge study are given in Table 2.

<table>
<thead>
<tr>
<th>An. Nr.</th>
<th>Status</th>
<th>Route of challenge</th>
<th>V/N titre At challenge10 days p c</th>
<th>Clinical signs</th>
<th>Post Mortem lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/0</td>
<td>vacc.</td>
<td>Not challenged</td>
<td>3.4 3.3</td>
<td>none</td>
<td>none 5wks p c</td>
</tr>
<tr>
<td>2/1</td>
<td>vacc.</td>
<td>oral</td>
<td>3.1 3.2</td>
<td>none</td>
<td>none 5wks p c</td>
</tr>
<tr>
<td>2/2</td>
<td>vacc.</td>
<td>oral</td>
<td>3.1 3.1</td>
<td>none</td>
<td>none 5wks p c</td>
</tr>
<tr>
<td>5/0</td>
<td>vacc.</td>
<td>Not challenged</td>
<td>3.2 3.9</td>
<td>none</td>
<td>none 5wks p c</td>
</tr>
<tr>
<td>5/1</td>
<td>vacc.</td>
<td>I/M</td>
<td>2.8 3.2</td>
<td>none</td>
<td>none 5wks p c</td>
</tr>
<tr>
<td>5/2</td>
<td>vacc.</td>
<td>I/M</td>
<td>3.2 3.5</td>
<td>none</td>
<td>none 5wks p c</td>
</tr>
<tr>
<td>6/0</td>
<td>unvacc</td>
<td>Not challenged</td>
<td>&lt;1.2 &lt;1.2</td>
<td>none</td>
<td>none 5wks p c</td>
</tr>
<tr>
<td>6/1</td>
<td>unvacc</td>
<td>I/M</td>
<td>&lt;1.2 Dead</td>
<td>Acute death</td>
<td>Acute myocarditis, severe lung oedema</td>
</tr>
<tr>
<td>6/2</td>
<td>unvacc</td>
<td>I/M</td>
<td>&lt;1.2 3.2</td>
<td>none</td>
<td>Severe myocardial scarring, 5wks p c</td>
</tr>
<tr>
<td>7/0</td>
<td>unvacc</td>
<td>not challenged</td>
<td>&lt;1.2 &lt;1.2</td>
<td>none</td>
<td>none 5wks p c</td>
</tr>
<tr>
<td>7/1</td>
<td>unvacc</td>
<td>oral</td>
<td>&lt;1.2 Dead</td>
<td>Acute death</td>
<td>Acute myocarditis with ecchymoses</td>
</tr>
<tr>
<td>7/2</td>
<td>unvacc</td>
<td>oral</td>
<td>&lt;1.2 2.2</td>
<td>Diarrhoea, malaise, abnormal ECG</td>
<td>Severe myocardial scarring, 5wks p c</td>
</tr>
</tbody>
</table>

The abnormal ECG from animal 7/2 is demonstrated in Figure 1.

Discussion

Encephalomyocarditis viruses are classified in the family Picornaviridae, genus Enterovirus, species cardiomyovirus. It was first isolated and identified in 1940 and has a worldwide distribution in mammals and arthropods.\(^5\)
Encephalomyocarditis has been reported in captive African elephants, in a Sumatran orangutan and in eight non-human primates, a Thompson gazelle and one dromedary camel in the Audubon Park Zoo in Louisiana.

This was the first recorded outbreak in a free-ranging elephant population and the first study where the vaccine was extensively tested in African elephants. This study showed that significant antibody titres developed after 7 - 10 days post vaccination. All vaccinated elephants were totally protected from challenge with virulent virus by this vaccine.

Three out of four unvaccinated elephants challenged with virulent virus developed clinical disease, and 2 out of 4 died from EMC. Since all four of these elephants had characteristic myocardial lesions at autopsy, it follows that one of them (Nr 6/2) had developed subclinical disease. No horizontal transmission of EMC infection occurred, and no virus could be isolated from the faeces at any stage in any elephant. EMC virus was isolated from the blood and myocardial tissue of the two elephants that died. Elephants can be infected by both the oral (probably natural) route and parental route. The incubation period of EMC after oral infection is 9 - 10 days, but after parental infection, is as short as 4 - 5 days. Significant antibody titres to this disease develop within 7 to 10 days after vaccination or infection. Significant electrocardiographic changes are present during the clinical phase of the disease.

No significant febrile reactions were noted in any of the elephants, whose rectal temperatures remained predominantly in the range of 34,0 - 36,0°C and seemed to be mainly affected by environmental temperature and the animal's activity.

Clinical symptoms seen were depression, malaise, reduced activity, unwillingness to walk, loose stool, sucking tip of the trunk and increased trunk activity (to and fro swinging of the trunk). The animal still eats!! Death is usually acute, and there are sometimes signs of terminal paddling movements in the soil or vegetation in the immediate area around the carcass. White froth (from pulmonary oedema) may be present, exuding from the external nares of the trunk.

ACKNOWLEDGEMENTS

The authors would like to thank the National Parks Board for the registration of this project and the use of their facilities. The analyses of the samples at the Foot and Mouth Institute at Onderstepoort is greatly appreciated.

LITERATURE CITED

SURGICAL REMOVAL OF INFECTED PHALANGES FROM AN ASIAN ELEPHANT
(Elephas maximus)

Laurie J. Gage, DVM* and David Blasko
Marine World Foundation, Marine World Parkway, Vallejo, California 94589, USA

Murray E. Fowler, D.V.M., and John Pascoe, B.V.Sc., Ph.D.
University of California, School of Veterinary Medicine, Davis, California 95616, USA

A forty year old female Asian elephant (Elephas maximus) developed a draining tract behind the lateral nail of her left front foot. There had been an infection in the pad in this area several months previously that had resolved. There had been a crack in that nail that had been present for several years. The day after the discovery of purulent material coming from the lesion in the pad, the left front limb became swollen. The cuticle was swollen around the left lateral nail. The foot was soaked in disinfectant solutions and epsom salts. An incision was made into the cuticle at the proximal-most portion of the nail, and a tract was found that measured 10 cm and extended distally and slightly medially. The distal region of the cracked nailbed was blocked with 2% lidocaine and a 6 cm diameter hole was cut. A tract was found that communicated with the previously discovered tract. The lesion was treated by aggressive irrigation using a variety of standard disinfectant solutions. The elephant was placed on 100 cc benzathine penicillin i.m. s.i.d. x 5 days, then 25 grams ampicillin i.m. s.i.d. x 10 days. Radiographs were taken and degeneration was evident in the third phalanx (P-3) of the fifth digit, and there was evidence of osteomyelitis in P-2. The tract was flushed with a variety of disinfectant solutions for four months, however, radiographs indicated the infection was progressing. The infected portions of P-2 and P-3 were removed surgically.

Six months after surgery the incision had healed, but a fistulous tract remained behind the nailbed, exiting in the pad tissue below the lateral nail. There was radiographic evidence of osteomyelitis that had progressed to the distal portion of P-1. Aggressive irrigation and antibiotic therapy did not resolve the problem. A second surgery was performed, during which the remainder of P-2, and the distal portion of P-1 were removed. Aggressive aftercare included 16 grams gentomycin diluted in one liter lactated ringers i.v. s.i.d. x 10 days, sterile wrap changes s.i.d. x 13 days, and a 34-day around-the-clock training staff member present to ensure the elephant did not remove the wraps. The elephant was maintained on 67 grams trimethoprim-sulfa p.o. s.i.d, for two weeks after the i.v. gentomycin treatment was discontinued. Pseudomonas sp. was cultured from the lesion two weeks post-surgery, and the lesion was then packed with sterile gentocin-soaked gauze sponges each day when the bandage was changed. Three weeks after this treatment had started, the cultures were negative for pseudomonas, however, this treatment was continued for a total of 12 weeks. The foot bandage was changed daily for a total of three months post-surgery. The elephant would occasionally remove the wraps when the elephant personnel were not present, however, the foot continued to heal without incident, and was completely healed four months post-surgery.
Filarial nematodes have been reported in a number of exotic felid species. Of specific clinical importance, *Dirofilaria immitis*, which is pathogenic in domestic cats and was the cause of death in a Bengal tiger (*Panthera tigris*), has also been reported in the jaguar (*Felis onca*), tigers (*F. sondaicus* and *F. tigris*), wild cats (*F. bangsi costariensis*), jagouarundi (*F. yagouarundi*), a snow leopard (*Panthera uncia*), and bobcats (*Lynx rufus*) from Florida. Other filarial species known to occur in exotic felids are *Dirofilaria repens* which has been reported in the lion (*Panthera leo*) and *Dirofilaria striata* which was first reported in pumas from Brazil (*Felis concolor* and *Felis macroura*) and since has been found in the ocelot (*Felis pardalis*), the margay (*Felis tigrina*), the bobcat, and the Florida panther (*Felis concolor coryi*).

As part of routine clinical evaluation of the critically endangered Florida panther, biological samples were collected and examined for a number of pathogens. Protocol included the collection of whole blood and its preservation in 2% formalin for microfilarial analysis. This present study was carried out to determine the incidence of microfilariae in the Florida panther population, to assess the microfilaremias of individual animals, and to analyze morphological characteristics (head and tail shape) of individual microfilariae in an attempt to discern if they were all of one species and if that species was indeed *D. striata* as previously reported.

Thirty-five of 47 (74.5%) adult (2+ years) Florida panthers were positive for microfilariae (mff+) and all but two panthers remained positive on subsequent tests throughout their lives. No panthers tested at ≤ 6 months of age were mff+ (n=5); 2 of 10 (20%) tested positive in the 1 year class, and 15 of 23 (65%) of panthers were positive at 2 to 4 years of age. Of the 23 panthers sampled at ≥ 10 yrs. of age, 22 (96%) were mff+.

Microfilarial counts ranged from 10 to 7,380 mff/ml of whole blood. Numbers showed fluctuations with no apparent trends when comparisons were made over periodic sampling of individual panthers; nor were there any patterns with regard to panther sex, location, or genetic make-up. Analysis of microfilarial length measurements showed that there was no
statistically significant difference (p<0.05) in the length of the microfilariae with regard to filarid morphology supporting the hypothesis that there is only on species of microfilariae in the Florida panther. The average length of panther microfilariae (n=280) was 320 µm (273-370 µm) and the average width was 4-5 µm. Comparison of microfilarial length values and morphological characteristics with those published for various Dirofilaria sp. shows that they are most similar to D. striata which was the only species of adult Dirofilaria sp. found infrequently in necropsied panthers. To date, no pathology has been attributed to infection with circulating microfilariae or to the presence of adult filarids (usually located in the peritoneum or in the fascial planes of the extremities.

LITERATURE CITED


Tuberculosis, caused by \textit{Mycobacterium avium}, is a major cause of death in captive marsupials, including various species of tree kangaroos.\textsuperscript{3,4} Avian tuberculosis (ATB) in tree kangaroos has proven very difficult, if not impossible, to diagnose especially in early cases. Further complications are that the organism is common in the environment and very resistant to treatment. In a survey of 42 zoological collections in 1990, mycobacterial disease was found in 8\% of the adult tree kangaroo deaths.\textsuperscript{6} In 1992-95 there have been nine additional diagnoses of ATB in tree kangaroos with five deaths. We currently have three Matschie’s Tree Kangaroos under treatment for ATB.

The clinical problems associated with ATB in Matschie’s Tree Kangaroos are:
1 - \textit{M. avium} is a common environmental organism commonly cultured from many sources (water and soil).
2 - \textit{M. avium} has been cultured from individuals which have remained free of clinical signs of disease for more than two years.
3 - Diagnosis of ATB in an individual is very difficult since intradermal tuberculin skin test are inconclusive and culturing \textit{M. avium} from a tree kangaroo does not distinguish between infection and commensal colonization.
4 - Treatment of ATB is difficult and prolonged since \textit{M. avium} is very resistant to most antitubercular drugs.
5 - Individuals with ATB appear immunologically compromised.

\textit{M. avium} is a prevalent organism in the environment, therefore it is almost impossible to eliminate exposure to this potential pathogen.\textsuperscript{4} When \textit{M. avium} is isolated from a tracheal wash of a clinically normal tree kangaroo it is likely that the organism is colonizing the animal; However, infection with \textit{M. avium} may occur if the animal’s immune system becomes compromised. We are attempting to develop and validate an immunological profile for
"normal" tree kangaroos by measuring the competency and quantity of subsets of lymphocytes that transform when co-cultured with lectin mitogens, various mycobacterial antigens, and mixed lymphocyte cultures. We have also evaluated their immunoglobulins. This profile can be useful as both a diagnostic and prognostic tool for ATB. Initial results indicate that 'normal' captive Matschie's Tree Kangaroos have about 20% of the cellular immune competence of other mammals studied. Tree kangaroos with ATB have even a lower level. There is evidence of a possible deficiency of cell-mediated immunity in other marsupials with ATB, and immunosuppression in Koalas (Phascolarctos cinereus) with opportunistic infections may be associated with a retrovirus.

Currently we suspect ATB in all ill tree kangaroos until we are able to prove otherwise. Commonly, ATB animals present with weight loss, pneumonia, osteomyelitis, abscesses and/or neurological signs. Radiography is an aid to detect pneumonia and osteomyelitis. A definitive diagnosis requires positive cultures or DNA probe for M. avium from the lesion. We have found that suppressed immunological profiles correlates well with tree kangaroos with ATB.

We are evaluating treatment regimens which include multiple antitubercular drugs (amikacin [3 mg/Kg BID], rifabutin [20 mg/Kg oral SID], myambutol [20 mg/Kg oral SID], enrofloxacin [2.5 mg/Kg BID]), newer drugs (azithromycin [20 mg/Kg oral SID]), and immunotherapy with M. vaccae. Two tree kangaroos have responded to this therapy. Evaluation of their initial immunological profile showed immunosuppression in both cellular and humoral immune responses. Their clinical status and immunological competence improved concurrently with the therapy; They are active and eating well. The aged female with pulmonary disease still has radiographic evidence of pulmonary disease and continues to sheds acid fast organism in her tracheal lavage. This generates major clinical questions: 1)when do we stop treating? 2)is ATB only under control while she is being treated? 3)when is it safe to pair this animal with another animal? In contrast, the animals with osteomyelitis appear to be a better candidate for a cure from ATB which has been also seen by others.

In an attempt to prevent or minimize ATB in captive tree kangaroos we are evaluating killed M. vaccae as a vaccine. Our initial results of vaccinating "normal" tree kangaroos has shown an overall enhanced immunological response in lymphocyte transformation studies. This includes increased reactivity to M. vaccae and concurrently M. avium, which most likely represents cross-reactivity.

LITERATURE CITED

AN APPARENT EPIZOOTIC OF NECROTIZING PHARYNGITIS AND MUCOSAL
ULCERATION IN THE MHORR GAZELLE

Michael B. Worley, DVM*, Linda J. Lowenstine, DVM, PhD
Virology Laboratory and Department of Pathology, Center for Reproduction of Endangered Species, Zoological
Society of San Diego, P.O. Box 551, San Diego, CA 92112, USA

Kent G. Osborn, DVM
The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA

Jack Allen, DVM, and James E. Oosterhuis, DVM
Department of Veterinary Services, San Diego Wild Animal Park, 15500 San Pasqual Valley Road, Escondido, CA 92024, USA

Introduction

The Mhorr gazelle (Gazella dama mhorr), a valid subspecies of the Dama gazelle (Gazella dama), historically occurred in southwestern Morocco and the Rio de Oro region of Western Morocco. Now extinct in the wild, the subspecies is listed as CITES Appendix I and Critical (50% probability of extinction within 5 years or two generations, whichever is longer) according to Mace-Lande criteria for categories of threat. Hunting, habitat loss, and genetic isolation were factors that contributed to the loss of the population in its natural habitat. The captive stock is descendant from 19 animals imported to Almeria, Spain, in 1971 and 1975. As the search for proper areas for reintroduction continues, captive propagation of the global population of approximately 170 individuals has been given a high priority.

This study describes an epizootic of a probable infectious disease in a captive breeding program for the Mhorr gazelle and the impact on that program.

Description of outbreak

Mhorr gazelle enclosure. On January 23, 1988, an adult female Mhorr gazelle, noted to be thin and depressed for several days, was immobilized for examination. A necrotic exudate was present along the dorso-lateral and caudal aspect of the oral cavity. After hospitalization and supportive care including penicillin injections, the animal recovered and was returned to the exhibit. This animal had shared an enclosure, designated HTHP 32, with five adult and one juvenile (its own calf) Mhorr gazelles, one Maxwell’s duiker (Cephalophus monticola maxwelli), and, until January 7, eight slender-horned gazelles (Gazella leptoceros). Within eight days of the first signs of disease in the enclosure, the juvenile and two adult Mhorr gazelles became ill. The onset of the illness was acute and manifested by respiratory distress, head and neck extension, salivation, fever, and rough hair coat. Physical examination revealed oral lesions with heavy, white diphtheritic membranes in the pharynx. One adult and the juvenile had complete airway obstruction requiring emergency tracheostomies. In spite of supportive care and antibiotic therapy, both of these animals eventually succumbed while the second adult, with less severe lesions, recovered.
Approximately four weeks after the recognition of illness in the first animal, an apparently asymptomatic Mhorr gazelle died as a result of a fracture of the third and fourth cervical vertebrae. Examination of the oral cavity revealed a single healed ulcer on the hard palate and an active ulcer on the buccal mucosa.

Including the slender-horned gazelles, a total of 17 animals had been potentially exposed to an infectious agent in this enclosure. A review of the gross and histopathologic records of the three Mhorr gazelle deaths in HTHP 32 in 1987 did not reveal the presence of any resolved or active oral lesions. These three animals were five to seven months of age and all were offspring of females that were affected in early 1988.

**North Africa boma and enclosure.** On January 7, 1988, five slender-horned gazelles were transferred from HTHP 32 to the North Africa boma. On January 25, four of these animals and an additional slender-horned gazelle from HTHP 32 were placed in the North Africa enclosure. One of the gazelles housed in the boma was experiencing acute respiratory distress on January 25 and was hospitalized alone and indoors. Physical examination revealed pharyngeal edema and diphtheritic membranes causing partial airway obstruction. After apparent recovery following treatment, the animal was found moribund four days after admission and died within several hours from peritonitis secondary to perforating rumen ulcers. Examination of the oral cavity revealed resolving lesions.

On February 18, an adult Addra gazelle (*Gazella dama ruficollis*) was found dead in the North Africa enclosure. Examination revealed severe ulcerative tonsilitis and pharyngitis and evidence of severe diarrhea. A total of 34 animals had been potentially exposed in this enclosure.

**Infant isolation unit (IIU).** On February 1, the infant born in HTHP 32 on January 24 was moved to the IIU due to the illness of its mother. After fourteen days of isolation without the development of any clinical symptoms, this animal was let into an outside yard with six additional infants of mixed antelope species. The next day, the Mhorr gazelle was salivating and its appetite was decreased. Physical examination of the oral cavity revealed lesions typical of those found in the previously affected animals and the calf was placed back in isolation on antibiotic therapy. After seven days of therapy, no oral lesions were observed. Eleven days after exposure to this Mhorr gazelle infant, a Thomson's gazelle was observed with a runny nose and decreased appetite. Necrotic pharyngitis and buccal mucosal ulcers were observed and antibiotic therapy for five days led to resolution of the lesions. The successful treatment of this case occurred approximately one month after initial clinical symptoms were observed in the index case.

**Additional Mhorr gazelle deaths**

Animals that recovered from their original illness were returned to HTHP 32 where breeding continued. Offspring and some adults were eventually moved to another enclosure. In September, 1988, the infant that went to the IIU in early February was euthanized at 8 months of age after several episodes of chronic pharyngitis. In February-March 1990, three
gazelles were euthanized with active disease or for herd health reasons. These animals included the originally affected female, one of her offspring, and a six-month old calf born in HTHP 32. In late November 1990, a young adult female with a history of four episodes of pharyngeal abscesses over an 18 month period was euthanized as was her dam one week later. Because of recurrent illness in the herd possibly due to a carrier state in some individuals and the inability to exchange animals with other institutions, the decision was made in mid-March 1991 to euthanize the three remaining Mhorr gazelles at the Wild Animal Park. These included two previously affected adults enclosed in HTHP 32 during the original outbreak and the offspring of one of them. No attempts have been made to establish another herd at the Wild Animal Park, although an unaffected, unexposed herd is maintained at the San Diego Zoo.

Pathology findings

Gross lesions consisted of varying degrees of hyperemia, erosion or overt ulceration and necrosis of buccal, pharyngeal and rumenal mucosa. Tonsilar necrosis was a common finding and in one animal the tongue was also affected. The most severe lesions were covered by a caseous diphtheritic membrane. Lung abscesses were present in some animals. Lesions elsewhere were inconsistent. Healed lesions were seen in some of the animals that were euthanized as suspected carriers.

At the time of necropsy all lesions were heavily contaminated by mixed bacteria including mats of filamentous rods arrayed perpendicular to the ulcer surface (morphology suggestive of \textit{Fuso bacterium}). Fibrin thrombi were often present in the inflamed lamina propria beneath the ulcer. Marked granulation was present in many animals. Edema and inflammation extended into the underlying pharyngeal salivary gland lobules and connective tissue.

No epithelial lesions suggestive of viral infection, such as individual cell necrosis (acantholysis), vesiculation, ballooning degeneration or inclusion bodies, were recognized in the oral or rumenal epithelium. In salivary glands, however, ductular epithelium often was altered by segmental individual cell necrosis suggestive of viral etiology. Squamous metaplasia was present in some ducts. The lesions resembled those seen in rats infected with sialodacryoadenitis virus (a coronavirus).

Microbiology

\textbf{Bacteriology results.} Swabs of the pharynx, hard palate, and buccal mucosa collected during antemortem examination as well as specimens of various tissues collected at necropsy were submitted for bacterial isolation. Routine culture techniques resulted in the identification of a variety of organisms from these specimens including alpha and beta hemolytic \textit{Streptococcus}, \textit{Pseudomonas aeruginosa}, \textit{Rhodococcus equi}, \textit{Escherichia coli}, and \textit{Proteus} sp. Although \textit{Bacteroides fragilis} was independently isolated from the tongue and buccal mucosa of two affected Mhorr gazelles, attempts to isolate \textit{Fusobacterium necrophorum} or fungi were unsuccessful.
**Virus isolation and serology results.** Samples of various tissues, whole blood, serum, and feces were sent to the National Veterinary Services Laboratory (NVSL), Ames, Iowa and the California Veterinary Diagnostic Laboratory (CVDL), Davis, for virus isolation and detection of virus-specific serum antibodies. Dr. Anthony Castro at CVDL visualized coronavirus particles in cell cultures independently inoculated with pharyngeal tissue from one Mhorr gazelle and fecal homogenate from a second Mhorr gazelle. All other attempts to detect the presence of virus in clinical specimens were unsuccessful. Sera from five affected Mhorr gazelles were tested against a panel of viruses including bluetongue (BT) virus, epizootic hemorrhagic disease virus, malignant catarrhal fever (MCF) virus, bovine viral diarrhea (BVD) virus, multiple serotypes of calicivirus, bovine enteric coronavirus, encephalomyocarditis virus, vesicular stomatitis (VS) virus, infectious bovine rhinotracheitis (IBR) virus, bovine herpesvirus-4, bovine parainfluenza-3 virus, bovine enterovirus, and bovine respiratory syncitial virus for the presence of specific antibodies. Antibody only to bluetongue virus was detected in three of these animals.

**Discussion**

During the protracted course of the disease syndrome eleven Mhorr gazelles were involved; three died in the original outbreak and eight were later euthanized because of active lesions or management decisions. Animals with active lesions ranged in age from three weeks to over six years. In addition to the Mhorr gazelles, a total of three slender-horned gazelles and two Addra gazelles died or were euthanized.

The causative agent(s) responsible for the outbreak were never identified. The differential included viral, bacterial or toxic (plant or fungal toxin) etiologies. The presence of filamentous organisms in the histologic lesions strongly supports *Fusobacterium necrophorum* as a key factor, although the organism was never cultured. *Fusobacterium necrophorum* has been implicated in necrotic stomatitis in other non-domestic ruminants. An outbreak of systemic necrobacillosis in Thomson’s gazelles resulted in the deaths of 20 animals from September 1977-February 1978. Initial attempts to isolate *F. necrophorum* were unsuccessful until the optimal culture conditions were determined.

Nine cases of necrotic stomatitis occurred in a captive herd of Dorcas gazelles in the winter of 1982 and spring of 1983. Swabs and direct smears from the mouths of the affected animals yielded a variety of bacteria, not including *Fusobacterium* species. The possibility existed that fungal toxins might have played a role in the development of the disease.

A major consideration in the epizootic at the Wild Animal Park was whether a virus acted as an initiating factor. Viruses considered in the differential were the vesicular and ulcerative viral diseases of ruminants including VS, BVD, IBR, bovine papular stomatitis, BT, MCF, foot-and-mouth disease (FMD), rinderpest, and peste des petits ruminants (PPR).

The detection of serum antibody to BTV in three of five affected animals examined may not be significant. A relatively high prevalence of antibody to BTV in nondomestic ruminants has been detected with little, if any, association with clinical disease.
typical of BT were not seen histologically. Serology was not performed against FMD virus, rinderpest virus, or PPR virus; however no histologic lesions suggestive of these infections were seen.

Thus, tests done at the time seemed to exclude the usual vesicular viruses. However a coronavirus was isolated from pharyngeal tissue homogenate. Although far from suggestive of any etiological role, this finding is still interesting. The histologic changes in the salivary glands do suggest the possibility of a coronavirus infection in some of the gazelles. Unfortunately, the cultured coronavirus was lost and stocks do not exist with which to conduct seroprevalence studies.

Although the exact etiology was never determined, an infectious etiology is almost certain. This outbreak highlights the impact that infectious disease can have on limited stocks of highly endangered species. Since many of these captive raised species are part of a global conservation strategy that includes reintroduction, it is imperative that we better understand the spectrum of diseases affecting their survival.
ACKNOWLEDGEMENTS

The authors thank Drs. Montash Banjeree, Anthony Castro, James Pearson, Meg Sutherland-Smith and Werner Heuschele, and Robert Klieforth for their assistance during various stages of the epizootic and this study.

LITERATURE CITED

MYCOPLASMA PODODERMATITIS (BUMBLEFOOT) IN A SIBERIAN CRANE (Grus leucogeranus)

Julie Langenberg, VMD
International Crane Foundation, Box 447, Baraboo, WI 53913-0447, USA

A six year old male Siberian Crane (Grus leucogeranus) was presented for inflammation of the right foot. For the previous two months he had been lame on the left leg due to chronic non-infectious tendonitis in the left hock.

On physical examination, the central area of the foot was markedly swollen, and a 2cm pigmented scab was present in the center of the ventral weight-bearing surface. The white blood cell count was elevated (20,640/mm$^3$) due to a heterophilia, and a serum chemistry profile was unremarkable. Radiographs of the foot showed soft tissue swelling, but no evidence of bone involvement. The ventral scab was surgically removed and a swab was taken of the underlying necrotic tissue. On aerobic culture, four coliforms grew: two unspeciated Enterococcus, Enterobacter agglomerans, and Pseudomonas aeruginosa.

Initial treatment of the foot included debridement of necrotic tissue, packing of the foot with betadine-soaked gauze, and wrapping with a modified "ball" bandage. Treatment over the next three weeks included gentamicin (6mg/kg) and ampicillin (150mg/kg) SQ BID, flunixin meglumine (3.5mg/kg) SQ SID as needed to control lameness, and daily debridement, soaking, and bandaging of the foot wound. Parenteral antibiotics were discontinued after three weeks; topical wound treatment and bandaging were continued on a schedule of decreasing frequency for the next month. Two months after initial presentation, the foot wound was healed, the bird was no longer lame, and nothing was found on aerobic culture of a fine needle aspirate from the foot. The affected right foot was still larger than the left, but the swelling appeared to be fibrous scar tissue.

Three months after initial presentation, the crane was evaluated for recurrence of reluctance to walk on the right foot. On physical examination, the right foot was significantly more swollen and warm. The total white blood cell count was elevated (29,502/mm$^3$), but other CBC and serum chemistry analyses were unremarkable. Radiographs once again showed only soft tissue swelling. Two cc's of tan fluid were aspirated from the swelling. The cytologic diagnosis was "pus": sheets of degenerating heterophils; no bacteria were seen. No significant growth occurred on aerobic and anaerobic cultures. Mycoplasma alkalescens was isolated from the pus.

The foot was surgically drained and flushed with 2% chlorhexidine through 4 small incisions. The incisions were partially closed around 3 Penrose drains, and the foot was bandaged. Post-operatively, the crane was treated with tylosin (25mg/kg) IM BID, and flunixin meglumine (7.5mg/kg) SQ BID as needed to keep the bird comfortable enough to bear weight on the foot. The inflammation of the foot decreased dramatically over the next week, and no new pus was seen after the first 24 hours. Eight days after surgery, the bird was found dead. The cause of death was severe visceral and renal gout, likely due to...
Pathology in the foot included cellulitis, tendonitis and septic arthritis of one interphalangeal joint. Post-mortem cultures of the foot were not done.

Discussion

Mycoplasmosis, including respiratory, genital, and joint infections, is well documented in poultry, but not well-known in other species of birds. Several investigators have searched for mycoplasmas in cranes, but there is only one previous report, *M. gallinarum* in a Demoiselle crane (*Anthropoides virgo*), with no description of associated clinical disease. At the International Crane Foundation, cranes of 4 species have been screened for mycoplasmas (using tracheal wash culture, and *M. gallisepticum*, *M. synoviae*, and *M. meleagridis* hemagglutination-inhibition serologic tests), with no positive results.

Mycoplasmal synovitis is well-known in chickens and a variety of other gallinaceous birds, waterfowl, and pigeons infected with *M. synoviae*. Mycoplasmal joint infections have also been described in raptors, including a case of pododermatitis in a Red-tailed Hawk which was associated with an unspeciated mycoplasma.

*Mycoplasma alkalescens* has not been previously associated with disease in birds. It was first isolated in Australia from the nasal passages of normal cattle, and has caused mastitis and calf arthritis in Holstein herds in the US and Australia.

The causal relationship between the isolated *M. alkalescens* and the crane's pododermatitis is suggested by the similarity of the pathology to that described in cattle joints, a failure to demonstrate other disease-causing organisms, and the apparent response to tylosin. A particularly striking feature of the pododermatitis in this case was the accumulation of "mammalian-type" pus, rather than the more typical caseonecrotic inflammatory exudate usually seen with bacterial pododermatitis in cranes. "Creamy" exudate is described in early stages of mycoplasmal synovitis in chickens, but the exudates associated with mycoplasmal synovitis in other avian species were not well described in the reports.

The source and transmission route for the *M. alkalescens* in this case are unknown. On necropsy there were no signs of airsacculitis or involvement of other joints which might be expected if the infection was systemic; transmission via the respiratory tract and systemic spread to the joints are the norm for mycoplasmal synovial infections in poultry. It seems most likely that the *M. alkalescens* was directly inoculated into the foot from a soil source or fomite. The *M. alkalescens* infection most likely was established in the compromised tissues remaining after treatment of the initial bacterial pododermatitis. Mycoplasma are thought to be important as secondary invaders in respiratory and ocular conditions in other species.
ACKNOWLEDGEMENTS

The author thanks Ruth Duncan, National Wildlife Health Center, for the microbiological investigations.

LITERATURE CITED

RAPTOR REHABILITATION – PRACTICAL EXPERIENCES FOR THE EVALUATION OF INJURED ANIMALS

Jean-Michel Hatt, Dr.med.vet.*, Ruth Baumgartner, Dr.med.vet. and Ewald Isenbügel, Prof.Dr.med. vet.
Clinic for Zoo Animals and Exotic Pets, University of Zurich, Veterinary Faculty, 8057 Zurich, Switzerland

Introduction

Urbanization and the consequent loss of natural habitat continue. This has led to an increasing number of confrontations between wildlife and man. On the other hand, an increased public awareness of the need to “help” can be noted. As a result veterinarians will have to deal with injured wildlife and their rehabilitation more often. However the public motivation to save every organism has made rational decision-making difficult, as has been well documented in the media.

Because of their beauty and biology birds of prey enjoy a status of high priority. In the treatment of sick and injured raptors, techniques such as complex orthopedic surgery are relatively recent and they have certainly made new possibilities accessible. However it has to be emphasized that the ultimate goal of any veterinary treatment of birds of prey, as it is for any other kind of wildlife, must be to return to a suitable environment an animal that will be able to survive. The effort to achieve this aim has to be considered from different points of view. There are legal, financial as well as ethical or moral aspects.

A very important step to successful rehabilitation is a correct assessment of the animal, as has been described by various authors. Furthermore the presentation of data on the diseases of birds of prey and the success of treatments is very valuable in enhancing the knowledge of raptor biomedicine.

The Clinic for Zoo Animals and Exotic Pets of the University of Zurich has been involved in the treatment and rehabilitation of birds of prey since 1968. The data and experiences have been published regularly.

Material

Between 1 January 1985 and 31 December 1994, 554 birds of prey (Tab.1) have been admitted to our clinic by private people and different public institutions (e.g. police, airport staff, etc.). The reasons were, in order of number of individuals: 1. Accidents (car, electric cable, window etc.), 2. starvation, 3. diseases, 4. orphaned birds, and 5. problems due to wrong management.

After admission to the clinic, every bird goes through a general check-up involving: thorough anamnesis, adspection, physical examination, radiography, weight- and wing-measurement. If necessary further diagnostic procedures such as hematology, blood chemistry, parasitology of the feces, endoscopy etc. are performed. The decision on the releasability and therefore on treatment versus euthanasia is made on the basis of the following information: 1. general
health status of the raptor, 2. type of disease or injury (fracture, luxation, open or closed, etc.), 3. species (very common, endangered, etc.).

Due to the restricted space available at the clinic, birds are only kept there for emergency and surgical treatment. For the postsurgical treatment and preparation for rehabilitation birds are moved to a raptor rehabilitation center. Every bird of prey that is released gets an identification-ring. Before release, birds are trained in cages of different size. The time until a bird is fit for rehabilitation depends very much on the duration of medical treatment. The chosen site of release is either, where the animal was found originally or, if this is not possible, an alternative site where the animal could survive for several days. It has been shown that rehabilitated birds spend 3-5 days on the site of release before continuing to a new territory.5,11

Results

From 554 birds of prey admitted to the clinic, 170 (31%) could be successfully rehabilitated (Tab.1). The average duration of time spent in captivity was 28 days, ranging from 1 to 260 days. Three birds, two peregrine falcons (Falco peregrinus) and one eagle owl (Bubo bubo) went to a breeding center.

Because of debilitation or the severity of disease or injury, 384 (69%) of all birds had to be euthanized or they died immediately after admission.

A total of 261 (47%) birds had one or more fractures (Tab.2), with the wings (71%) being involved most often (Tab.4). Only 49 (19%) fractures concerned the legs and 13 (5%) the coracoid bones. Fractures were treated in different ways as has been described in the literature; such as: External coaptation, internal fixation, external skeletal fixation.2,13 Only 89 (34%) of the fractures could be considered as being worth attempted treatment (Tab.5 and Tab.6). From those, 47 (53%) could be successfully released. A total of 72% from the 32 fractures that were treated nonsurgically led to successful rehabilitation. Generally, closed fractures of either ulna or radius could be left to natural healing.

Two common buzzards (Buteo buteo) were recaptured 3 and 8 years after bone surgery. They obviously had survived well and could be identified because of their ring.

Fractures of the wing were treated surgically where possible. The method of choice depended on the type and localization of the fracture (intramedullary pin, external fixation, plate, etc.). After surgery, the birds were kept in darkness for 1-2 weeks before being placed in larger cages.

Fractures of the legs usually have a good prognosis in birds of prey. In most cases surgery was the method of choice.

Fractures of the coracoid bone occur with birds flying headlong into windows or other solid objects. Usually these birds have an anamnesis of not gaining any height when flying. The fracture of the coracoid bone leads to an instability in the pectoral girdle.
All birds with fractures of the pelvis or vertebrae showed paralysis of the legs and the innervation of the cloaca appeared to be severely disturbed. None of these birds could be released.

**Feather fractures** were due to either wrong handling and keeping, or had a direct connection to the accident. If treatment and rehabilitation of the bird was decided, the feathers were repaired using the imping technique.¹⁹

**Foreign bodies**, besides gunshot, were seldom. A black kite (*Milvus migrans*) died from a condom that it had swallowed.

The common buzzards (*Buteo buteo*) and the European kestrels (*Falco tinnunculus*) with **metabolic disease** had all been kept on wrong diets for several weeks and showed signs of metabolic bone disease. A red kite (*Milvus milvus*) had a massive struma.

**Hand-rearing** of young birds that appeared to have been orphaned is not encouraged and was only performed in 11 cases (2%) and mainly in European kestrels (*Falco tinnunculus*) (Tab.3). Major attention was paid to avoid the animal becoming imprinted on humans.⁸ From the **infectious agents** endoparasites were the most frequently diagnosed, especially *Capillaria sp.*, *Porrocaecum sp.* and Coccidia. However it has to be mentioned that not every bird was screened for parasites since most of them were treated routinely with 50 mg/kg fenbendazole p.o. (Panacur®, Suspension 10%, Hoechst, 8044 Unterschleissheim b. München, Germany) for 5 days and a pyrethrum spray (Vinx, A. Ziegler AG, 8143 Stallikon, Switzerland) against internal and external parasites, respectively. One peregrine falcon (*Falco peregrinus*) died from Trichomoniasis. Bacterial (*E. coli*), viral (one suspected case of a adenovirus-infection) and mycotic (*Aspergillus sp.*) agents were rarely identified. **Traumatic ocular lesions** were most frequent in common buzzard (*Buteo buteo*) and tawny owl (*Strix aluco*). It is important to look for ocular lesions as early as possible since late diagnosis might prolong the treatment unnecessarily and this can have a negative influence on the rehabilitation.¹⁰

Because of the light and unstable bones of the skull, birds are very vulnerable to head trauma. Typical symptoms of birds with head trauma were superficial lesions on the cere or around the eyes, apathy and sometimes CNS symptoms such as torticollis. Minor cases would usually resolve within 3 days, with the bird being kept in darkness. More severe head trauma would lead to the death of the animal.

Ninety-four (17%) of all the birds admitted to the clinic showed signs of starvation. Degree of starvation is evaluated upon palpation of the breast muscles and published data on the ideal bodyweight for the species.²⁰ A weight loss of more than 30% in diurnal raptors and 20% in nocturnal birds appears to be fatal as has been stated by others, too.¹⁴ These birds have to be artificially fed on a special diet. The reasons for an excessive loss of weight are either lack of food during sudden climatic changes (e.g. heavy snow fall) or a prolonged interval between accident and the time when the bird was found. It has also been mentioned
that moult might be a reason as well as inability to establish territory and thus secure adequate food supply during their early independence from adults. 9

Discussion

Raptor rehabilitation is subject to high costs due to special housing requirements and a frequently long convalescence, which usually includes flight training. Unfortunately the costs must often be absorbed by the rehabilitators. Therefore a selection between releasable and non-releasable birds has to be made during the first days after admission to the clinic. It also is a matter of animal welfare that birds that are non-releasable should be euthanized as soon as possible.

The decision on euthanasia versus treatment has to be made on the basis of the results of the examination but also on the knowledge of the biology and behaviour of the bird. In the above mentioned results it appears that injuries in birds of prey are more frequent than are diseases. One has to consider, however, that disease, such as heavy parasitic infestation, might be the reason that a bird came to be injured.

In our experience birds with a bad prognosis for rehabilitation show symptoms such as: 1. Starvation with loss of more than 30% of the ideal bodyweight in diurnal raptors and more than 20% in owls. 2. Old, open fractures especially in the wings (in this data nearly 2/3 of the fractures involved the wing!). Fractures that involve the articulation are particularly serious. 3. Paralysis of an extremity such as the legs.

The path from a sick raptor to its successful rehabilitation is a complex puzzle. Research on the disease and the biology of birds of prey is still needed to enhance the chances of survival of these impressive animals.

ACKNOWLEDGMENTS

The authors would like to thank Mrs. Veronika von Stockar from the Greifvogelstation Berg am Irchel (Switzerland) for her assistance in reviewing the case histories in this report.

LITERATURE CITED

Table 1. Species and number of birds of prey admitted to the Clinic for Zoo Animals and Exotic Pets, University of Zurich, Switzerland from 1 January 1985- 31 December 1994 (* 1 eagle owl and 2 peregrine falcons went to a breeding center). The species numbers 1-15 are used as identification in Tab. 2-6.
Table 2. Injuries and musculo-skeletal problems in birds of prey.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>Σx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractures</td>
<td>125</td>
<td>36</td>
<td>35</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>12</td>
<td>7</td>
<td>3</td>
<td>261</td>
</tr>
<tr>
<td>Luxations</td>
<td>13</td>
<td>10</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gunshot</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Impact</td>
<td>22</td>
<td>11</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oil</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Superf.</td>
<td>26</td>
<td>5</td>
<td>7</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Internal</td>
<td>16</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arthritis/osis</td>
<td>4</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ligament rupt.</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Feather</td>
<td>8</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3. Infectious diseases and internal problems in birds of prey.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>Σx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand-rearing</td>
<td>1</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>Endoparasites</td>
<td>14</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>Bact. infection</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Viral infection</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Aspergillosis</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Ocular lesions</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>24</td>
</tr>
<tr>
<td>Bumblefoot</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>CNS</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Starvation</td>
<td>46</td>
<td>11</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>94</td>
</tr>
<tr>
<td>Foreign body</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Metab. disease</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 4. Localization of fractures in birds of prey.

<table>
<thead>
<tr>
<th>Fracture</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>Σ%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humerus</td>
<td>25</td>
<td>21</td>
<td>11</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>85</td>
</tr>
<tr>
<td>Radius / Ulna</td>
<td>30</td>
<td>10</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>60</td>
</tr>
<tr>
<td>Metacarpus</td>
<td>15</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>41</td>
</tr>
<tr>
<td>Femur</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>18</td>
</tr>
<tr>
<td>Tibiotarsus</td>
<td>18</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>28</td>
</tr>
<tr>
<td>Tarsometatarsus</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Scapula</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Clavícula</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Coracoid</td>
<td>7</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13</td>
</tr>
<tr>
<td>Vertebræ</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Pelvis</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 5. Resolution of surgically treated fractures in birds of prey.

<table>
<thead>
<tr>
<th>Fracture</th>
<th>Total Attempts</th>
<th>Number Healed</th>
<th>Number Released</th>
<th>Nonunions</th>
<th>Died or Destroyed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Humerus</td>
<td>25</td>
<td>44</td>
<td>10</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td>Radius and Ulna</td>
<td>9</td>
<td>16</td>
<td>3</td>
<td>33</td>
<td>3</td>
</tr>
<tr>
<td>Metacarpus</td>
<td>10</td>
<td>17</td>
<td>7</td>
<td>70</td>
<td>6</td>
</tr>
<tr>
<td>Femur</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Tibiotarsus</td>
<td>10</td>
<td>17</td>
<td>5</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>Tarsometatarsus</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td>100</td>
<td>27</td>
<td>47</td>
<td>24</td>
</tr>
</tbody>
</table>

1995 PROCEEDINGS JOINT CONFERENCE AAZV / WDA / AAWV
Table 6. Resolution of nonsurgically treated fractures in birds of prey.

<table>
<thead>
<tr>
<th>Fracture</th>
<th>Total Attempts</th>
<th>Number Healed</th>
<th>Number Released</th>
<th>Nonunions</th>
<th>Died or Destroyed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Humerus L, lW</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>Radius or Ulna</td>
<td>15</td>
<td>47</td>
<td>14</td>
<td>93</td>
<td>14</td>
</tr>
<tr>
<td>Metacarpus</td>
<td>10</td>
<td>32</td>
<td>5</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>Femur</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tibiotarsus</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Tarsometatarsus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coracoid</td>
<td>3</td>
<td>9</td>
<td>3</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>100</td>
<td>26</td>
<td>81</td>
<td>23</td>
</tr>
</tbody>
</table>
VERTEBRAL CHONDROSARCOMA IN A CORN SNAKE

Michael M. Garner, DVM, DACVP
Northwest ZooPath, 15326 Broadway Avenue, S.E., Snohomish, WA 98290-7042, USA

Darin Collins, DVM, Janis Joslin, DVM
Woodland Park Zoo, 5500 Phinney Avenue North, Seattle, WA, 98103-5897, USA

Introduction

Primary neoplasms involving the spinal column are rare in reptiles.1 Stolk described multiple chondromas arising in the vertebral cartilage of the tail of an Emerald lizard (Lacerta viridis).2 Wadsworth described a osteochondrosarcoma in the vertebra of a black cobra (Naja melanoleuca).3 Hill described osteosarcomata of the spinal column in a rufous-beaked snake (Ramphiophis rostratus).4 A slow growing chondrosarcoma of the vertebrae in a corn snake (Elaphe guttata) with visceral metastasis was reported by Dawe et al. A viral etiology for the neoplasm was suspected but not proven in that case, based on light and electron microscopic examination of the primary tumor and serial cultured cells from the tumor.5 This report describes an additional chondrosarcoma of the vertebrae in an aged corn snake.

Case Report

A nine year old female cornsnake was euthanized due to a slowly developing focal, proliferative and lytic lesion of approximately 2 years duration. The lesion involved two adjacent vertebrae, approximately 57 centimeters from the tip of the tail. Events leading to euthanasia included a pathologic fracture of the affected region of spine and subsequent posterior paresis. At necropsy, gross abnormalities were limited to the vertebral lesion, in which adjacent vertebrae were obliterated by a firm, smooth, white soft tissue mass that on cut surface had a caseous and cystic core. No other gross abnormalities were detected.

Microscopic examination of sagittal sections of the affected vertebrae revealed focally extensive obliteration of vertebrae by a multilobulated, expansile and invasive neoplastic mass. Neoplastic lobules were comprised of sheets of relatively well differentiated but disorganized chondrocytes in a chondromatous stroma. The neoplastic cells were located within variably sized clear lacunae surrounded by concentric zones of basophilic granular material. The cells had scant to moderate amounts of pale eosinophilic cytoplasm and moderately anisokaryotic round hyperchromatic to vesicular nuclei containing one or two small nucleoli and occasional mitotic figures. Some lacunae contained two or three cells or necrotic cell debris. The neoplasm was invading marrow spaces, cortical and trabecular bone, adjacent skeletal muscle and connective tissue, and in many areas was contiguous with remaining histologically normal articular cartilage of lateral facets. Vascular invasion and metastatic foci were not detected.
Discussion

The slow progression of this malignant neoplasm was attributed to its well differentiated cytomorphology and invasive rather than metastic behavior. A weakening of the vertebrae by the neoplastic infiltrate eventually resulted in the pathologic fracture and paresis caudal to the fracture site. It is interesting to note that in the previously reported vertebral chondrosarcoma in a corn snake, the progression of tumor growth was also relatively slow, with that snake surviving approximately two years from the time the tumor was first noted clinically. Although multiple visceral metastatic foci were detected in that case, the snake of this report had no detectable metastases. Cytoplasmic inclusions detected in the previous case of chondrosarcoma were not a feature of the tumor in the snake of this report.

Chondrosarcomas originate from existing normal cartilage or from perichondrium. Primary tumors of hyaline cartilage reportedly do not occur. In this case, neoplastic cells were clearly contiguous with well differentiated hyaline cartilage of lateral facets, suggesting that in this case the tumor may have originated from hyaline cartilage. Conversely, the tumor may have originated in fibrocartilage or perichondrium adjacent to the joint and subsequently invaded the hyaline cartilage.

Differential diagnoses for proliferative or lytic lesions involving the spinal column of snakes include trauma, osteomyelitis and osteitis deformans. History is helpful in establishing trauma as a causitive event. In the latter two conditions, the lesions generally are segmental and may involve several contiguous vertebrae. Although apparently rare, neoplasia should be considered in the differential diagnosis of focal lesions in the spinal column of reptiles.

ACKNOWLEDGMENTS

The authors thank Susan Walls for gross photographs obtained at necropsy and Phoenix Central Laboratory, Everett, WA, for loaning the slides for photomicroscopy.

LITERATURE CITED

ACORN TOXICITY IN A GRANT'S ZEBRA (Equus burchelli)

Terry M. Norton, DVM, Dipl. ACZM*
North Carolina Zoological Park, Hanes Veterinary Medical Center, 4401 Zoo Parkway, Asheboro, NC 27203, USA

Ted Mashima, DVM
Department of Companion Animal and Special Species Medicine, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606, USA

In October 1994, a 21 year old, male grant's zebra (Equus burchelli) developed slight lethargy, decreased food consumption, and loose stool with large quantities of acorn parts. Fecal examination for parasites was negative, a stained smear for fecal white blood cells was negative, and feces were collected and submitted for salmonella culture. The next day the zebra was extremely lethargic, consuming even less food and very little water, and was salivating excessively. Urination was not observed and no urine was present in the zebra's stall. The zebra/giraffe exhibit had large quantities of acorns on the ground compared to previous years. Other zebra housed next to this animal appeared to be normal with only small quantities of acorns in the stool.

Acorn toxicity was suspected and it was elected to do a complete diagnostic workup and treat the zebra with aggressive fluid therapy. The zebra was immobilized with detomidine, butorphanol, etorphine, and ketamine.

Blood was obtained prior to fluid therapy for a complete blood count, serum biochemical profile, and serum banking. Abnormal serum biochemical parameters included hypercalcemia, hypoalbuminemia, and increased blood urea nitrogen and creatinine.

Physical examination revealed a grade I-II/V systolic heart murmur, auscultation of the lungs was normal, rectal examination was normal other than diarrhea and numerous acorn parts in the feces, the body temperature was low and continued to decline throughout the procedure. Sixteen liters of warmed lactated ringer's solution was administered over 2.5 hours. There was no obvious urine production throughout the procedure. The zebra went into respiratory arrest 2.5 hours into the procedure. Resuscitation efforts were unsuccessful.

Postmortem findings were consistent with acute anuric renal failure secondary to acorn toxicosis. Urine collected at the postmortem exam was submitted for gallic acid equivalent content and is still pending.

Measures taken to prevent further ingestion by the zebra herd included raking acorns from the exhibit and providing ad lib hay on exhibit in areas devoid of acorns. No further morbidity or mortality were encountered.
PARALYSIS OF THE SUPRASCAPULAR NERVE (SWEENY) IN A JACKSON'S HARTEBEEST (Alcelaphus buselaphus jacksoni)

Terry M. Norton, DVM, Diplomate ACZM
North Carolina Zoological Park, Hanes Veterinary Medical Center, Asheboro, NC 27203, USA, and the Wildlife Conservation Society, St. Catherines Island, Midway, GA 31320, USA

Jennifer Garber, DVM, Murray P. Brown, DVM, MSc
University of Florida, College of Veterinary Medicine, Department of Large Animal Clinical Sciences, Gainesville, FL 32610-0136, USA

Jeff S. Spratt, MS, John F. Iaderosa, and Dan D. Beetem
Wildlife Conservation Society, St. Catherines Island, Midway, GA 31320, USA

A 7 year old female Jackson's hartebeest (Alcelaphus buselaphus jacksoni) presented with a sudden onset of left front limb lameness with abduction of the shoulder during full weight bearing.

Twenty-nine days from the onset of lameness, the hartebeest was immobilized with carfentanil and supplemental ketamine. The muscles of the left scapula were severely atrophied. The left shoulder had decreased range of motion. A complete blood count, serum chemistry profile, and radiographs of the shoulder were within normal limits. A tentative diagnosis of suprascapular nerve paralysis (sweeny) was made based on the clinical findings. The effects of carfentanil were reversed with naltrexone.

On day 53 from the initial onset of lameness, the antelope was anesthetized with carfentanil, supplemental ketamine, and maintained on isoflurane. Physical examination revealed severe atrophy of the infraspinatus and supraspinatus muscles. Electromyographic (EMG) findings were consistent with denervation of these muscles. Based on this diagnosis, a surgical approach to treatment similar to that used in equine patients with this condition was elected. The suprascapular nerve was identified and freed up by transecting the ligament surrounding the nerve and blunt dissection of fibrous tissue in the area. It was elected not to notch the bone of the scapula.

The hartebeest was housed alone for 5 months post surgery and only mild improvement was noted. The animal was subsequently placed with 4 younger hartebeests in a larger enclosure which dramatically increased her physical activity level. Over the next 3 months significant improvement was noted and by 8 months postoperatively there was no visually detectable muscle atrophy or gait abnormalities. A physical exam and EMG were performed under carfentanil and ketamine anesthesia and the results were considered to be within normal limits. Two years after surgery the animal continues to do well.
THE USE OF A MODIFIED, LARGE CERVID HYDRAULIC SQUEEZE CHUTE FOR RESTRAINT OF EXOTIC UNGULATES

Scott B. Citino, DVM, Dipl. ACZM*
White Oak Conservation Center, White Oak Plantation, 726 Owens Road, Yulee, Florida 32097, USA

Introduction

Maintenance of healthy collections of captive, exotic ungulates requires development and implementation of rigid, intensive preventive medicine programs. Preventive medicine programs often require frequent handling of animals for such things as quarantine, annual, and preshipment health screening, disease surveillance, parasite control, and dental and hoof prophylaxis. State and federal veterinarians are also beginning to impose more extensive testing requirements for interstate and international shipments of exotic ungulates which entail increased animal handling and manipulation. Progressive collections are utilizing captive ungulates for numerous studies addressing the medical, management, behavioral, and reproductive concerns of various species in captivity and in the wild which, again, often requires frequent handling of animals. The development of good chute systems and restraint devices can streamline these procedures and reduce the stress and possible morbidity associated with frequent and/or multiple anesthesias on individual animals. Drop-floor chutes have been used effectively at numerous facilities for the restraint of several ungulate species; however, these systems appear to have some drawbacks and lack versatility.\(^1\) This report describes the in-factory modification of a large cervid hydraulic Tamer\(^R\) (Fauna Research, Inc., 8 Bard Avenue, Red Hook, New York 12571) into a versatile restraint chute which has been effective for restraint of several medium to large ungulate species at White Oak Conservation Center.

Design and Modification

The standard hydraulic Tamer\(^R\) consists of 4' x 8' sides with high and low 4" padding separated by bare metal. One side is moved horizontally by a large hydraulic piston and supplies the crushing/restraint action of the unit. The sides can open wide to form a 6' x 8' open stall-like structure. Both sides can be moved independently of one another in the vertical direction to allow elevation of the restrained animal. The operator stands on a platform on the horizontally stationary side and controls the unit with 3 levers while watching the animal in the chute. The crushing force of the unit is controlled by an adjustable bleed valve near the control levers, and the hydraulics are powered by a commercial two horsepower motor and heavy duty hydraulic pump.

Several important in-factory modifications were made to the standard hydraulic Tamer\(^R\) to improve its versatility and effectiveness for the varied ungulate species housed at White Oak Conservation Center. The height of its sides was extended by 3' with reinforced solid steel walls with slide access doors to prevent escape by jumping species while still allowing good access to the animal. The sides of the unit were hinged on moving arms to allow independent angulation of the sides. The sides can be angled together at the bottom to act...
as a V-shaped impingement chute for small species (similar to a drop-floor chute) or angled together at the top for better control of the head and neck of large, powerful species. Two inch padding was added to the center section of both sides to improve safety and prevent horn entrapment of smaller species. Two hinged access doors were placed in the center section of both sides to increase access to animals for bleeding and injections.

Results and Discussion

The most important feature of this modified hydraulic TamerR chute is its great versatility. It can easily be changed from a raised, drop impingement chute for small and medium ungulates to an active, crush chute for large ungulates. The operator has quick, precise control over the action of the chute and can quickly squeeze an animal for safe restraint or can "back-off" and allow the animal to re-position itself. If the animal is powerful and struggling, its legs can be lifted off the ground to take away its advantage. The unit can be opened wide to allow an animal to turn around, to allow an animal to calm down, or for use in sorting several animals. With smaller species, 2 to 3 animals can be restrained together in the chute. The unit allows good access to animals from the top through the sliding access doors, from the sides through the hinged access doors, or from the front and rear. Access is good for performing most procedures. If the animal is lifted off the ground, access to the feet for trimming or treatment can occur. To reduce noise levels, the hydraulic pump and motor can be moved away from the chute.

This modified hydraulic TamerR has been used successfully to restrain lowland nyala, greater kudu, bongo antelope, giant eland, and banteng so far at White Oak Conservation Center. The unit has been extremely useful for preventive medicine procedures and for repetitive, daily, hands-on treatment of cases which would otherwise require immobilization. Its most important future use will be for research applications.

LITERATURE CITED

METABOLIC BONE DISEASE IN DROMEDARY CAMELS (*Camelus dromedarius*)

Michael Lynch, BSc, BVSc, MVS
*MellxJurne Zoo, P.O. Box 74, Parkville, Victoria 3052, Australia*

Ronald F. Slocombe, BVSc, MS, PhD, Dip ACVP
*Professor Veterinary Pathology, University of Melbourne, Parkville, Victoria 3052, Australia*

Karl E. Harrigan, BVSc
*Veterinary Pathologist, Veterinary Clinical Centre, Werribee, Victoria 3030, Australia*

This report describes the occurrence between August 1989 and July 1993 of a severe metabolic bone disease characteristic of a fibrous osteodystrophy in 4 adult non-breeding female Dromedary Camels (*Camelus dromedarius*) housed at Werribee Open Range Zoo in Southern Victoria.

All animals were parent-raised and their ages ranged between 2 and 12 years of age. Cases 1 and 2 both presented with multiple limb lameness and general stiffness unresponsive to treatment with oral phenylbutazone. Necropsy findings were identical in these two animals and consisted of multiple, severe articular cartilage erosions affecting multiple joints and an extensive deposition of proliferating fibrous connective tissue that almost completely filled their nasal cavities. Case 3 presented with bilateral epiphora and a stiff gait that had developed rapidly. Treatment with nonsteroidal antiinflammatories, oral limestone and lucerne (alfalfa) hay was unsuccessful and necropsy findings were identical to the first two cases. Case 4 presented with an enlarged rostral mandible and was in only fair condition, despite abundant fodder. A mandibular bone biopsy showed bone resorption and deposition of non-mineralised fibrous connective tissue consistent with fibrous osteodystrophy. This animal was treated with dicalcium phosphate powder over a six week period but was eventually euthanised due to the rapid development of depression and muscular rigidity. At necropsy extensive fibrous connective tissue was found in the nasal cavities and mandible. This animal had only slight articular erosions in the major limb joints, compared to the previous 3 cases.

Table 1 shows selected serum biochemical values for Cases 2, 3 and 4 in comparison to published normal values for Dromedary Camels. Data is also shown from 2 additional male camels from the Werribee Zoo that remained clinically normal. In addition to total serum calcium, a calcium value adjusted for binding to serum albumin is shown, in order to better estimate the level of ionic calcium in serum. Serum ionised calcium tends to be maintained within normal levels in preference to protein-bound and bone calcium. This is demonstrated by these cases, in that camels had serum phosphorus and adjusted serum calcium levels within the normal range, despite their severe bone disease. A pre-euthanasia sample taken from Case 4 had a low serum level of calcium and a phosphorus level above normal. At the time of the sample collection this animal was displaying depression and tetanic spasm of neck musculature which were presumed to indicate profound disturbances in its calcium:phosphorus homeostasis rather than reflect a dietary calcium deficiency. All affected camels had lower than normal serum albumin levels and greater than normal levels of
alkaline phosphatase (ALP). The significance of the former is unclear while high ALP values probably reflect increased bone turnover.

Werribee Zoo is located in Southern Victoria and has a temperate, predominantly winter rainfall climate. The camels at Werribee have been maintained on a variety of pasture types, all of which appeared to provide a balanced mineral composition. Supplemental feeding with pellets, lucerne and meadow hays was provided whenever pasture quantity was insufficient for maintenance. From June 1988 to October 1992 all female camels, including the 4 cases affected with fibrous osteodystrophy, were held on lush irrigated enclosures consisting of mixed grass species and some lucerne. All camels when in these enclosures had irregular but frequent reports of loose faeces. Case 1 presented in August 1989 and the remaining cases between December 1992 and July 1993. These latter cases occurred after the animals had been moved to a drier enclosure, where it was noted that they did not often suffer from diarrhea.

Post mortem and histopathological findings in all cases were consistent with severe fibrous osteodystrophy and osteochondrosis. The aetiology of this condition remains unclear. At the time of clinical presentation all camels were on a diet presumed to be adequate in calcium as indicated by the basic pasture analysis presented in table 2. Cases 3 and 4 received calcium supplementation before and after displaying clinical signs of fibrous osteodystrophy but histopathological observations on these 2 animals suggested that the lesions were active and ongoing. In addition, serum electrolytes in Case 4 deviated further from normal as the disease progressed and samples taken at the time of euthanasia were markedly abnormal, despite continued calcium supplementation.

Fibrous osteodystrophy is most often described as a disease of horses and swine resulting from the feeding of high phosphorus-containing rations such as grains. Fibrous osteodystrophy of unknown aetiology has been seen in horses pastured in the Werribee area. This condition has previously been reported in 2 Dromedary Camels and was thought to be caused by a predominantly grain diet. Affected animals displayed multiple limb lameness and facial deformity. Deranged dietary calcium:phosphorus ratios appear unlikely in Werribee Zoo camels, because animals were seldom fed concentrates. Oxalate-containing plants may also induce fibrous osteodystrophy in horses because of oxalates complexing with dietary calcium. Examination of pastures at Werribee Zoo failed to identify large quantities of plants reported to contain significant amounts of oxalates. The possibility of other calcium-binding pasture intoxicants cannot be excluded. Ruminants do not show the same susceptibility as monogastric animals when grazing oxalate-rich pastures, because ruminal micro flora catabolize oxalate thereby increasing calcium availability. The camel fore-gut presumably functions in a similar fashion as the rumen and camels therefore are likely to be insensitive to complexing of dietary calcium by oxalates. Osteoporosis in cattle is reported due to hyperoestrogenism associated with functional follicular cysts. Pastures at Werribee Zoo have not been assessed for the presence of oestrogen-like compounds. Phosphate levels were also measured in the water supply following the development of clinical signs in Case 4 and were found to be minimal (1 mg/L).
Diarrhoea is a common disorder of camels in captivity and is often attributed to diets high in green material particularly in association with parasite burdens. Fecal flotations and post mortem examinations showed that camels at Werribee Zoo had moderate nematode burdens (genera *Trichostrongylus* and *Camelostrongylus*) and the group frequently grazed upon quite lush pastures between June 1988 to October 1992. It is possible that frequent diarrhoea in the group over this time induced a malabsorption syndrome that interfered with calcium absorption. Decreased serum calcium values in association with parasitism have been reported in camels although no bone lesions were noted in these animals. Significant malabsorption is likely to lead to other clinical signs, including emaciation, ascites and hypoproteinemia, prior to clinical evidence of bone disease. All affected camels showed serum albumin levels below normal but it is difficult to attribute this finding to a malabsorption syndrome without functional studies of the gastrointestinal system.

Malabsorption of calcium may also arise because of abnormal vitamin D metabolism. The camels had adequate exposure to sunlight, no significant lesions were found in gut, kidney or liver were found and serum 25 hydroxychlecalciferol levels appeared normal.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Judy Slocombe for clinical pathology, Professor Ivan Caple for advice on calcium metabolism and Gary Vaughan for husbandry history of the camels at Werribee Zoo.

LITERATURE CITED

Table 1: Serum mineral analysis and measurements of serum albumin, 25 hydroxycholecalciferol and alkaline phosphatase of case studies compared with 2 unaffected animals and published normal values

<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>Ca mmol/L</th>
<th>Albumin g/L</th>
<th>Adjusted Ca mmol/L</th>
<th>P mmol/L</th>
<th>25 OHCC nmol/L</th>
<th>ALP IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 2</td>
<td>2.3</td>
<td>28</td>
<td>2.4</td>
<td>2.53</td>
<td>124</td>
<td>250</td>
</tr>
<tr>
<td>Case 3 2/3/93</td>
<td>1.68</td>
<td>17</td>
<td>2.1</td>
<td>1.96</td>
<td>182</td>
<td>185</td>
</tr>
<tr>
<td>23/3/93</td>
<td>1.95</td>
<td>16</td>
<td>2.4</td>
<td>1.40</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>Case 4 28/7/93</td>
<td>2.17</td>
<td>28</td>
<td>2.3</td>
<td>1.76</td>
<td>233</td>
<td>172</td>
</tr>
<tr>
<td>2/12/93</td>
<td>1.30</td>
<td>25</td>
<td>1.55</td>
<td>3.10</td>
<td>197</td>
<td></td>
</tr>
<tr>
<td>Male 1</td>
<td>2.44</td>
<td>40</td>
<td>2.32</td>
<td>1.09</td>
<td>236</td>
<td>89</td>
</tr>
<tr>
<td>Male 2</td>
<td>2.1</td>
<td>33</td>
<td>2.15</td>
<td>2.38</td>
<td>607</td>
<td>68</td>
</tr>
<tr>
<td>Normal Range</td>
<td>1.58</td>
<td>30</td>
<td>2.30</td>
<td>1.26</td>
<td>43</td>
<td>49*</td>
</tr>
<tr>
<td></td>
<td>to 2.75@</td>
<td>to 44@</td>
<td></td>
<td>to 2.19@</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

@ Reported by Higgins and Kock (1986)
+ Beef Cattle Normals (Trube pers.comm.)
* Reported by Caligiuri et al. (1989)

Table 2: Analysis of 2 pastures grazed by affected animals

<table>
<thead>
<tr>
<th>Pasture 1</th>
<th>Pasture 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>82.3</td>
</tr>
<tr>
<td>Dry Matter (%)</td>
<td>17.7</td>
</tr>
<tr>
<td>* Crude Protein (%)</td>
<td>17.4</td>
</tr>
<tr>
<td>(Nitrogen x 6.25)</td>
<td></td>
</tr>
<tr>
<td>* Digestibility (%)</td>
<td>73.7</td>
</tr>
<tr>
<td>* Phosphorus (%)</td>
<td>0.35</td>
</tr>
<tr>
<td>* Calcium (%)</td>
<td>0.44</td>
</tr>
</tbody>
</table>

* Calculated on dry matter basis
BILIARY CARCINOMA AND HEPATOCELLULAR CARCINOMA IN AN ASIATIC BLACK BEAR

Scott D. Fitzgerald, DVM, PhD, Dipl ACVP & ACPV
Animal Health Diagnostic Laboratory, and Department of Pathology, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824, USA

Richard Bennett, DVM
Animal Clinic, P.C., 133 - 28th Street, S.E., Grand Rapids, MI 48824, USA

A captive 25-year-old male Asiatic black bear (Selenarctos thibetanus) had a 2 year history of periodic inappetence with elevated liver enzymes. Following a recent prolonged period of inappetence, icterus, and elevated liver enzymes (Table 1), the bear was euthanitized and submitted for necropsy. At necropsy, the peritoneal cavity contained 4 to 5 liters of orange ascitic fluid. A 6 cm diameter, firm mass was present involving the extrahepatic bile duct. The liver was small, firm, and had dozens of disseminated nodular masses varying from 0.5 to 6 cm in diameter. Microscopically two distinct types of neoplasms were present. The extrahepatic bile duct mass and several of the intrahepatic masses were composed of ducts lined by a single layer of cuboidal epithelial cells, and surrounded by moderate amounts of fibrous connective tissue, consistent with biliary carcinomas. Other intrahepatic masses consisted of haphazardly arranged cords of polygonal epithelial cells with round vesicular nuclei, moderate amounts of eosinophilic cytoplasm, and a moderate mitotic index, consistent with hepatocellular carcinomas.

Various types of hepatic tumors have been frequently reported in captive bears, particularly Asian bear species. However, this appears to be the first report of biliary and hepatocellular carcinomas in a bear. The clinical signs, clinicopathologic abnormalities, gross and microscopic lesions are typical for hepatic neoplasia in bears.

Table 1. Serum chemistry data from an Asiatic black bear.

<table>
<thead>
<tr>
<th>Test values</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST - Aspartate aminotransferase (IU/L)</td>
<td>119</td>
</tr>
<tr>
<td>ALT - Alanine aminotransferase (IU/L)</td>
<td>77</td>
</tr>
<tr>
<td>LDH - Lactate dehydrogenase (IU/L)</td>
<td>1772</td>
</tr>
<tr>
<td>ALP - Alkaline phosphatase (IU/L)</td>
<td>388</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>4.1</td>
</tr>
</tbody>
</table>
ZINC-RESPONSIVE DERMATOSIS IN RED WOLVES (*Canis rufus*)

Karen S. Kearns, DVM*, Jonathan M. Sleeman, MRCVS
Department of Comparative Medicine, University of Tennessee, College of Veterinary Medicine, Knoxville, TN 37901, USA

Linda A. Frank, MS, DVM
Department of Small Animal Clinical Sciences, University of Tennessee, College of Veterinary Medicine, Knoxville, TN 37901, USA

Linda Munson, DVM, Ph.D, Phil Bochsler, DVM, PhD and Karrie Brenneman, DVM
Department of Pathology, University of Tennessee, College of Veterinary Medicine, Knoxville, TN 37901, USA

An 18 month-old male red wolf (*Canis rufus*) from the Great Smoky Mountains Red Wolf Recovery Project presented with a history of generalized pododermitis which was first observed after 11 months in captivity. Initial physical exam findings revealed footpad hyperkeratosis, suppurative paronychia, inflamed interdigital skin, and deep pyoderma distal to the carpus or tarsus on all limbs. In addition, the popliteal lymph nodes were enlarged bilaterally.

Results of a complete blood count showed a mild neutrophilic leukocytosis with moderate eosinophilia. A mixed population of opportunistic bacteria were cultured from draining tracts on the distal limbs. Histopathology of skin biopsies collected from digital skin revealed eosinophilic inflammation, acanthosis, and parakeratosis with bacterial furunculosis.

These findings were consistent with a diagnosis of chronic dermatitis with secondary bacterial infection. Possible causes for the primary dermatitis included zinc deficiency, allergic skin disease, or ectoparasitism.

A presumptive diagnosis of zinc-responsive dermatosis was reached based on response to treatment. Therapy included zinc sulfate supplementation at a dosage of 10 mg/kg orally once daily, as well as antibiotics for secondary pyoderma. Clinical signs began improving within two weeks of the initiation of therapy. Following clearing of the secondary pyoderma, the hyperkeratosis resolved and the feet appeared normal within ten weeks, after which time zinc supplementation was discontinued.

The cause of zinc-responsive dermatosis in this case is undetermined. In dogs, certain breeds including Siberian huskies and malamutes have been shown to have a genetic deficiency in zinc absorption. In addition, dogs on cereal-based, high-calcium diets or on other mineral supplementation may have difficulty absorbing zinc due to binding of this mineral or competition for absorption sites. This wolf was receiving a calcium-based mineral supplement; however, other wolves on the same diet and supplementation were not showing clinical signs.
Generalized pododermatitis with hyperkeratotic footpads has been observed in two other red wolves in Washington state. Zinc deficiency was suspected and clinical signs resolved when wolves were placed on a higher quality dog food (Mike Jones, DVM personal communication).

LITERATURE CITED

GASTRIC DILATATION-VOLVULUS AND BELT-LOOP GASTROPEXY IN A JAGUAR
(Panthera onca)

Donna M Isleggio*, DVM
The Philadelphia Zoo Department of Animal Health, 3400 W Girard Avenue, Philadelphia, PA 19104, USA

Daniel J Brockman, BVSc, CVR, CSAO, MRCVS
Department of Clinical Studies, University of Pennsylvania Veterinary School, 3900 Delancy Street, Philadelphia, PA 19104-6010, USA

A 56.5 kg nine-year-old sexually intact male jaguar (Panthera onca) housed in the Carnivore Kingdom exhibit at the Philadelphia Zoo developed hematemesis of acute onset. The cat was lethargic but responsive, appeared to be weak, and maintained a hunched posture suggestive of abdominal discomfort. All other animals housed in this exhibit area were normal.

Physical examination of the cat, under anesthesia, was unremarkable. Following gastric insufflation for gastroscopy, the abdomen remained distended and turgid, despite attempts at evacuation of the introduced air. The gastric dilation was associate with a fall in mean arterial blood pressure. Attempts at orogastric intubation were unsuccessful and gastric torsion was suspected. A right lateral abdominal radiograph confirmed gastric dilatation-volvulus (GDV).

A cranoventral midline laparotomy was performed, and a clockwise gastric rotation manually reduced. A gastrotony was performed in order to evaluate the gastric mucosa. Although the mucosa initially appeared edematous, it improved in color and appearance throughout the surgical procedure. There were no other abnormalities seen within the stomach, caudal esophagus, or cranial duodenum. A belt-loop gastropexy was performed between the pyloric antrum and the right body wall. This gastropexy was sutured using 0-polydioxanone (PDS, Ethicon, Inc. Somerville, NJ 08876-0151).

Recovery was uneventful. One year post-operatively, there has been no recurrence of signs consistent with GDV.

ACKNOWLEDGEMENTS
The authors wish to thank Drs Monika Griot, Cindy Ward, and Patty Impietro, all of the University of Pennsylvania, School of Veterinary Medicine, and Sandy Skeba, CVT, Philadelphia Zoo Department of Animal Health, for their assistance. Histopathologic evaluation of gastric biopsy samples was performed by Dr Virginia Pierce, Philadelphia Zoo Department of Research and Pathology.
Physiotherapy and rehabilitation of a spectacled langur (Presbytis obscurus) with bilateral flexion contractures of the carpometacarpal musculature, secondary to uncomplicated metabolic bone disease

Donna M. Ialeggio*, DVM, Beth A. Schwenk, Lead Keeper; Spectacled Langur Studbook Keeper
The Philadelphia Zoo, 3400 W Girard Avenue, Philadelphia, PA 19104, USA

Introduction

Metabolic bone disease (MBD) may refer to any of a number of conditions affecting bone primarily or secondarily, including rickets and nutritional secondary hyperparathyroidism. In common usage in reference to young animals, the term has become nearly synonymous with nutritional secondary hyperparathyroidism (NSH). Despite many advances in husbandry and nutrition, MBD continues to present so significant a problem to zoo veterinarians, animal managers and nutritionists, that as one of its first actions the AZA Nutrition Advisory Group impaneled a subcommittee on MBD in juvenile primates.

This case study does not purport to augment available literature regarding the signalment, presentation, diagnosis, treatment, or prevention of MBD, per se. Rather, it briefly describes the process by whereby an animal severely affected by the disease and its sequelae was successfully rehabilitated.

Case Report

A thirteen month-old female spectacled langur (Presbytis obscurus) housed in the Rare Animal House at the Philadelphia Zoo was presented for examination with a history of weakness, poor growth, and abnormal locomotion. Pertinent historical information included: that this was the fourth offspring born to the same female in four years; that there had been a change in the dam’s nursing diet (from a soft concentrate cake to hard biscuit) approximately one-and-one-half years prior to this animal’s birth; that weaning was ongoing; and that natural skylights above the 10-foot exhibit had ben partially covered for several months during the weaning period following a cagemate’s cataract surgery. Initial physical findings included low body weight (1.58 kg), muscle wasting, palpable enlargements of the physeal regions of the distal humerus and femur, and proximal and distal radius, ulna, and tibia, bilaterally. The distal right hindlimb was grossly curved. The range of motion in the femorotibial and tarsal joints was remarkably restricted bilaterally. There was radiographic evidence of widening of the physeal regions of all long bones and curvature of the proximal tibiae. Serum alkaline phosphatase was 3822 IU/L (ISIS 352 IU/L, sd 307 IU/L); all other serum chemistries were within ISIS limits. Hematology was unremarkable. Vitamin D3 levels were not determined. Vitamin D deficiency rickets was considered the primary differential diagnosis, although NSH was not ruled out, as parathyroid response to perceived hypocalcemia could have resulted in normal serum calcium and phosphorus levels.
Following subcutaneous administration of injectable vitamin D₃ (Injacom, 10000 U vitamin A and 1000 U vitamin D₃), the animal was returned to the exhibit. Oral multivitamin suspension with calcium was dispensed for daily administration, and keepers were instructed to attempt hand feeding in order to insure adequate intake of appropriate food items. Although the exhibit skylights are not believed to transmit the light wavelengths appropriate for vitamin D conversion, skylight covers were removed.

Attempts at hand feeding and medication were minimally effective despite intensive keeper efforts and a history of regular individual interaction of keepers with the animals in this exhibit. The animal became increasingly inactive and immobile. Four days after initial examination, it was reported that the animal appeared to be losing the use of its hands, that it was no longer extending all of the fingers. Three days later, it was removed from the exhibit displaying signs of carpopedal tetany. Due to the severity and rapid progression of signs, euthanasia was considered. Because we believed it likely that primary nutritional deficiency and/or imbalance was the ultimate cause of the signs appreciated, we elected to attempt nutritional and physical therapy. For humane reasons, we agreed that if the monkey's condition deteriorated or if, on weekly re-evaluation, improvement was not noted, the animal would be euthanatized.

Three concurrent approaches to rehabilitation were attempted: nutritional, social, and physiotherapeutic. It was known that nutrient intake during the period between initial presentation and removal from the family group had been inadequate; it was assumed that prior nutrient intake had been inappropriate, despite the ready availability of appropriate food items. Nursing had been observed as recently as two weeks prior to presentation. Similac (Ross Products Division, Abbott Laboratories, Columbus, OH, 43215-1724) diluted 1:1 with Pedialyte (Ross Product Division, Abbott Laboratories, Columbus, OH, 43216) was offered and readily accepted from a kitten nurser. In addition, a variety of familiar food items, including both produce and a proprietary concentrate (Mazuri Leafateer Primate Diet, Product no. 5M02, PMI Feeds, PO Box 66812, St Louis, MO 63166-6812) (primate biscuit), were made available. Formula and solids were provided in four daily feedings offered between 07:00 and 23:00.

As the initial goal was to insure voluntary intake, the animal was allowed to select diet items to supplement ad libitum Similac/Pedialyte. It was apparent within one week's time that there was a marked preference for white and sweet potato, both of which are high in phosphorus and low in calcium content. In addition, what appeared to be food item color preferences were observed. That is, on a given day a significant majority of food items selected were of one color, usually orange or white, regardless of the variety of items presented. Greens were rarely selected and in very limited amounts; concentrates were not selected. Novel food items, including those that had occasionally been offered to the exhibit group as treat items, were not accepted.

Interactions between caregiver and patient were initially limited to feeding and physiotherapy sessions, in between which the animal would be returned to its crate, where it would sit or lie quietly; vocalizations were rare. As strength improved, vocalizations,
progressing from mother location "hoots" to screams, increased in both frequency and volume, disrupting both feeding and rest periods. Biting, swatting, grabbing, and tongue flicking accompanied the vocalizations. Although limited grooming by the caretakers during feeding appeared to have a calming effect, agitation was evident and interfered with both feeding and physical therapy. The animal was therefore afforded unlimited access to its two primary caregivers, one each for day-time and off-hours. Appropriate solicitations for contact, grooming, feeding, etc. received positive responses. Inappropriate behaviors were gently but firmly disciplined.

Three of the scheduled feedings coincided with usual caretaker mealtimes. Langur feeding preceded human meals. As social intimacy increased, the animal began to solicit and accept such novel food items as pasta with clam sauce, cooked beef, tuna salad, and french fries from its caregivers. These items were accepted in one trial, provided that the animal had first observed their consumption by the caregiver. One-and-one-half months after the animal was removed from exhibit, the daytime caretaker (BAS) was able to introduce an appropriate concentrate (Mazuri Leafeater Primate Diet Mini Biscuit, Product no. 5672, PMI Feeds, PO Box 66812, St Louis, MO 63166-6812) into the diet by first consuming it herself. Within one week's time, the langur was consuming sufficient quantities of concentrate to meet nutritional requirements for protein, fiber, calcium and phosphorus, as determined by the Zoo's nutritionist.

Passive physiotherapy was initiated upon removal from exhibit. Diazepam (0.3 mg/kg) was administered subcutaneously twice daily the first day to provide temporary relief of carpopedal tetany. Thereafter, 0.8 mg/kg diazepam was administered orally four times daily, at the beginning of each feeding session. Passive physiotherapy, consisting of manual extension and flexion the phalanges and metacarpi, was performed after each feeding. Within four days diazepam was no longer effective in relieving metacarpal and phalangeal muscle contraction, although muscle relaxation in other body parts was appreciated; that is, tetany had progressed to flexion contracture. Diazepam therapy was discontinued after another three days of medication.

Passive manipulation was continued following each feeding, but although flexibility appeared to improve during therapy sessions, improvements were not maintained between sessions. After two weeks' manipulations, splints were introduced. A large ball of cotton cast padding was wrapped into the palm of each hand to achieve approximately 50% extension. Splints were applied each evening and removed the following morning. Improved flexibility was appreciated for several hour after splint removal, but was not apparent at reapplication. It was therefore elected to maintain the splints for two to three days, changing them as necessitated by fecal soiling. Passive manipulation continued after feeding whenever splints were removed.

Active physiotherapy, in the form of limited assisted play (climbing low objects, rope manipulation), was initiated two weeks after the animal was removed from exhibit. As strength and dexterity improved, frequency and duration of play sessions were increased, as were the physical demands placed upon the animal.
Rigid splints were introduced five weeks after treatment began. Made of low-temperature thermoplastic (Preferred, North Coast Medical, Inc., 187 Stauffer Blvd, San Jose, CA, 95125-1042), the splints were fashioned by Zoo staff with the guidance of a hand therapist from The Philadelphia Hand Center; 90% metacarpal and phalangeal extension was desired. Splints were applied in the evening and removed the following morning. Flexibility improved and limited, but significant, active extension and flexion of the phalangeal joints was appreciated. After two weeks, application was temporarily discontinued due to the development of epidermal erosions ("tape burns") on the distal forearms. Active (play) therapy and passive manipulation continued. Despite a decrease in flexibility of the right hand appreciable on passive manipulation, climbing abilities and agility continued to improve.

Ten weeks after it had been removed from its family group, the langur was returned. Reintroduction was uneventful, with no group disruption apparent within five minutes of its release into the exhibit. Although at the time of return the monkey's hands were held in a flexed position when walking (knucklewalking), instead of in the normal palmargrade (metacarpal hyperextension, phalangeal extension) posture, it was able to extend and flex the metacarpal and phalangeal joints voluntarily with sufficient strength and ease to negotiate the exhibit safely. Keepers continued to hand feed the animal after its return. One year after reintroduction, forelimb posture is normal, and although range of motion of the femorotibial and tarsal joints is restricted, it does not affect the langur's ability to participate fully in play and other typical activities.

Discussion

When calcium and phosphorus intake are appropriate, simple rickets (vitamin D deficiency) should respond rapidly to vitamin D supplementation. In light of the apparent lack of response to vitamin D₃ supplementation alone, calcium-phosphorus imbalance and NSH (normal serum calcium and phosphorus levels notwithstanding) became the more likely primary differential diagnosis. Serum calcium and phosphorus analyses performed during the rehabilitation period are consistent with this suggestion. Renal disease, liver disease, diabetes mellitus, and possible hereditary vitamin D-resistant rickets (reported in humans) were considered and ruled out on the basis of laboratory findings and ultimate response to therapy.

Many factors may have contributed to the development of MBD in this animal, here likely a combination of NSH and vitamin D deficiency. Given known food item selection following removal from the family grouping, it is likely that inappropriate food choices during weaning were of primary significance. The animal's rapid response to physical therapy once adequate intake of appropriate levels of calcium and phosphorus could be maintained supports this suggestion.

Rapid progression from carpopedal tetany to flexion contracture is a characteristic of the course of disease in human patients affected by rickets or similar diseases, as well as by severe carpal/tarsal and metacarpal/metatarsal injuries (personal communication, Dr Greg
Keenan, Department of Pediatric Rheumatology, Children's Hospital of Pennsylvania (Children's Seashore House). As was appreciated in this case, muscle relaxants will allow limited manipulation of affected joints and digits until flexion contracture occurs. In retrospect, it might have been appropriate to supplement passive manipulation with rigid splinting during the first few days after the animal had been removed from the exhibit, in an effort to forestall this progression. However, given the radiographic appearance of skeletal tissues at that time, phalangeal and/or distal radial fractures would likely have resulted.

We believe that the intimate social interaction fostered between caregiver and patient was essential to the success of this endeavor. It is probable that the prior close relationship of one of us (BAS) with the animal when in its family group facilitated the development of the caregiver relationship during rehabilitation. Behaviors such as screaming, swatting, grabbing, biting, and tongue-flicking that had been observed frequently prior to the assumption of the role of surrogate parent by two primary caregivers were rarely observed thereafter. Stereotypy was not observed at any point. Vocalizations usually associated with mother location ceased when either surrogate responded. Novel food items were accepted in one or two trials (feedings) if first ingested by the surrogate. The close physical contact maintained between the surrogates and the langur allowed for immediate reinforcement of appropriate behaviors and discipline of inappropriate behaviors, which may have facilitated the animal's reintroduction into its family group following a two-and-one-half-month absence.

ACKNOWLEDGMENTS

We offer heartfelt thanks to Hand Therapist Trish Byron, MA, OTR, CHT, of The Philadelphia Hand Center, for sharing her expertise. Thanks, too, to Dr Keith Hinshaw, Vice President for Animal Health; Dr Andy Baker, Curator of Primates and Small Mammals; the members of the Philadelphia Zoo Primate Team; and especially to Sandy Skeba, CVT, who assumed the thankless duties of "baby-sitter", absent the surrogates.
CLINICAL MANAGEMENT OF LYMPHOMA IN A BLACK AND WHITE COLOBUS MONKEY (*Colobus guereza*)

R. Avery Bennett, DVM, MS, Diplomate ACVS®, Freeland H. Dunker, DVM
San Francisco Zoological Gardens, San Francisco, CA 94132-1089, USA

*Dr. Bennett's current address is University of Florida, College of Veterinary Medicine, Department of Small Animal Clinical Sciences, Gainesville, FL 32610-0126, USA*

A 4 year old male black and white colobus monkey (*Colobus guereza*) was evaluated for an acute onset of swelling of the right inferior eyelid of 4 days duration. The swelling was initially diffuse over the right side of the face but over the 4 days localized to the eyelid. The colobus was alert and active with a normal appetite. The globe appeared normal. A review of the past medical history included health examinations and intradermal tuberculosis testing 1 and 2 years previous to this problem. Tuberculosis tests were negative and the radiographic and hematologic parameters were considered normal except for an anemia (PCV 31%). The monkey exhibited a dyssymmetry in inguinal lymph node size with the right larger than the left. The anemia and right inguinal lymphadenomegaly were noted on examination 1 year previously. A differential diagnoses for the focal eyelid swelling included insect bite, bite from a cage-mate, and tooth root infection. The keeper was instructed to monitor for continued resolution of the swelling.

The swelling gradually resolved but recurred acutely 10 days after the initial examination. The animal was reevaluated and the swelling was again confined to the right inferior eyelid. No therapy was administered and over the subsequent 10 days the swelling progressed to involve the superior eyelid obscuring visualization of the globe. The animal was then immobilized for a detailed evaluation.

No wounds were associated with the swelling. Dental examination and skull radiographs were normal. No bacterial growth was isolated from conjunctival scrapings and conjunctival cytology was consistent with a very mild neutrophilic/lymphocytic inflammation. An anemia (PCV 27%) and a thrombocytopenia (216,000/ul; ISIS reference range 253-429) were the only hematologic abnormalities. The animal weighed 11.5 kg and was treated empirically with benzathine penicillin (20,000 IU/kg benzathine procaine penicillin G + 20,000 procaine penicillin G SQ) and prednisone (10 mg prednisone carboxymethylcellulose SQ). The monkey refused oral antibiotics, but accepted oral prednisilone and was treated with prednisolone (0.5 mg/kg PO BID X 3 d then SID X 3 d).

Despite the treatment, the swelling progressed over the next two weeks. The animal was immobilized again and dental radiographs were made. No abnormalities were noted. Ophthalmic and ultrasound examinations revealed a normal globe with periorbital swelling. The globe was retropulsed by the eyelid swelling. There was no evidence of retrobulbar nor periorbital abscess. Follow-up hematologic evaluation revealed a persistent anemia (PCV 28%) and thrombocytopenia (144,000/ul). An eyelid punch biopsy revealed a diffuse monomorphic lymphoid infiltrate with no follicular formation. The lymphocytes were
intermediate in size with few small lymphocytes. Cytology collected by fine needle aspirate showed marked lymphoid hyperplasia with all stages present but lymphoblasts were the predominant type. The pathologist felt that the cytology and histopathology were more consistent with a lymphoproliferative disease than lymphoid infiltration, but was reluctant to make a definitive diagnosis of lymphoma. Based on discussions with the pathologist and an oncologist, it was elected to treat the condition as a periocular lymphoma with radiation therapy. The oncologist offered a good prognosis for tumor control based on experience with companion animals.

The first dose of radiation was administered 10 days following biopsy collection. The affected tissue measured 3.0 cm diameter by 1.5 cm deep. An orthovoltage external beam radiation therapy unit applied a dose of 4 Gy through a 6 cm cone. Therapy was applied every 3-4 days for 7 treatments with each of the subsequent doses being 5 Gy. The cone size decreased as the tumor size decreased.

One week following the first treatment the tissue had decreased in size to 2.5 cm diameter and 1.0 cm deep. After 2 weeks of therapy the cornea was visible and appeared normal. The tissue had decreased in size to 2.0 cm diameter and 0.8 cm deep.

After 2 weeks of therapy, the keeper reported that the animal was anorectic and heat seeking. A CBC reveal a worsening anemia (PCV 21%) and thrombocytopenia (98,000/ul). The biochemistry profile revealed a hypoproteinemia (4.5 gm/dl). When the animal was immobilized for the next radiotherapy, anemia (21%) and thrombocytopenia (127,000/ul) persisted. Serology was negative for viruses that might cause lymphoproliferative disease. FDP was negative. Bone marrow aspirate revealed an over representation of lymphoblasts and prolymphocytes with few mature lymphocytes in the bone marrow aspirate. The M:E ratio was 13:1 and the lymphocytic:granulocytic ratio was 30:1. A diagnosis of early myelophthisic lymphoid neoplasia was made. The anemia and thrombocytopenia were attributed to this process.

Because the monkey had biological data consistent with systemic lymphoma, it was decided to institute chemotherapy to the treatment regimen. L-asparaginase (10,000 IU/m$^2$ IM once weekly) and prednisolone (20 mg/m$^2$ PO BID) were used because of their ease of administration. The plan for the chemotherapy was to treat for 8 weeks, then repeat a bone marrow cytology to assess response to therapy. The monkey received one additional dose of radiotherapy before radiation burn of the eyelid prompted termination of this portion of the therapy.

At the time the first chemotherapy was administered, blood was collected for CBC and biochemistry analysis. The hematocrit had decreased to 18% and for the first time a leucocytosis (13,200/ul) and lymphocytosis (9,504) were present.

Oral prednisolone therapy was successfully administered throughout the first week. The monkey received the second dose of L asparaginase by dart on day 7 of treatment. Two days later, the keeper reported the monkey was lethargic and there was evidence of diarrhea.
and vomiting. The hematocrit was 18% at this time. In light of the monkey's deteriorating condition in the face of therapy, euthanasia was elected.

At necropsy the monkey weighed 9.0 kg and was thin and dehydrated. In addition to the swollen eyelid, masses were identified in the mesenteric root, within the stomach, and surrounding each ureter from the caudal pole of the kidney to the inguinal canal. The bone marrow appeared white and solid.

Histologically, the tumor was characterized as a large cell lymphoma consistent with lymphoblastic lymphoma. Organs affected included the gastrointestinal tract, mesentery, retroperitoneal perirenal and periureteral regions, right inferior eyelid, renal hilus, lymph nodes, and bone marrow.

ACKNOWLEDGEMENTS
The authors thank Drs. Jane Turrel, Cynthia Cook, Ken Mero, and Paul Brown, DDS for their assistance on this case.
CEREBROSPINAL NEMATODIASIS IN A WHITE-HANDED GIBBON (Hylobates lar) DUE TO Baylisascaris procyonis

Ray L. Ball, DVM*
Resident Veterinarian, Topeka Zoo/College of Veterinary Medicine, Kansas State University, Manhattan, KS, 66502, USA

Sandra Wilson, DVM
Exotic Animal, Wildlife, and Zoo Animal Service, Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS, 66502, USA

Mike Dryden, DVM, PhD, and Johna Veatch, DVM, PhD
Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, 66502, USA

Introduction

Cerebrospinal nematodiasis in primates caused by the migrating larvae of the raccoon (Procyon lotor) ascarid, Baylisascaris procyonis, has been experimentally induced in squirrel monkeys (Saimiri sciureus) and cynomolgus monkeys (Macaca fascicularis). The present report describes a case of cerebrospinal nematodiasis presumably due to migrating B. procyonis larvae in a white-handed gibbon (Hylobates lar) at the Topeka Zoo.

Case Report

A female 17 year-old white handed gibbon had a history of neurologic signs for 22 months. These signs included a progressively worsening head tremor, generalized motor incoordination, and partial loss of the use of the right arm. Mentation was judged to be normal. Diagnostic findings during this time revealed radiographic evidence of a malaligned sixth cervical vertebra that considered incidental, an EMG of the right pectoral region that was non-diagnostic, and a MRI of the head that revealed a lesion in the left cerebral hemisphere. A slight relative monocytosis and eosinophilia was seen at the onset of the neurologic signs. Treatment included antibiotics and supplemental feedings as deemed necessary. In October 1994 the gibbon was found down in the exhibit and was non-responsive to keepers as they entered the exhibit. Emergency and supportive measures were performed until the gibbon’s condition stabilized. Neurological exam revealed bilateral hyperreflexive patella, positive bilateral Babinski reflex, and enlarged optic discs. Cerebral spinal fluid analysis revealed acellular fluid with a protein level of 22 mg/dl. Because of the longstanding neurological condition, failure to produce a definitive diagnosis, and poor prognosis for a return to an acceptable quality of life, euthanasia was elected.

On gross necropsy there were no gross lesions in the brain. Histologically, the sections from the cerebrum and cerebellum had multiple, random foci of granuloma formation. In the center of the inflammation were sections of nematodes that had prominent, single lateral alae. There were small areas of malacia randomly throughout the cerebral cortex, and occasional perivascular cuffs of lymphocytes and monocytes. No evidence of larvae was
found in the other tissues examined. Raccoon feces were found on the island on which the gibbon was previously housed and an additional 12 raccoon latrines were located on zoo property. Qualitative fecal flotations revealed infective larva at all sites. Four of eleven soil samples collected on the island contained *B. procyonis* eggs. Necropsy of one raccoon live trapped on the shore nearest the island revealed 56 adult *B. procyonis* (36 female; 20 male) in the small intestine.

Discussion

This report describes the natural occurrence of cerebrospinal larval nematodiasis presumably caused by *Baylisascaris procyonis* in a white-handed gibbon. The circumstances and epidemiology of the infection rule out other *Baylisascaris* species. *B. columnaris* of skunks (*Mephitis*) have been incriminated in the death of several marmosets. While skunks are found routinely on the zoo property, there is no evidence that they have been on the island. Elevated eosinophil counts are common in the CSF of infants with *B. procyonis* cerebrospinal nematodiasis, as is visceral granulomas. The gibbon in this report had none of these findings. This may be explained by the fact that the larvae was well encapsulated and that the sample was obtained late in the course of the disease. The chronicity of the infection and the lack of extraneural lesions may indicate a low level of infection. Infections with low numbers of infective larvae have been incriminated in humans with diffuse unilateral subacute neuroretinitis (DUSN). Ocular pathology was observed in squirrel and cynomolgus monkeys. Optic disc swelling was a clinical finding in this gibbon, but no larvae were found in the retina. Hyperreflexia and positive Babinski reflexes found in the gibbon of this case are consistent with findings in children with *Baylisascaris* cerebrospinal larval migrans. MRI in human infants with *Baylisascaris* cerebrospinal nematodiasis may be normal. The experimentally infected squirrel monkeys were found to have hemorrhagic tracts in the brain at necropsy. Such tracts could potentially produce lesions on a MRI similar to the ones seen on the gibbon.

Recommendations for the island exhibit included removing 15 centimeters of soil, placing hot wire around the perimeter of the island enclosing the area under the shelter, and daily cleaning of left over food items. An additional live trapping program has been implemented as well.
ULTRASONOGRAPHY AS A TOOL IN PROPAGATION OF ZOO ANIMALS

Thomas B. Hildebrandt, DVM* and Frank Göritz, DVM
Institute for Zoo Biology and Wildlife Research Berlin (IZW), Alfred-Kowalke-Str. 17, D-10315 Berlin, Germany

Reproductive disorders are a major problem in wild animals held in captivity and have a significant impact on many captive breeding programs. Basic scientific information regarding reproduction is missing or incomplete for most animal species. Questions regarding sex determination, sexual maturity, cycle length, sperm viability in the female genital tract, embryonic development and gestation length remain unanswered for the majority of zoo maintained animals and this is especially true for those species under the threat of extinction. As the maintenance of viable populations of these animal species in captivity becomes increasingly important, knowledge of the unique, reproductive biological features of a species and information concerning the reproductive status of the individual members of a breeding group is essential for successful captive breeding. The development of endocrine monitoring within the last years is an important first step toward increasing our knowledge of basic reproductive physiology in different animal species. Furthermore, the introduction of non-invasive techniques for measuring sex hormones in urine and faeces has been of considerable practical value in the reproductive management and treatment of diverse zoo species by avoiding the need for capture or restraint conforms with animal protection efforts.

The potential of real time ultrasound as an alternative approach for non-invasive exploration of reproductive biology and pathology of zoo and wild animals has not been fully utilised. Despite tremendous progress in the development of sonography and its incorporation into human medicine during the 1960's and veterinary medicine in agricultural and laboratory animals at the end of the 1970's, until now, ultrasound has only played a limited role in wildlife medicine.

The general advantages of ultrasonography as opposed to other imaging techniques are: i) it is a non-invasive technique, ii) provides real time, visual and reproducible information regarding organs, iii) produces cross-sectional images of tissues and organ structures, as well as organs in motion which is difficult or not feasible with other techniques, iv) enables morphometric measurements of examined objects to be obtained, v) facilitates documentation and preservation of primary data on storable cassettes, vi) can be easily integrated with other examination methods.

The use of ultrasound imaging in reproductive biology provides a better understanding of early embryonic development and contributes essential, new knowledge regarding the function of the uterus and ovaries. As a non-invasive imaging technique, ultrasound delivers new information concerning reproductive biology, which has not been accessible to the researcher using classical methods of experimental embryology and endocrinology. However application of ultrasonography to zoo and wild animals does have a number of specific difficulties which do not exist, or at least are less problematic, in human or classic veterinary medicine. Furthermore, knowledge obtained in humans or domestic animals, although
useful, is usually not directly transferable to wild or zoo animals. In Table 1, the principle types of difficulties in applying ultrasound technology to zoo animals and wildlife are shown together with some specific examples.

Table 1. Problems in applying ultrasound technique in zoo and wild animals

<table>
<thead>
<tr>
<th>No.</th>
<th>Problem</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Physical or chemical restraint of subject</td>
<td>Most species; some birds may be held by the wings and legs in dorsal recumbency; investigation of cold-blooded animals at 0°C; tranquilization necessary for carnivores.</td>
</tr>
<tr>
<td>2a</td>
<td>Acoustic coupling of the ultrasound probe on the skin</td>
<td>Shells, plates, feathers, fur</td>
</tr>
<tr>
<td>2b</td>
<td>or insufficient penetration of ultrasound waves through the tissue investigated</td>
<td>Avian air sacs, thick skin, large subcutaneous fat pads, long distances in megavertebrates</td>
</tr>
<tr>
<td>3</td>
<td>Modification of ultrasound system components for zoo and wild animals</td>
<td>Portable system, accumulator pack, cable extension for probes, specifically designed scan heads</td>
</tr>
<tr>
<td>4</td>
<td>A minimum of reference data on ultrasonographic appearance of different organs</td>
<td>Gonads and kidneys in birds, ovaries of elephants, etc.</td>
</tr>
<tr>
<td>5</td>
<td>Limited experience of investigator in a large number of species</td>
<td>About 5,000 mammalian species</td>
</tr>
<tr>
<td>6</td>
<td>High cost of ultrasound equipment</td>
<td>Approximately $55,000</td>
</tr>
</tbody>
</table>

In order to find some solutions to these problems in applying ultrasound to zoo and wild animals, the ultrasound research group of the Institute for Zoo Biology and Wildlife Research (IZW) has, during the last three years, performed approximately 1,100 individual ultrasound exams in over 100 animal species including amphibians, reptiles, birds and mammals. As part of this effort, ultrasound technology has been integrated into our studies on reproductive disturbances in zoo and wild animals to address the following issues.
1. Sex determination of monomorphic species

For many animal species, it is not possible for an animal keeper to make appropriate management decisions regarding breeding due to the fact that monomorphic species show no, or only slight, phenotypic differences which can be used to distinguish between the sexes. In addition, differences in behaviour based on gender are frequently not evident in captivity. There are many species of monomorphic birds and, until now, sex determination has mainly been achieved with invasive techniques such as laparoscopy or by chromosomal evaluation, which requires capture of the bird to obtain a blood sample. Ultrasonography is a non-invasive and accurate technique which can be used to detect gonadal or intragenital structures at various topographical locations. The unique anatomical characteristics of birds, however, may initially pose problems for successful sex determination using ultrasonography. The air sacs and the compact intestinal convolution prevent transmission of the ultrasound waves. In our studies, these difficulties have been overcome by the development of specialised probes allowing trans-intestinal or trans-cloacal examination. The gonads can be visualized by inserting a miniaturised probe with a frequency of 7.5 to 15.0 MHz and definitive sex determinations can be made based on the appearance of the structures seen.\textsuperscript{13,19,23,24,26}

Sonographic sex determination can also be applied to several mammalian species such as the beaver, sloth and spotted hyena. Sex determination in the two-toed sloth (\textit{Choloepus didactylus}, \textit{Choloepus hoffmanni}) and in the spotted hyena (\textit{Crocuta crocuta}) have been successful using trans-rectal ultrasound.\textsuperscript{10,40}

2. Determination of sexual cycle in females

Detailed information regarding the female sexual cycle of most animals is limited. Repeated examinations performed throughout the cycle are necessary to make statements regarding the length of the sexual cycle and its component phases. Procedures in zoo animals are accomplished in most cases by physical or chemical restraint, although methods for urinary or faecal hormone analysis provide a useful non-invasive alternative. Invasive manipulations are not without risk and can cause stress which may harm the individual animal or interfere with its reproduction. We repeatedly examined chemically immobilised lowland anoa (\textit{Bubalus depressicornis})\textsuperscript{11} and were able to determine for the first time that the length of the estrus cycle is 22 days. In some species, it is preferable to perform an ultrasound examination without physical or chemical restraint. For example, we have performed trans-rectal ultrasound examinations in trained elephants at the Hagenbeck Animal Park in Hamburg, Germany without any means of chemical restraint. It is possible to visualize structures on the ovaries with a high-frequency probe and a specially designed ultrasound configuration system.\textsuperscript{22} A single examination of the female genital tract does not offer any indication of cycle length, but may provide information on the phase of the cycle since in many species it is possible to distinguish corpora lutea from the various follicular stages using a high frequency ultrasound probe.
3. Monitoring of gestation with possible prediction of day of parturition or laying term

For a long time, biologists, zoo keepers and veterinarians have relied on observations of sexual behaviour, mating and birth to determine the average gestation length for different animal species. With these methods, the day of delivery can be estimated and management and feeding of the gravid animal can be adjusted accordingly. The introduction of sonography to veterinary medicine allows monitoring of the early stages of gestation and fetal development. Measurement of fetal structures and comparison of these findings with reference data allow the stage of gestation to be estimated and, therefore, prediction of approximate date of delivery. Indirect methods of pregnancy determination, such as progesterone and/or oestrogen metabolites in faeces or urine can be highly informative but are often species-specific due to differences in hormone metabolism and physiology. Pregnancy diagnosis is possible in a large number of wild and zoo animals (amphibians, reptiles and mammals including elephant and rhinoceros) with a one time trans-intestinal or trans-cutaneous ultrasound examination. Another possible option is fetal sex determination by ultrasonography. Sexing requires a definite age, position of the fetal and sufficient amniotic fluid. Therefore, there are only few opportunities to perform foetus sex determination successfully. With this method, statements regarding number, vitality and developmental stage of the embryo may also be possible. Trans-cloacal and trans-intestinal sonography can be employed to image the yolk and ovary in birds or reptiles and based on the extent of calcification, a prediction regarding the laying term is possible. Since ultrasound is a non-invasive, direct method which allows imaging of embryonic/fetal morphologic structures, it has therefore many advantages as a method for pregnancy diagnosis in zoo animals.

4. Identification of pathological alterations to the inner genital tract

Knowledge concerning the appearance of healthy genital structures is necessary for clear recognition of pathological alterations. Given the wide variability of genital tract structures within mammals, it is understandable that this is not always possible.

Some pathological changes are relatively easy to identify such as cysts or tumors. For example, cystic degeneration of the ovaries and endometrium in old carnivora and especially in great cats is frequently reported. Alterations in the testes, such as calcification or intra-parenchymal cysts, enables healthy and pathological structures to be relatively easily distinguished. It is more difficult to distinguish between physiologic uterine fluid and a pathological condition. For example, purulent endometritis in the monkey is not easily distinguished from the monthly menstruation.

Assessment of the reproductive capacity of the individual is based on the health of the internal genital tract. It is therefore important to detect pathological alterations of the inner genital tract and to determine the meaning or influence it may have on reproductive performance before forming breeding groups.
5. Identification of pathological disorders of embryo genesis

Information regarding the viability of the early embryo is relatively quickly obtained with trans-cutaneous or trans-intestinal sonographic examination. In some species, the time frame in which pathological changes are detected is mainly in the first trimester of gestation in mammals, although this may extend until the end of the second trimester. In most cases, it is not possible to obtain detailed diagnostic images with a high-frequency ultrasound probe late in gestation due to the formation of fetal fur and increased calcification of the skeleton.

However, it is possible to assess heart activity, volume of embryonic fluid and condition of the placenta during mid-to-late pregnancy. In addition, some abnormalities such as schistosoma reflexa can be detected by the appearance of coils of intestine floating in the embryonic fluid. Growth disorders (e.g., severe retardation) are also apparent with sonography during this stage of gestation.

Most pathologic disorders of embryogenesis occur during the time of implantation. Early embryonic death is a frequent occurrence which can be retrospectively detected with ultrasound, and structural changes within the endometrium of the slightly enlarged uterus can be visualized. Changes indicating early embryonic death include the detection of an approximately 1 mm large undifferentiated echogenic form in the uterine lumen and a corpus luteum on at least one ovary. Embryonic membranes and fluid are frequently still present in the uterus.

In some cases, identification of fetal malformations can be problematic. With ultrasound, we detected an abnormality in a Przewalski horse fetus. Only after examination of the isolated uterus could we clearly see that it was a symmetrical malformation of the fetus. Malformations causing accumulation of fluid such as ascites, cystic kidneys, hydrocephalus, etc. are clearly imaged with ultrasonography. As previously indicated in section 4, knowledge regarding the normal appearance of the different gestational stages is absolutely necessary to detect pathological changes.

6. Identification of maternal pathological processes with potential effects on structure and function of genital organs or embryo genesis

Sonographic examination of the major abdominal organs, such as liver, spleen, kidney, adrenal and pancreas may provide useful criteria to appraise the fitness or breeding potential of an animal. Detection of subclinical changes in these organs indicates that a clinically apparent metabolic disorder may occur under the physiological burden of pregnancy which could have possible lethal consequences for the mother and/or fetus. Pathological changes of the liver are most frequently found in the form of cysts or nodes (e.g., lowland anoa, dhole). Tumors in the kidney, spleen, intestine and greater omentum have also been discovered (generalised sarcoma in great cats). Excessive intra-abdominal accumulation of fluid was detected in two elephants by sonographic investigation of the
genital tract, which proved to be secondary to a subclinical cardiac irregularity. Findings regarding the general health of the animal and those specific to the genital tract should be considered in order to make appropriate decisions regarding which animals to breed.

7. Support of diagnostic procedures, therapy or assisted reproduction

Ultrasound as a non-invasive imaging technique in real-time mode can provide visualization of diverse diagnostic procedures and treatments. Direct visualisation provided by ultrasound aids the investigator in performing insertion or manipulation of instruments in patients, such as flushing of the urinary bladder and uterus, collection of salivary fluid or obtaining diagnostic biopsy specimens. Ultrasonography can also be of assistance by providing intra-operative orientation for the castration of elephant bulls. Sonographic assisted follow-up evaluations make it easier to monitor the recovery of patients or to optimize ongoing treatment. Ultrasonographic supported embryo collection in Macaca fascicularis is a non-surgical way to collect blastocysts from the uterus by trans-cervical insertion of a two-way catheter. Equivalent methods are useful for intrauterine artificial insemination or non-surgical embryo transfer in small sized mammals.

8. Control of health status of reproductively inactive individuals

A stable hierarchy is the basis for establishing a successful breeding group in animals with a highly developed social structure. Especially in species like elephants or great apes, old individuals not employed in reproduction take up a special social position within the group. These animals fulfil special tasks in the raising of infants. They play an essential role in passing on social behaviour. The loss of high ranking animals leads to a stressful reorganisation of the social structure of the whole breeding group. In these phases of social instability, reproduction decreases or tapers off. Therefore, we believe that it is essential to integrate individuals not employed in reproduction into the ultrasound supported health control program along with the fertile members of the group. Pathological alterations in organs can immediately be detected and adequately treated when sonographic examination series are established.

Summary

Ultrasonography is an alternative approach for non-invasive exploration of reproduction and reproductive pathology of zoo and wild animals which is still under-utilized in wildlife medicine. It is a non-invasive technique, which provides reproducible real time images, cross-sectional imaging of tissues, organ structures and motions, morphometric measurements, documentation and preservation of data. The advantages of this technique favour its more intensive use. Ultrasound imaging in reproductive biology delivers new information regarding embryonic development, function of the uterus and ovaries where experimental embryology and endocrinology fail. In order to solve the problems which occur with the application of
ultrasonography in zoo and wild animals, we performed 1100 individual examinations in over 100 species. Our interest was focused on:

1. Sex determination of monomorphic species to make appropriate breeding management decisions and to avoid invasive sexing techniques. We performed successful examinations in reptiles, birds and in mammals such as beaver, sloth and spotted hyena.

2. Ultrasonography is an alternative or supportive method to urinary or faecal analysis in the determination of sexual cycle in females. We determined the cycle length in lowland anoa (22 d) and performed trans-rectal examinations on trained elephants without sedation to determine the phase of the reproductive cycle.

3. Pregnancy diagnosis is possible in many species using ultrasound. Monitoring early stages of gestation, fetal development and degree of maturation of the placenta allow a prediction of the date of delivery. Estimation of laying term is possible in reptiles and birds through trans-cloacal and trans-intestinal ultrasound as well.

4. Understanding pathological alterations within the inner genital tract and their influence on reproductive performance is important in order to consider reproductive capacity of the individual.

5. Of interest in embryogenesis is the detection of heart activity, volume of embryonic fluid and condition of the placenta. Pathological disorders in embryogenesis such as growth disorders, malformations and early embryonic death can be visualized.

6. Another criteria for appraisal of the fitness or breeding potential of an animal is the investigation of the major abdominal organs. Pathological alterations sonographically detected such as cysts, nodes, tumors or intra-abdominal fluid can be a secondary cause for infertility.

7. Sonography can be used as a supportive technique for the insertion or manipulation of instruments in the patient, as an additive help for orientation in the operating area or in the field of assisted reproduction like artificial insemination and embryo collection. Therapies can be optimised by sonographical follow-ups.

8. The control of the health status of socially high-ranking individuals not employed in reproduction is of significance in successful breeding. Non-reproductive animals play a responsible role in the socialisation of infants. Early diagnosis of diseases by ultrasound can, therefore, prevent social instability or stress in reproduction.

Application of ultrasound to the systematic examination of selected animal groups as opposed to case-related investigations is necessary in the frame of zoo and wild animal research to offer new solutions to manage reproductive problems. Improvements in ultrasound technology, as well as the development of new types of application techniques for wild animals will support this process.
ACKNOWLEDGMENTS

The authors thank Richard J. Montali for his support.

LITERATURE CITED

ESTROUS CYCLE INDUCTION IN A WHITE RHINOCEROS (*Ceratotherium simum simum*) AND CONCOMITANT EIA FECAL PROGESTAGEN ANALYSIS

Christian Walzer, Dr.med.vet
Salzburg Zoo Hellbrunn, A-5081 Anif, Austria

Franz Schwarzenberger, Dr.med.vet
Institut für Biochemie and L.Boltzmann-Institut für Veterinärmedizinische Endokrinologie, Vet. Med. Universität, A-1030 Vienna Austria

Introduction

Captive breeding of the African rhinoceroses, the black (*Diceros bicornis*) and the white (*Ceratotherium simum simum* and *C.s.cottoni*) could become essential for their long-term survival. At present the reproductive rate in captivity is very poor. Sixty seven percent of captive rhino are essentially in a non-breeding situation. Only 8.10 animals in 7 European facilities are breeding regularly. Aggravating this situation is the fact that 66 percent of these European animals are wild-caught and over 19 years of age. If these potentially valuable animals are to make a contribution to the captive rhinoceros population, intensive management procedures must be initiated immediately. The reasons for the poor breeding performance are unclear. Management factors such as the size of the enclosure, the group size and composition, and the position of a given animal in the social hierarchy are important influences.

Literature concerning the estrous cycle in white rhinoceroses is controversial. Behavioral observations of captive white rhinoceroses have indicated various estrous cycle lengths, ranging from 30 to 90 days. Using rectal palpation, vaginal cytology and urinary steroid analysis a cycle length of 42 days has been suggested. Hindle et al. using urinary steroid analysis have suggested an estrous cycle length of 25 and 32 days for the northern and southern subspecies. While investigating fecal progestagens in 5 white rhinoceroses over a period of 14-24 months Schwarzenberger et al. described missing or erratic cyclicity as a considerable problem, and regular estrous cycles (9) of approximately 10 weeks duration in only 1 of these 5 animals. The cycle duration was 68.4 ± 3.4 days, follicular and luteal phases (LP) were 12.2 ± 0.8 and 56.5 ± 3.3 days, respectively. Progestagen levels in 2 of the 5 white rhinoceroses also indicated luteal activity, but the intervals between LPs and duration of LPs were irregular.

Salzburg Zoo Hellbrunn started holding white rhinoceroses (2.2) in 1991. The animals are held in a very large, natural outdoor facility (15,000 m²) together with oryx (*Oryx gazella gazella*). Since fecal analysis in one of the two female rhinoceroses indicated missing luteal activity over a period of >2 years, it was decided to attempt cyclic activity induction in this animal. A synthetic progestagen (Chlor Madinon Acetate, CMA) was applied for 1.5 months, and subsequently hCG was injected. The dose and treatment interval were calculated allometrically using the horse mare as a model.
Materials and Methods

Fecal samples were collected 2-3 times/week for 28 months from the female white rhinoceros "Baby". The animal was wild-caught in 1971 and has been kept with a second white rhinoceros "Kathy" and two alternating males at the Salzburg Zoo since 1991. Feces (0.5 g) were extracted with methanol as described by Schwarzenberger et al.®, an additional 1.0 g of powdered aluminum oxide was added prior to extraction. Methanol aliquots were analyzed with an enzyme-immunoassay (EIA), using an antibody against 5 alpha-pregnane-3β-ol-20-one 3HS:BSA. The assay is considered as group-specific and quantifies total immunoreactive progestagens containing a 20-oxo group. Preliminary data using this assay were described. 10,13,17

Since the animal "Baby" showed no behavioral estrous and fecal progestagen analysis indicated missing luteal activity for >2 years, induction of cyclic activity was attempted. Long-term fecal progestagen analysis of the second female from the Salzburg Zoo, "Kathy", indicated a regular estrous cycle length of ~10 weeks (follicular phase 12.2 ± 0.8 and luteal phase 56.5 ± 3.3 days). Due to this long-term data we considered the normal estrous cycle length in the white rhinoceros to be 10 weeks.

The treatment protocol was based on the horse mare as a model animal. The CMA (Synchrosin®, Fa. Werft-Syntex, Vienna) dose for the mare is 0.02 mg/kg body weight, and the application interval is 24 hours. The hCG (Chorulon®, Intervet, Boxmeer) dose for a mare is 2500 IU (5 IU/kg body weight). The dose was scaled allometrically as described by Sedgwick. 13 The body weight was estimated to be 2500 kg and the constant (K) for the calculation of the Minimum Energy Cost was 70. Using these factors, a CMA dose of 35 mg (0.014 mg/kg) and a dose interval of 35 hours was computed. The hCG dose for the white rhinoceros was calculated as 8400 IU (3.36 IU/kg). CMA was applied perorally in 32 doses in 35 hour intervals (a period of 45 days); hCG was given as a single intramuscular injection at the base of the ear by means of a Jab Stick (Dan-Inject Denmark) 5 days after the last CMA application.

Results and Discussion

Fecal progestagens in the white rhinoceros "Baby" were analyzed for >2 years; the results of the progestagen evaluations during 1994 are shown in Fig. 1. Progestagens were low before and during the application of CMA. CMA metabolites did not affect the fecal progestagen measurements. Ten days after the hCG application the fecal progestagen concentrations increased and thus indicated luteal activity. The length of this induced luteal phase was about 20 days. Estrous behavior lasting 48 hours was observed 70 days after the hCG application, and thus for the first time since the animal was at the Salzburg Zoo (from 1974-1991 the animal was kept at the Munich Zoo were estrous behavior was also not observed). Mating was not observed, which may be due to the inexperience of the male placed with the female at the time.
The present study indicates that induction of luteal activity in white rhinoceroses is possible. Although the described treatment protocol failed to induce continuous cyclicity, results are encouraging. Luteal activity was seen 10 days after the hCG application and thus within range of the follicular phase (12.2 ± 0.8) described in a previous study. These results differ from those obtained by Godfrey et al., who attempted to superovulate a white rhinoceros prior to euthanasia. Their study failed to induce ovulation and subsequent luteal activity. Estrous behavior 70 days after hCG injection is within the range we suggest to be the "normal" duration of the estrous cycle in the southern white rhinoceros. Induction of behavioral estrous was also achieved at the Dvur Kralker Zoo using altrenogest (Regumate) and hCG. Similar to our study none of the treatments at the Dvur Kralker Zoo resulted in a subsequent pregnancy.

In conclusion our results demonstrate that luteal activity can be induced using a synthetic progesterone derivative followed by an injection of hCG. Treatment efficiency and results can be monitored using EIA fecal steroid analysis. Since one of the reasons for poor breeding results in captive white rhinoceroses is missing or erratic luteal activity, it is suggested to continue attempts to induce cyclicity. Further treatment protocols could also include GnRH, FSH or PMSG. In order to find optimal treatment protocols, fecal monitoring will be necessary. It should, however, not be overlooked that the various breeding technologies can only be considered as an adjunct to the implementation of adequate management and holding structures.

ACKNOWLEDGMENTS

The authors thank the keepers F. Messner and P. Hollweger for the conscientious collection of fecal samples; Ms. C. Beck-Granninger for the organization of fecal collection in 92-93. Dr. E. Möstl for preparation of antibodies and biotinylated labels; Ms. A. Aichinger and Ms. E. Leitner for the excellent technical assistance.

LITERATURE CITEd


ENDOCRINE RESPONSES TO EXOGENOUS PROGESTOGEN AND PROSTAGLANDIN ADMINISTRATION IN THE SCIMITAR-HORNED ORYX

Catherine J. Morrow, MSc* and Steven L. Monfort, DVM, PhD
Smithsonian Institution, National Zoological Park, Conservation and Research Center, 1500 Remount Road, Front Royal, Virginia 22630, USA

Introduction

Scimitar-horned oryx (SHO) reproduce well in captivity and populations of this endangered desert antelope are dispersed worldwide. Assisted reproductive techniques such as estrous synchronization, semen cryopreservation, artificial insemination (AI) and embryo transfer (ET) have potential for facilitating maintenance of genetic diversity in this species. However, successful assisted reproduction requires a fundamental understanding of species-specific physiology and methods for monitoring hormonal patterns during treatment.

The ultimate aim of estrous synchronization procedures is to consistently induce a high incidence of estrus and ovulation, with minimal variance in the time to onset of estrus/ovulation with normal or improved fertility. Historically, estrous synchronization in domestic ungulates has been achieved using intravaginal, oral or subcutaneous delivery of exogenous progestogens. Progesterone (P₄), or synthetic progestogens, such as norgestomet and melengestrol acetate (MGA), are administered for extended intervals to mimic the function of the corpus luteum (CL) by inhibiting ovulation until the progestogen is withdrawn. Intravaginal CIDR (Controlled Internal Drug Releasing) devices containing P₄ have been effective for synchronizing estrus and ovulation in domestic species including cattle, sheep, goats and deer. After CIDR device insertion, there is a rapid elevation in peripheral blood P₄ that gradually declines over 2-3 wk. A second synchronization method utilizes 2 intramuscular (i.m.) injections of prostaglandin F₂α (PGF₂α) administered 10-12 d apart to induce premature luteolysis in species such as cattle, sheep and deer.

In general, estrous synchronization overcomes difficulties associated with the detection of natural estrus, and fixed-time insemination after estrous synchronization is useful for routinely producing offspring in sheep, goats and deer. Numerous published reports have described estrous synchronization, semen cryopreservation, AI, superovulation, embryo collection and transfer in SHO. Results indicate that assisted reproduction can be successful (1 offspring by ET; 2 offspring by AI), but current protocols are inadequate for routinely producing offspring, in part, because basic knowledge is lacking. Thus, one of the challenges for developing assisted reproductive techniques continues to be devising simple, reliable and cost-effective methods of synchronizing estrus and ovulation. Unfortunately, the development of these procedures has been hampered by an inability to monitor ovarian responses to hormonal treatments. Our goals were to: 1) develop an effective non-invasive reproductive-endocrine monitoring technique; 2) characterize the natural reproductive cycle; and 3) compare the potential of PGF₂α and/or CIDR-G devices (0.365 g P₄, InterAg, Hamilton, New Zealand) for artificially synchronizing estrus and ovulation.
Methods

Baseline jugular venous blood samples (10 ml) and fecal samples were collected 3 times/wk for 4 wk from 5 mature (>3 yr old) female SHO (mean liveweight, 150.5 kg) manually restrained in a drop-floor device (The Tamer®, Fauna Products, Red Hook, NY). Treatment (see schematic below) consisted of 2 injections of a PGF$_{2\alpha}$ analog (Estrumate, Cloprostenol; Miles, Inc., Shawnee Mission, KS, 66201) administered 10 d apart. At the time of the second injection, MGA silastic rubber implants (7.8-8.0 g MGA, provided by Dr. E.D. Plotka) were placed subcutaneously over the scapula. Blood and fecal samples were collected weekly and twice weekly, respectively, for 3 wk after MGA implant insertion. With MGA still implanted, a single intravaginal CIDR-G device was inserted for an additional 20 d while blood and fecal samples were collected. Blood samples were collected at the time of CIDR-G device insertion (day 0) and +1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 21, 22 and 24 d post-CIDR-G device insertion (CIDR-G devices were withdrawn on day +20). Fecal samples were collected non-invasively 1-3 times/wk for 4 wk following CIDR-G device withdrawal. Serum P$_4$ concentrations were measured in duplicate using a direct, solid-phase $^{125}$I RIA (Coat-A-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA) that was validated for SHO serum. Fecal progestogens were extracted from 0.025 g of dried feces and assayed using an RIA validated for SHO feces.

Results

During the initial 4 wk of the study, 4 females exhibited evidence of spontaneous estrous cyclicity and luteolysis. Serum P$_4$ and fecal progestogen concentrations ranged from 0.05-9.69 ng ml$^{-1}$ and 0.82-42.80 µg g$^{-1}$ dry feces, respectively. Mean (±SEM) peak luteal serum P$_4$ and fecal progestogen concentrations ($n = 4$) were 4.85 ± 0.93 ng ml$^{-1}$ (range, 2.47-7.39 ng ml$^{-1}$) and 23.70 ± 4.71 µg g$^{-1}$ (range, 11.60-36.00 µg g$^{-1}$), respectively. One of the 4 females exhibited a complete luteal cycle (Fig. 1) that lasted ~24 d (interval between inter-cyclic serum P$_4$ nadirs). Among all females (Fig. 2), serum P$_4$ ranged from 0.08-3.31 ng ml$^{-1}$ at the time of PGF$_{2\alpha}$ administration, and for females with elevated serum P$_4$ (>1.0 ng ml$^{-1}$), concentrations declined

![Schematic. Experimental treatment protocol](image-url)
within 24 h post-PGF$_{2\alpha}$ administration to <0.1 ng ml$^{-1}$. Basal serum P$_4$ (0.08 ± 0.01 ng ml$^{-1}$) and fecal progestogen (1.01 ± 0.19 µg g$^{-1}$) concentrations at CIDR-G device insertion suggests ovulation was effectively suppressed during the 3 wk MGA pre-treatment. Within 24 h of CIDR-G device insertion, serum P$_4$ (0.54 ± 0.15 ng ml$^{-1}$) and fecal progestogens (4.04 ± 0.28 µg g$^{-1}$) were elevated ($P < 0.05$) above basal concentrations. Both serum P$_4$ (0.33 ± 0.04 ng ml$^{-1}$) and fecal progestogen (2.06 ± 0.11 µg g$^{-1}$) concentrations steadily declined by the end of the 20 d insertion interval. By 48 h after CIDR-G device withdrawal serum P$_4$ and fecal progestogens declined 0.18 ± 0.05 ng ml$^{-1}$ and 1.86 ± 0.21 µg g$^{-1}$, respectively (Fig. 2). Longitudinal fecal progestogens (data not shown) monitored in a single female (2-4 times/wk) for 6 mo revealed 5 spontaneous luteal cycles with a mean estrous cycle duration of 28 ± 2.6 d (range, 24-32 d).

Figure 1. Serum P$_4$ (closed circles) and fecal progestogen (open circles) concentrations from a single SHO female during 1 complete estrous cycle and after treatment with PGF$_{2\alpha}$, a subcutaneous MGA implant and an intravaginal CIDR-G device.

Figure 2. Mean (±SEM) serum P$_4$ (closed circles) and fecal progestogens (open circles) concentrations from 4 female SHO after treatment with PGF$_{2\alpha}$, subcutaneous MGA implants and an intravaginal CIDR-G devices. Data for 1 female was excluded because the serum P$_4$ profile indicated an absence of luteal activity before PGF$_{2\alpha}$ and MGA treatment.
Discussion

The 24-32 d estrous cycle duration in the present study is slightly longer than previously reported for this species (mean 21-22 d, range 17-25 d)[1], but comparisons must be made cautiously due to the small number of animals in the present study. However, mean luteal phase serum P₄ concentrations are within the range reported by others (4.28 ± 1.08 ng ml⁻¹). Our data are the first to report the effectiveness of fecal progestogen monitoring for tracking both spontaneous and manipulated estrous cycles in the SHO, indicating that this non-invasive method may aid in the development of assisted reproduction in this species. As previously reported in SHO[2], PGF₂₈ induces a decline in progestogen metabolites. However, our data confirm that PGF₂₈ is an effective luteolytic agent in SHO only when administered in the presence of luteal activity (i.e., serum P₄ concentrations >1.0 ng ml⁻¹), and like cattle[3], PGF₂₈ must be administered on or after day 5-6 of the estrous cycle.

The MGA implant is the most widely used contraceptive in North American zoos[4]. Because ovariectomy was not feasible, MGA in the present study was used to hormonally 'castrate' females, thereby suppressing ovarian-derived P₄. This manipulation permitted testing the efficacy of CIDR-G devices for inducing and maintaining elevated P₄ concentrations, in the absence of endogenous P₄. Results indicate that subcutaneous MGA implants effectively suppressed endogenous P₄ secretion and inhibited ovulation and CL formation. Although inducing elevated serum P₄, CIDR-G devices alone are ineffective for inducing and maintaining blood P₄ at concentrations equal to, or greater than, those secreted by a natural corpus luteum. As a result, a single CIDR-G device is unlikely to be effective for inducing estrus and ovulation in SHO because maintenance of sustained elevations in P₄ is essential for successful estrous synchronization. Although more work is required, the qualitative and temporal patterns of CIDR-G device-induced P₄ release suggests that CIDR devices containing increased quantities of P₄ may be more suitable for synchronizing estrus in SHO.

ACKNOWLEDGMENTS

We thank Larry Collins and the animal keeper and veterinary staff of the National Zoological Park's Conservation and Research Center (CRC) for providing assistance with animal handling, blood sampling and husbandry. We also thank Kendall Mashburn for laboratory assistance and the CRC maintenance and fence crews for their expertise in designing and constructing the animal handling facilities. The MGA implants were kindly provided by Dr. E.D. Plotka, the Marshfield Medical Research Foundation. Financial support was provided by Friends of the National Zoo and the Scholarly Studies Program of the Smithsonian Institution.

LITERATURE CITED

REPRODUCTIVE SURVEY OF ENDEMIC FELID SPECIES IN LATIN AMERICAN ZOOS: MALE REPRODUCTIVE STATUS AND IMPLICATIONS FOR CONSERVATION

William F. Swanson, DVM, PhD*; David E. Wildt, PhD; Richard C. Cambre, DVM
Conservation and Research Center and National Zoological Park, Smithsonian Institution, Washington D.C. 20008, USA

Scott B. Citino, DVM
White Oak Plantation, Wildlife Conservation Center, Yulee, FL 32097, USA

Kathy B. Quigley, DVM
Hornocker Wildlife Research Institute, Moscow, ID 83843, USA

MVZ Dulce Brousset
Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyocán 04510, México D.F.

Rosana Nogueira de Morais, MV, Msc; Nei Moreira, MV, MSc
Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, PR (RNM) and Curso de Medicina Veterinária, Universidade Federal do Paraná, Palotina, PR (NM), Brasil

Stephen J. O'Brien, PhD; Warren E. Johnson, PhD
Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, MD 20205, USA

Introduction

Ten felid species are endemic to Latin America, specifically, the jaguar (Panthera onca), puma (Felis concolor), ocelot (Felis pardalis), margay (Felis wiedi), tigrina (Felis tigrina), Geoffroy's cat (Felis geoffroyi), pampas cat (Felis colocolo), kodkod (Felis guigna), Andean mountain cat (Felis jacobita) and jaguarundi (Felis yagouaroundi). All are listed on Appendix I or II of CITES, and five species (jaguar, ocelot, margay, tigrina, Andean mountain cat) and six other subspecies (jaguarundi, puma) are listed as endangered by the U.S. Fish and Wildlife Service. Latin American felids face an uncertain future in the wild due to continued habitat destruction and poaching pressures. As cat populations become more geographically fragmented, further restriction of genetic exchange may contribute to inbreeding. Effective conservation strategies involving habitat preservation, natural breeding in zoos and assisted reproduction may be critical for maintaining genetic viability of both captive and wild populations.

Understanding the reproductive status of captive populations can provide direction in planning overall conservation strategies. In turn, the reproductive health of zoo-maintained populations has a pronounced impact on the success of natural breeding programs and assisted reproductive technologies, like artificial insemination (AI) and genome resource banking. However, for most Latin American felid species, baseline information on reproductive characteristics is limited, with only the puma receiving more than cursory attention. Additionally, although most felids in Latin American zoos are wild-

1995 PROCEEDINGS JOINT CONFERENCE AAZV / WDA / AAWV
caught and represent potentially valuable founders, little information is available on the overall reproductive status of these cats. Therefore, the objectives of the present study were to: 1) assess the past breeding success of male cats maintained in Latin American zoos; 2) conduct detailed evaluations to determine current reproductive status; and 3) whenever possible, conserve their future reproductive potential by cryopreserving high quality sperm in a genome resource bank.

Materials and Methods

The opportunity to conduct large-scale reproductive assessments of male felids in Latin America arose from the initiation of a parallel survey of the molecular systematics of felid species in this region (conducted by Johnson and O'Brien of the National Cancer Institute). For the genetics and reproductive survey, male and female cats held in 44 Latin American zoos and private cat facilities were anesthetized for the collection of blood and skin samples for later analysis, with the primary focus on wild-caught animals of known origins. For most species, males were anesthetized using a tiletamine-zolazepam combination (5-10 mg/kg; telazol, Fort Dodge Laboratories, Inc., Fort Dodge, IA 50501) administered i.m. by hand or pole syringe, blow pipe or CO₂-powered pistol, depending on exhibit design and animal accessibility. This injectible anesthetic provided an adequate plane of anesthesia for electroejaculation procedures, except in the puma and jaguarundi. To achieve adequate relaxation in these two species, male pumas were anesthetized with an initial injection of ketamine hydrochloride (8-10 mg/kg; Ketaset, Fort Dodge Laboratories, Inc., Fort Dodge, IA 50501) followed 10-15 minutes later with an injection of tiletamine-zolazepam (2-3 mg/kg) whereas jaguarundis were injected with a combination of ketamine (8-10 mg/kg) mixed with xylazine (0.2-2 mg/kg; Rompun, Mobay Corp., Shawnee, KS 66205). If possible, males were fasted 12-24 hours prior to anesthesia.

Zoo veterinarians, biologists, keepers and/or directors at collaborating institutions provided information on the captive history of each male, including origin (wild-caught or captive born; location in the wild or origin of parents), length of time in captivity, previous breeding success, main components of diet and, at some institutions, exhibit characteristics (presence of cage mates, public accessibility). Reproductive evaluations followed a standardized protocol, consisting of pre- and post-electroejaculation blood sampling, evaluation of penile morphology, measurement of testicular volume and a regimented electroejaculation protocol comprised of 80-120 electrical stimuli delivered in three or four series. Following each series of stimuli, semen was evaluated for volume, pH, presence or absence of sperm and sperm motility (percent motile and rate of progressive motility on scale of 0 - 5, with 5 being optimal). If an ejaculate contained sperm, an aliquot (5-10 μl) was fixed in 0.3% glutaraldehyde and later evaluated using phase contrast microscopy (1000x) to determine percentage of normal sperm morphology. In addition, motility traits were used to calculate a sperm motility index [SMI = % motile + (20 x rate of progressive motility)/2] for each ejaculate. The remaining raw semen was diluted with equal volumes of warm (37°C) Ham's F10 culture medium (Irvine Scientific, Santa Ana, CA 92705), supplemented with 5% fetal calf serum. Diluted spermic ejaculates from each series were combined and sperm
concentration was determined using a red blood cell determination kit/hemacytometer method.\(^5\)

If total sperm recovery and sperm motility were acceptable (generally \(\geq 10 \times 10^6\) motile sperm), sperm were processed for cryopreservation. Sperm were centrifuged at 200 \(\times\) g to remove seminal fluid and culture medium, and the resulting sperm pellet was resuspended in cryoprotectant medium (consisting of 11% lactose, 20% egg yolk and 4% glycerol) to a target concentration of 50-100 \(\times\) 10^6 motile sperm/ml. Extended sperm were cooled in a refrigerator (or ice chest) for 30 minutes to 5°C and, depending on the availability of dry ice and/or liquid nitrogen (LN\(_2\)), frozen by one of two methods: 1) pelleting onto indentations in dry ice\(^6\); or 2) straw freezing at a controlled rate over LN\(_2\) vapor.\(^4\) Frozen pellets and straws were sealed in cryovials and stored in LN\(_2\) dry shippers for transport.

**Results**

Felids in 11 countries and 44 zoological parks and/or private cat facilities were assessed during the ~2 year survey period. A total of 186 male cats representing 8 felid species (Table 1) were evaluated for reproductive status. The kodkod and Andean Mountain cat were not encountered. Of the 186 males, 173 (93%) were reportedly wild-caught, with the remaining 13 individuals (4 jaguars, 5 pumas, 3 ocelots and 1 jaguarundi) being captive-born to wild-caught parents. Across species, the level of successful captive breeding was low, with only 37 males (20%) classified as proven breeders (Table 1). Excluding the jaguar, puma and ocelot, only 13% of the smaller-sized cats (margay, tigrina, Geoffroy’s cat, pampas cat, jaguarundi) ever had reproduced in captivity.

Reproductive evaluations of the 186 males revealed that 132 (71%) had sperm present within their ejaculates. However, only 87 (47%) had at least 1 \(\times\) 10^6 total sperm/ejaculate (Table 1) and just 53 (28%) had \(\geq 10 \times 10^6\) total sperm/ejaculate. Mean (± SEM) values for semen volume, sperm concentration, total sperm/ejaculate, sperm motility index, % normal sperm morphology and testicular volume were calculated for each species (Table 2). As expected, semen volume, total sperm/ejaculate and testicular volume generally varied with species size, with the larger species typically having larger testicular volumes and producing more voluminous ejaculates containing more total sperm. Sperm motility indices were similar among species, but the puma and several small-sized cat species (margay, tigrina, jaguarundi) had low percentages (<40%) of normal sperm morphology (Table 2).

Reproductive traits also varied within species, possibly due to differences in husbandry practices among zoos, especially related to diets and potential levels of captive stress. Of the 44 facilities, the diets at 29 (representing 139 cats in the survey) consisted almost entirely of red meat (horse or beef) or chicken heads and necks. Fifteen (34%) institutions provided fairly routine (> once per week) diet supplements of whole prey carcasses, organ meat or commercial vitamin/mineral mixtures. When results were evaluated based on nonsupplemented versus supplemented diets, distinct trends were observed in total sperm recovery for several species. For example, jaguars (n=18) and pumas (n=22) on nonsupplemented diets averaged 11.0 \(\times\) 10^6 and 24.8 \(\times\) 10^6 total sperm/ejaculate, respectively.
compared to values of $34.3 \times 10^6$ and $87.6 \times 10^6$ total sperm/ejaculate for jaguars (n=3) and pumas (n=13), respectively, on supplemented diets. For comparison, ejaculates from jaguars (n=5) and pumas (n=12) maintained in U.S. zoos (generally maintained on Nebraska Feline Diet) averaged $32.4 \times 10^6$ and $56.6 \times 10^6$ total sperm/ejaculate, respectively. Among the small cats, margays (n=16) and Geoffroy's cats (n=21) on nonsupplemented diets had mean values of $5.8 \times 10^6$ and $7.4 \times 10^6$ total sperm/ejaculate, respectively, compared to margays (n=11) and Geoffroy's cats (n=3) on supplemented diets with $8.0 \times 10^6$ and $14.2 \times 10^6$ total sperm/ejaculate, respectively. For comparison, ejaculates from margays (n=5) and Geoffroy's cats (n=12) in U.S. zoos averaged $16.0 \times 10^6$ and $60.0 \times 10^6$ total sperm/ejaculate, respectively.

Exhibit conditions also may have influenced male reproductive traits. For example, presence or absence of cage mates was known for 146 surveyed males. Of these, most (69%, n=101) were housed alone or paired with a single conspecific female. Seventy-nine (79%) of these males had spermic ejaculates, 48 (48%) had $\geq 1 \times 10^6$ total sperm/ejaculate, and 33 (33%) had $\geq 10 \times 10^6$ total sperm/ejaculate. In contrast, 45 males were maintained with either multiple conspecific females (n=4), with other conspecific male(s) (n=21), with conspecific males and females (n=13) or with heterospecific males or females (n=7). Only 23 (51%) of these individuals had spermic ejaculates, 14 (31%) had $\geq 1 \times 10^6$ total sperm/ejaculate and 7 (16%) had $\geq 10 \times 10^6$ total sperm/ejaculate.

Despite variability in sperm recovery and quality, 63 ejaculates (34%) from 8 species met the general criteria for sperm cryopreservation. Most (n=50) were frozen by pelleting onto dry ice with the remainder (n=13) frozen in straws over liquid nitrogen vapor. These cryopreserved samples currently are stored in the National Zoological Park's Felid Genome Resource Bank. Based on known pre-freeze motility and sperm concentration of each stored sample and a minimal inseminant dose of $10 \times 10^6$ motile sperm per AI, these cryopreserved samples potentially represent $\sim 145$ AI doses.

Discussion

This represents the first broad-based survey of the reproductive status of felid species maintained in Latin American zoos and private cat facilities. The information generated from this survey has helped define the current reproductive health of the captive population and has important implications for zoo-based conservation in these countries. Although only male cats were evaluated, the same factors may impact female reproductive success. Therefore, these data may provide a reasonable approximation of the general reproductive health of the entire captive population at these institutions. Zoos throughout Latin America have demonstrated a strong commitment to the conservation of these cat species and, during our collaboration, zoo veterinarians and staff consistently expressed a strong desire for information about the reproductive status of their collections and measures needed to improve breeding success. Hopefully, the results of this survey will provide incentive and guidance to enhance breeding success of these cat species.
Our findings indicated that few males (~20%) ever had reproduced in captivity, some from lack of opportunity but many perhaps due to suboptimal husbandry. The lack of captive breeding coupled with the low percentage of males (< 50%) exhibiting even minimal sperm production (i.e., ≥ 1 x 10⁸ sperm/ejaculate) indicate that the reproductive health of this population was not ideal. Multiple variables can affect male reproductive status, including nutrition, stress, seasonality and inbreeding. Our opportunistic, 'one-time' approach did not allow assessing seasonality, and we expect that inbreeding was a non-factor because most males originated from the wild. Rather, poor reproductive characteristics appeared related to diets and/or stressful housing conditions. Specifically, the nutritional status of many cats was inadequate because of the exclusive use of nonsupplemented, all-meat or all-chicken neck diets. Furthermore, even some cats on 'supplemented' diets may not have been receiving adequate nutritional support because interviews revealed that whole carcasses or commercial vitamin/mineral formulations rarely were consistently provided.

All-meat (and all-chicken neck) diets are known to be deficient in essential vitamins and minerals, including vitamins A, D and E, and have unbalanced calcium:phosphorus ratios. A recent study in pumas (JG Howard, M Roelke and C Glass, unpublished data) supports our contention that suboptimal diets affect male reproductive traits. Male pumas (n=6) were maintained solely on chicken neck diets for periods of at least ten months prior to reproductive evaluation and then switched to Nebraska Feline diet for the subsequent six months before being re-evaluated for the same reproductive parameters. With the new diet, sperm quality only was slightly improved, but the total number of sperm per ejaculate increased substantially (from a mean of 3.5 x 10⁸ sperm up to 32.9 x 10⁸ sperm). Because all other management factors remained constant, these findings provide strong evidence that nutrient-deficient diets impact directly on semen parameters, especially sperm production.

In addition, some cats in the survey, especially many of the less adaptable, smaller-sized species, may have been housed under potentially-stressful exhibit conditions. While most males were housed alone or paired with a single conspecific female, cats at some zoos were maintained in group exhibits, with multiple conspecific males and/or females in one enclosure, or occasionally with heterospecific males and females. Many exhibits also lacked den boxes or suitable hiding places which could potentially contribute to environmental stress. For example, absence of nest boxes or other hiding places and proximity of large cats to smaller cats induces persistent elevations in excreted cortisol, one indication of chronic stress. Additionally, housing small cats in groups (i.e., multiple males and/or females in same exhibit) has been associated with reduced reproductive success compared to cats maintained as heterosexual pairs.

The current reproductive status of these cats, as defined by this study, has several important conservation implications, especially in developing effective zoo breeding programs. For all of these species, systematic conservation logically should be located in these 'range' countries. If captive 'insurance' populations are warranted, then the zoological parks of Latin America would be expected to assume responsibility for the actual captive breeding of endemic species. In several Latin American countries, national and/or regional zoo associations are spearheading efforts to improve collaboration among zoos while providing
the organizational framework for cooperative breeding programs. Additionally, the Conservation and Breeding Specialist Group (CBSG) of the IUCN's Species Survival Commission has been active in fostering communication between zoos and encouraging the development of conservation assessment management plans (CAMPS) in Latin America (essentially estimates of species, husbandry and research priorities). It then becomes the responsibility of the regional zoo associations to transform this new information into 'regional collection plans' with the goal of producing healthy 'reservoir' populations that maintain all existing genetic diversity. It is these coordinated breeding programs that must succeed to a large extent based on the reproductive health of the extant captive population. Our survey results suggest that the reproductive health of the cats in Latin American zoos is suboptimal and could benefit through improved diets and/or exhibits. This situation provides incentive for more training and interregional cooperation.

We have asserted that assisted reproductive technology can augment natural breeding in the management of genetic diversity.\textsuperscript{10,12} While optimal protocols for artificial insemination (AI), in vitro fertilization, embryo transfer and the cryopreservation of germ plasm for genome resource banks still are being developed, certain aspects of this technology may be sufficiently advanced for application to present day management problems. For example, laparoscopic AI has been used successfully to produce offspring in two Latin American cat species, the puma\textsuperscript{2} and the ocelot\textsuperscript{7} with the ocelot pregnancy resulting from insemination with frozen-thawed sperm. Furthermore, although only one-third of the ejaculates collected in the reproductive survey met cryopreservation criteria, this frozen sperm still represents nearly 150 potential AI procedures. However, the effective utilization of this resource, as with natural breeding programs, greatly depends on the overall reproductive health of the captive population.

Although multiple variables may be involved, we believe that suboptimal diets and captive stress are two of the primary causes for the reproductive characteristics measured in Latin American felids. Fortunately, these factors can be corrected. Nutritional supplementation regimens for all-meat diets have been described previously,\textsuperscript{8} using relatively inexpensive, readily available multi-vitamin/mineral tablets and calcium mixtures. Alternatively, zoos can increase the proportion of whole animal carcasses provided in felid diets. Recommendations also have been made to improve captive management of smaller cats,\textsuperscript{3,4} the species most affected by stressful exhibit conditions. Easily applied corrective measures include providing hiding places (den boxes, vegetation) within exhibits, increasing exhibit complexity to encourage exploratory behavior and maintaining cats as heterosexual pairs or singly with periodic pairing for breeding purposes. The implementation of dietary and management changes may improve the reproductive characteristics of male cats at these institutions and, if reproductive success is enhanced, would represent another significant step toward achieving the tremendous conservation potential of Latin American zoos.

ACKNOWLEDGMENTS

The authors thank Joe Lopez, Dr. Marilyn Raymond, Melanie Culver and Dr. Jill Slattery of the National Cancer Institute for assistance with reproductive evaluations and thank the following institutions and/or individuals in Latin America for their cooperation: Universidad Nacional Autónoma de México, Africam Safari, Centro Zoológico de Sonora, Proyecto Balam, Zoológico de León, Zoológico el
Centenario, Zoológico San Juan de Aragon, Zoológico Tamatan, Zoológico Zoocilpan, Zoonat (Mexico); Parque Zoológico La Aurora, Autonafari Chapin (Guatemala); Parque Zoológico Edgar Lang, Zoológico de Juigalpa (Nicaragua); Werner and Lilly Hagnauer, Zoológico Simon Bolivar (Costa Rica); CEPPEPE o Zoológico del Summit (Panama); Emperor Valley Zoo, Guptee Lutchmedial (Trinidad); Parque Aquarum J.V. Seijas, Fundación Nacional de Parques Zoológicos y Acuarios, Jardín Acuario de Merida, Jardín Zoológico Las Delicias, Parque Zoológico Miguel Romero Antoni, Zoológico Leslie Pantin (Venezuela); Sociedad de Zoológicos Brasileras, Bosque dos Jequitibas, Fundacao Zoobotanica do Rio Grande do Sul, Itaipu Binacional, Jardim Zoológico de Brasilia, Parque Zoológico de Goiania, Parque Zoológico Municipal Quinzinho de Barros (Brazil); Zoológico Municipal Vesty Pakos, Zoológico Municipal Santa Cruz (Bolivia); Parque Pereira de Rossell, Estacion de Cria de Fauna Autoctona, Zoológico de Goiania, Zoológico Municipal Quinzinho de Barros (Brazil); CEPEPE o Zoológico del Summit (Panama); Fmperor Valley Zoo, Guptee Lutchmedial (Trinidad); Parque Pereira de Rossell, Estacion de Cria de Fauna Autoctona, Zoológico de Durazno, Zoológico de Mercedes (Uruguay); and Acuario Municipal de Mendoza, Jardín Zoológico de La Ciudad de Buenos Aires, Zoo Cordoba, Zoológico la Plata, Zoológico de Rawson (Argentina). This study was funded, in part, by the Philip Reed Foundation, Friends of the National Zoo (FONZ) and a grant to S.J. O’Brien from theRalston Purina Company/American Zoos and Aquarium Association’s Conservation Endowment Fund.

LITERATURE CITED

RESULTS FOR PRIMATES FROM THE AZA CONTRACEPTION DATABASE: SPECIES, METHODS, EFFICACY AND REVERSALS

Ingrid J. Porton, MS
St. Louis Zoological Park, Forest Park, St. Louis, MO 63110, USA

Introduction

The AZA Contraception Advisory Group has developed a Contraception Database that tracks the use of reversible birth control methods used in mammals housed in North American zoos and MGA use worldwide. One goal of the database is to provide animal managers with information on what species have been contracepted, as well as the efficacy, reversibility, and associated physical and behavioral effects of the different methods used.

Methods

Data for the AZA Contraception Database are obtained through two methods. The first is the MGA implant database started by Dr. U. Seal and continued by Dr. E. Plotka. Records are maintained on the distribution of all MGA (melengestrol acetate) implants including the institution and individual ordering the implant; the species, ID, weight, and birthdate of the individual for which the implant is intended; the implant ID number, weight, and date sent. Some follow-up information such as date implant inserted is added to the database. The majority of the follow-up data and data on all other reversible methods of contraception is collected through the annual AZA Contraception Survey. Table 1 details the information requested on the survey.

Results

Data on the use of reversible contraceptives in primates is detailed in Tables 2 - 12. The database shows that five types of reversible birth control have been used in non-human primates: the MGA implant, Norplant (levenorgestrel), Depo-Provera (medroxyprogesterone acetate), human birth control pills, and the oral form of megestrol acetate (Ovaban and Megace). Although the database also includes some information on contraceptives used in research trials (zona-pellucida, lupon, vas plug, two different formulations of hormonal implants) those data are not included in this report.

All the information pertaining to the number of species, methods used, duration of use, efficacy, results of contracepting females during pregnancy, and time to reversal is presented in the tables. Terms used in the tables are defined as follows:

MGA # sent: number of MGA implants sent to institutions that were designated for use in a primate species.
MGA data for: the number of implants for which there is follow-up information. Missing data is basically due to one of three reasons: 1. institutions/individuals have not responded to the request for follow-up data; 2. the implants were sent to foreign institutions to which we have only recently sent surveys; 3. the implants were distributed after the annual survey and will be included in the subsequent survey.

MGA fail: the MGA implant was confirmed in use at the time of conception.

Depo-P # females: number of females that were treated with Depo-Provera.

Depo-P # seasons (prosimians): prosimians are typically given two or three injections of Depo-Provera during the breeding season. Initially the dose recommendation distributed through the Black Lemur and Ruffed Lemur SSPs was 10 mg/kg body weight given at the initiation of the breeding season and repeated 90 days later. The following year the recommendation was changed to 5 mg/kg body wt at 2 month intervals. One institution (St. Louis Zoo) carried out trials with a 2.5 mg/kg body wt dose on both black and ruffed lemurs.

Depo-P # injects given: data on the use of Depo-Provera in non-seasonal primates is given as # of consecutive treatments.

Depo fail: Depo-Provera was confirmed in use at the time of conception.

Completed bouts: a completed contraceptive treatment, i.e.; when an MGA implant is removed, the bout is considered completed.

In progress: the contraceptive method is still in use and a final conclusion as to the effectiveness of the method can not yet be made; e.g., a failure may occur, an implant may be discovered lost.

Total lost/# pregnant: total number of implants lost (the implant was observed to fall out or was found missing during a physical examination)/ and of those lost, the number that resulted in pregnancies.

Discussion

The data show that the MGA implant remains the most frequently used contraceptive method in primates, particularly new and old world monkeys. In prosimians, old world monkeys and apes, the failure rate of the MGA implant is very low; 1 in 520 completed bouts (0.19%). In contrast, the failure rate in new world monkeys is 24 in 367 completed bouts (6.54%). The data suggest that certain new world monkeys may require species-specific dosage formulation. The significantly higher levels of endogenous steroids reported for some new world primates supports this proposition. Currently, research on the use of an estrogen/progesterone implant for cebids is being coordinated through the AZA Contraception Advisory Group.
In three cases (ruffed lemur, cotton-top tamarin, mandrill) it was not possible to classify an unplanned pregnancy as a true contraceptive failure. Using the gestation range reported for the species, conception could have occurred just before or just after implant insertion. The possibility that conception occurred directly after implant insertion does offer a reminder that if there is any possibility the female may be close to or in estrus she should be separated from a sexual partner to allow the contraceptive sufficient time to take effect.

Tables 2, 3, 7, 9, 10, 11, 12 provide information on the consequences of contracepting pregnant female primates. Excluding the callitrichids, most females were unknown to be pregnant at the time contraception was initiated. Because callitrichids exhibit a post-partum estrus, females in this primate family have been purposefully implanted during pregnancy to prevent a post-partum conception. The majority of young born to contracepted females were liveborn with no apparent abnormalities. In no case was parturition inhibited, and there were no reports of lactation problems. It should be presumed that the occurrence of first trimester miscarriages is under-represented in the dataset.

The database reveals that the use of Depo-Provera is most frequent in prosimians, largely because delivery can be restricted to the breeding season. Over the past several years dose and inter-dose interval adjustments have occurred in an attempt to reduce associated weight gain, and, in black and brown lemurs, darkening of the pelage. However, even the 2.5 mg/kg body wt dose at 60 day intervals has caused weight gain and pelage darkening. Documented failures have occurred only in ruffed lemurs, as detailed in Table 2. One case is particularly cautionary; a female ruffed lemur was given three injections of Depo-Provera at the appropriate time during the breeding season. That female conceived in May suggesting that her estrous cycle was pushed past the normal breeding season. Because detection of estrus is so easy in ruffed lemurs, it is recommended that caretakers continue to monitor a female's reproductive state during and after the contraception bout.

The database indicates that in non-seasonal primates, Depo-Provera is used more as an intermediate contraceptive method, most often to contracept a female inbetween implant orders.

With one exception (mandrill), human birth control pills have only been used in apes. Determining the failure rate in birth control pills is problematic because of the difficulty in confirming whether the female actually consumed the (entire) pill every day. When deciding which contraceptive method to use, the problem of treatment delivery in relation to the personality (tractability) of each individual has to be considered.

Only two primate species, the orangutan and the chimpanzee, have been contracepted with Norplant. Note that Table 12 shows two Norplant formulations for orangutans. The first formulation of 140 mg (two rods each containing 70 mg levonorgestrel) was distributed through the Population Council on a limited basis in the early 1980s. Nine orangutans were contracepted with the 140 mg Norplant, and the follow-up information for those contraceptive bouts is complete. In contrast, the 6 rod (36 mg per rod, total = 216 mg)
Norplant newly approved by the FDA for use in humans, has a recent history of use in apes. Consequently, information on the appropriate duration of use in the different species and the rate of failure will take years to amass.

Reversal data for all contraceptive methods is still very limited. This is most likely true because widespread contraceptive use in primates is relatively recent and, to date, few females have been taken off birth control for the express purpose of reproduction. The available data (see Tables 8, 9, 10, 12) does suggest individual variation is a significant factor in the rate at which females resume cycling and conceive.

ACKNOWLEDGEMENTS

I thank Betsy Hornbeck, Pablo Molino, and Ed Plotka for developing the Contraception Database program. The efforts of all the veterinarians, curators, keepers, and unsuspecting volunteers who completed the contraception questionnaires is greatly appreciated. We hope that seeing some results makes the process more bearable.

Table 1: Information requested on the AZA Contraception Advisory Group Survey.

<table>
<thead>
<tr>
<th>1. Species</th>
<th>14. Behavioral effects?</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. ISIS #, Animal name, Studbook #</td>
<td>15. Date BC ended to change method or implant, for medical reason, or other reason. If implant: was it recovered?</td>
</tr>
<tr>
<td>3. Sex &amp; Birthdate</td>
<td>16. Date BC ended for reproduction</td>
</tr>
<tr>
<td>4. Has individual ever produced offspring</td>
<td>17. Date given access to sexual partner</td>
</tr>
<tr>
<td>5. Date of most recent birth prior to initiation of BC</td>
<td>18. Date of planned birth &amp; viability of offspring</td>
</tr>
<tr>
<td>6. Contraceptive method</td>
<td>19. Unplanned birth: Date, viability of young: was BC method confirmed in use at conception?, at birth?</td>
</tr>
<tr>
<td>7. IF MGA implant: implant # and weight</td>
<td>20. If aborted: date</td>
</tr>
<tr>
<td>8. Birth control start date</td>
<td>21. Animal transferred: need date and address of new location</td>
</tr>
<tr>
<td>9. Dose</td>
<td>22. Death date</td>
</tr>
<tr>
<td>10. Animal weight</td>
<td>23. Comments</td>
</tr>
<tr>
<td>11. Date access to male after start of BC</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Reversible contraception methods reported used in prosimians: species, methods (MGA implant, Depo-Provera), sample size/method, and number of confirmed failures.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>MGA # SENT</th>
<th>MGA DATA FOR</th>
<th>MGA FAIL</th>
<th>DEPO-P # FEMALES</th>
<th>DEPO-P # SEASONS</th>
<th>DEPO FAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcebus murinus</td>
<td>4</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eulemur fulvus</td>
<td>76</td>
<td>60</td>
<td>0</td>
<td>22</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Eulemur macaco</td>
<td>40</td>
<td>25</td>
<td>0</td>
<td>30**</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td>Eulemur rubriventer</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Eulemur mongoz</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Lemur catta</td>
<td>65</td>
<td>40</td>
<td>0</td>
<td>7</td>
<td>12</td>
<td>0*</td>
</tr>
<tr>
<td>Varecia variegata</td>
<td>80</td>
<td>49</td>
<td>0*</td>
<td>39</td>
<td>80</td>
<td>5+</td>
</tr>
<tr>
<td>Galago crassicaudatus</td>
<td>10</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Female conceived just before or after initiation of contraceptive method.

** Two black lemur females were treated w/5 mg/kg body wt depo-provera in the early first and second trimester of pregnancy, young liveborn but died of maternal neglect.

+ DETAILS OF CONFIRMED FAILURES:
1. Female only given 1 treatment of Depo-Provera (10 mg/kg BW) on Oct.30; conceived 66 days post-treatment.
2. Female given 3 injections (5 mg/kg BW) at 55-59 day intervals during breeding season, conceived in May, 67 days after last treatment.
3. Female conceived 64 days after first treatment (10 mg/kg BW); given second treatment (unknown pregnant); livebirth.
4. Female conceived 54 days after first treatment (5 mg/kg BW); given second treatment; livebirth.
5. Female given 3 treatments of 5 mg/kg BW during breeding season, conceived 9 days after second treatment, infant found dead, unk if stillborn.
Table 3: Reversible contraception reported used in new world monkeys: species, method (MGA implant and Depo-Provera), sample size/method, and confirmed failures.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>MGA # SENT</th>
<th>MGA # DATA FOR</th>
<th>MGA # FAIL</th>
<th>DEPO-P # FEMALES</th>
<th>DEPO-P # INJECTS GIVEN</th>
<th>DEPO FAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leontopithecus rosalia</td>
<td>361</td>
<td>159</td>
<td>2</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Saguinus imperator</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Saguinus oedipus</td>
<td>124</td>
<td>27</td>
<td>*</td>
<td>1</td>
<td>1</td>
<td>1+</td>
</tr>
<tr>
<td>Callithrix argentata</td>
<td>2</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Callithrix jacchus</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Callithrix penicillata</td>
<td>2</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Callithrix pygmaea</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Callimico goeldii</td>
<td>127</td>
<td>95</td>
<td>1</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Callicebus moloch</td>
<td>10</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pithecia pithecia</td>
<td>13</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Aotus trirrgatus</td>
<td>12</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Saimiri sciureus</td>
<td>30</td>
<td>21</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cebus apella</td>
<td>22</td>
<td>13</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Alouatta caraya</td>
<td>11</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>6 &amp; 3</td>
<td>0</td>
</tr>
<tr>
<td>Alouatta villosa</td>
<td>2</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ateles geoffroyi</td>
<td>38</td>
<td>21</td>
<td>5</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ateles fusciceps</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Female conceived just before or after implant insertion.

+ Female treated with 10 mg/kg body wt dose of depo-provera 14 days post partum; conceived ~ 37 days post-partum; single liveborn offspring.
Table 4: Reversible contraceptive methods reported used in old world monkeys: species, method (MGA implant, Depo-Provera, birth control pills), sample size/method, and number of confirmed failures.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>MGA # SENT</th>
<th>MGA DATA FOR</th>
<th>MGA # FAIL</th>
<th>OTHER BC # FEMALES</th>
<th>OTHER BC # INJECT / DURATION</th>
<th>OTHER BC # FAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cercocebus aterrimus</td>
<td>13</td>
<td>11</td>
<td></td>
<td></td>
<td>Depo-P # inject 1</td>
<td></td>
</tr>
<tr>
<td>Cercocebus galeritus</td>
<td>6</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cercocebus torquatus</td>
<td>37</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cercopithecus aethiops</td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cercopithecus ascanius</td>
<td>18</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cercopithecus diana</td>
<td>7</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cercopithecus mitis</td>
<td>20</td>
<td>20</td>
<td>1*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cercopithecus mona</td>
<td>11</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cercopithecus neglectus</td>
<td>10</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macaca nigra</td>
<td>19</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macaca sylvanus</td>
<td>14</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macaca silenus</td>
<td>78</td>
<td>56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macaca fuscata</td>
<td>78</td>
<td>53</td>
<td></td>
<td></td>
<td>Depo-P # inject 1</td>
<td></td>
</tr>
<tr>
<td>Colobus guereza</td>
<td>72</td>
<td>43</td>
<td></td>
<td></td>
<td>Depo-P # inject 1</td>
<td></td>
</tr>
<tr>
<td>Presbytis cristatus</td>
<td>11</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presbytis entellus</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presbytis francoisi</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presbytis obscurus</td>
<td>22</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5: Results on the use of the MGA implant and Depo-Provera in apes: species, sample size/method and number of failures.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>MGA # SENT</th>
<th>MGA DATA FOR</th>
<th>MGA # FAIL</th>
<th>DEPO-P # FEMALES</th>
<th>DEPO-P # INJECTS</th>
<th>DEPO-P # FAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Erythrocebus patas</em></td>
<td>39</td>
<td>18</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Papio cyncephalus</em></td>
<td>62</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Papio hamadryas</em></td>
<td>92</td>
<td>54</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Papio sphinx</em></td>
<td>50</td>
<td>29</td>
<td>1**</td>
<td>BC PILL 1+</td>
<td># mos 61</td>
<td>0</td>
</tr>
</tbody>
</table>

* Failure; female implanted 20.5 months when she conceived.

** Female conceived just before or after implant inserted; offspring liveborn.

+ Ortho-novum 1/50; 1 tablet/day.
Table 6: Duration of MGA implant use in primates: bouts completed and still in progress (in months), total number of implants reported lost and of those lost, the number of resulting pregnancies.

<table>
<thead>
<tr>
<th>GENUS</th>
<th>COMPLETED BOUTS</th>
<th>TOTAL LOST/ # PREG</th>
<th>IN PROGRESS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-12</td>
<td>12-24</td>
<td>25+</td>
</tr>
<tr>
<td>Gala go</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lemur</td>
<td>12</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Eulemur</td>
<td>10</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Varecia</td>
<td>18</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Callithrix</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Saguinus</td>
<td>0</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Leontopithecus</td>
<td>10</td>
<td>29</td>
<td>32</td>
</tr>
<tr>
<td>Callimico</td>
<td>11</td>
<td>31</td>
<td>9</td>
</tr>
<tr>
<td>Cebus</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Saimiri</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Aotus</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Callicebus</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Alouatta</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ateles</td>
<td>2</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Cercocebus</td>
<td>4</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Cercopithecus</td>
<td>6</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Macaca</td>
<td>12</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>Colobus</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Presbytis</td>
<td>0</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Erythrocebus</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Papio</td>
<td>12</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td>Mandrillus</td>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Hylobates</td>
<td>3</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Pongo</td>
<td>17</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Pan</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Gorilla</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 7: Results of implanting female primates during pregnancy: species, trimester in which MGA implant inserted, and subsequent livebirth, stillbirth, or abortion.

<table>
<thead>
<tr>
<th>TAXA</th>
<th>TRIMESTER</th>
<th>BIRTH</th>
<th>TAXA</th>
<th>TRIMESTER</th>
<th>BIRTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. macaco</td>
<td>1st</td>
<td>Unk+</td>
<td>C. guereza</td>
<td>2nd</td>
<td>Live</td>
</tr>
<tr>
<td>V. variegata</td>
<td>1st</td>
<td>Live</td>
<td>C. guereza</td>
<td>3rd</td>
<td>Live</td>
</tr>
<tr>
<td>V. variegata</td>
<td>2nd</td>
<td>Live</td>
<td>C. guereza</td>
<td>3rd</td>
<td>Live</td>
</tr>
<tr>
<td>V. variegata</td>
<td>2nd</td>
<td>Live</td>
<td>P. obscurus</td>
<td>1st</td>
<td>Live</td>
</tr>
<tr>
<td>S. oedipus</td>
<td>2nd</td>
<td>Live</td>
<td>E. patas</td>
<td>2nd</td>
<td>Live</td>
</tr>
<tr>
<td>S. oedipus</td>
<td>3rd</td>
<td></td>
<td>P. hamadryas</td>
<td>1st</td>
<td>Live</td>
</tr>
<tr>
<td>L. rosali</td>
<td>1st</td>
<td>Live</td>
<td>P. hamadryas</td>
<td>2nd</td>
<td>Live</td>
</tr>
<tr>
<td>L. rosali</td>
<td>1st</td>
<td>Live</td>
<td>P. hamadryas</td>
<td>2nd</td>
<td>Live</td>
</tr>
<tr>
<td>L. rosali</td>
<td>1st</td>
<td>Abort</td>
<td>P. hamadryas</td>
<td>2nd</td>
<td>Live</td>
</tr>
<tr>
<td>L. rosali</td>
<td>2nd</td>
<td>Live</td>
<td>P. hamadryas</td>
<td>3rd</td>
<td>Live</td>
</tr>
<tr>
<td>L. rosali</td>
<td>2nd</td>
<td>Live</td>
<td>P. cynocephalus</td>
<td>2nd</td>
<td>Live</td>
</tr>
<tr>
<td>L. rosali</td>
<td>3rd</td>
<td>Live</td>
<td>P. cynocephalus</td>
<td>3rd</td>
<td>Abort</td>
</tr>
<tr>
<td>C. goeldii</td>
<td>1st</td>
<td>Live</td>
<td>P. cynocephalus</td>
<td>3rd</td>
<td></td>
</tr>
<tr>
<td>C. apella</td>
<td>1st</td>
<td>Live</td>
<td>M. sphinx</td>
<td>1st</td>
<td>Live</td>
</tr>
<tr>
<td>C. apella</td>
<td>1st</td>
<td>Live</td>
<td>M. sphinx</td>
<td>2nd</td>
<td>Live</td>
</tr>
<tr>
<td>Callicebus</td>
<td>3rd</td>
<td>Live</td>
<td>H. lar</td>
<td>1st</td>
<td>Still</td>
</tr>
<tr>
<td>A. goeffroyi</td>
<td>3rd</td>
<td></td>
<td>H. lar</td>
<td>2nd</td>
<td>Live</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H. syndactylus</td>
<td>1st</td>
<td>Live</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H. syndactylus</td>
<td>2nd*</td>
<td>Live</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H. syndactylus</td>
<td>2nd</td>
<td>Live</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H. syndactylus</td>
<td>3rd</td>
<td>Live</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P. pygmaeus</td>
<td>1st</td>
<td>Live</td>
</tr>
</tbody>
</table>

+ Infant found dead in am, unconfirmed if live/stillborn.
* Implant lost during pregnancy, duration of use unknown.
Table 8: Return to fertility (reversals) in female primates contracepted with MGA implants: species, duration (in months) of implant use, and time to reversal (in months).

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>DURATION OF USE</th>
<th>REVERSAL TIME</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eulemur fulvus</em></td>
<td>26</td>
<td>36</td>
<td>4th breeding season</td>
</tr>
<tr>
<td><em>Eulemur fulvus</em></td>
<td>26</td>
<td>1</td>
<td>Same breeding season</td>
</tr>
<tr>
<td><em>Eulemur macaco</em></td>
<td>50</td>
<td>22</td>
<td>Transferred overseas</td>
</tr>
<tr>
<td><em>Eulemur macaco</em></td>
<td>12</td>
<td>1</td>
<td>Same breeding season</td>
</tr>
<tr>
<td>Varecia variegata</td>
<td>11</td>
<td>13</td>
<td>2nd breeding season</td>
</tr>
<tr>
<td>Varecia variegata</td>
<td>11</td>
<td>1.5</td>
<td>Same breeding season</td>
</tr>
<tr>
<td>Varecia variegata</td>
<td>42*</td>
<td>19</td>
<td>* One implant, so expired at least 1 year</td>
</tr>
<tr>
<td>Leontopithecus rosali</td>
<td>30</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><em>L. rosali</em></td>
<td>12.5</td>
<td>29.5</td>
<td></td>
</tr>
<tr>
<td><em>L. rosali</em></td>
<td>21</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td><em>L. rosali</em></td>
<td>22.5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>L. rosali</em></td>
<td>26</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><em>L. rosali</em></td>
<td>18</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td><em>L. rosali</em></td>
<td>19</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>L. rosali</em></td>
<td>21</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Callicebus goeldii</td>
<td>19</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Ateles geoffroyi</td>
<td>4</td>
<td>.5</td>
<td></td>
</tr>
<tr>
<td>Colobus guereza</td>
<td>11</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Pongo pygmaeus</td>
<td>5</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Pongo pygmaeus</td>
<td>21</td>
<td>7.5</td>
<td>Access to male 3 mo post implant removal</td>
</tr>
<tr>
<td>Pongo pygmaeus</td>
<td>24.5</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>
Table 9. Use of birth control pills in *Pan troglodytes*: brands, number and duration of completed and ongoing bouts (in months).

<table>
<thead>
<tr>
<th>BRAND OF BIRTH CONTROL PILL</th>
<th>COMPLETED BOUTS</th>
<th>BOUTS IN PROGRESS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-18</td>
<td>19-36</td>
</tr>
<tr>
<td>Ortho-Novum 1/50</td>
<td>11 a</td>
<td>1</td>
</tr>
<tr>
<td>Modicon 28</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Loestrin 1/20</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Loestrin 1.5/30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FAILURES:
- b. Same female conceived after 73 months on Ortho-Novum 1/50. On pill through birth: liveborn.

TREATED WHILE PREGNANT:
- c. Female treated 2 mo during 3rd trimester w/ Ortho-Novum 1/50, stopped 1 mo before birth: stillborn.

REVERSALS:
- 1. Female treated 11 mo w/Modicon 28 and 14 mo w/Ortho-Novum 1/50; conceived 5.5 mo after treatment ended.
- 2. Female treated 25 mo w/Modicon 28; conceived 1 wk after treatment ended.
- 3. Female treated 19 mo w/Ortho-Novum 1/50; conceived 2 weeks after treatment ended.
Table 10: Use of birth control pills in *Pongo pygmaeus*: brands, number and duration of completed and ongoing bouts (in months).

<table>
<thead>
<tr>
<th>BRAND OF BIRTH CONTROL PILL</th>
<th>COMPLETED BOUTS</th>
<th>BOUTS IN PROGRESS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-18 19-36 37+</td>
<td>0-18 19-36 37+</td>
</tr>
<tr>
<td>Ortho-Novum 1/50</td>
<td>3 a 2 b</td>
<td></td>
</tr>
<tr>
<td>Lo-Ovral 28</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ovcon 35</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Norinyl 1+35</td>
<td>0</td>
<td>2 c</td>
</tr>
<tr>
<td>Ortho-cept</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Triphasil 28</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Cyclen 28</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

FAILURES:

a. Female conceived (16 mo into treatment) after refusing pills for 5 days; treated through mid-pregnancy; liveborn.

b. Female conceived after 34 mo treatment, discontinued BC 1 mo after found pregnant; liveborn.

c. Female conceived after 19 mo treatment; BC con’t. through birth; liveborn.

Female conceived after 26 mo treatment, BC con’t through mid-pregnancy; liveborn.

REVERSALS:

1. Female treated for 25 mo w/Ortho-Novum 1/50; conceived 4.5 mo after treatment ended.
Table 11: Use of oral contraceptives in *Gorilla gorilla* and *Hylobates* sp.*: brands, number and duration of completed and ongoing bouts (in months).

<table>
<thead>
<tr>
<th>BRAND OF BIRTH CONTROL PILL</th>
<th>COMPLETED BOUTS</th>
<th>BOUTS IN PROGRESS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-18</td>
<td>19-36</td>
</tr>
<tr>
<td>Overette</td>
<td>1/</td>
<td></td>
</tr>
<tr>
<td>Lo-ovral 28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovcon 35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovcon 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ortho-novum 1/35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ortho-novum 1/50</td>
<td>2/1a</td>
<td></td>
</tr>
<tr>
<td>Demulen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovaban</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Megace</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Provera</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data presented in the order: gorilla/gibbon

a Difficulty in getting female siamang to take pill, most likely conceived as a result of missed pills. Discontinued late second trimester; livebirth.

b White-cheeked gibbon conceived following 4.5 mo oral provera treatment. Discontinued provera during 1st trimester when pregnancy discovered; offspring liveborn.
Table 12: Data on the use of Norplant in primates: species, Norplant dose, duration of use, number reported lost, and number of pregnancies resulting from lost Norplants.

<table>
<thead>
<tr>
<th>SPECIES/ NORPLANT DOSE</th>
<th>COMPLETED BOUT (IN MONTHS)</th>
<th>BOUT IN PROGRESS (IN MONTHS)</th>
<th>LOST / PREG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-12</td>
<td>13-24</td>
<td>25+</td>
</tr>
<tr>
<td><em>Pongo pygmaeus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>140 mg dose</td>
<td>4</td>
<td>51*</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pongo pygmaeus</em></td>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>216 mg dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pan troglodytes</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>216 mg dose</td>
<td></td>
<td>1***</td>
<td></td>
</tr>
</tbody>
</table>

* One female conceived at 51 months; this data can more accurately be viewed as information on duration of efficacy than as a failure.
** Norplant removed after 14 mo to allow female to breed; transferred to a new institution, unk when introduced to male, conceived 22 mo post-removal.
*** Norplant inserted in female during her 2nd trimester (unknown she was pregnant); livebirth.
EFFICACY, SAFETY AND REVERSIBILITY OF A BISDIAMINE AS A MALE-DIRECTED ORAL CONTRACEPTIVE IN GRAY WOLVES (Canis lupus)

Cheryl S. Asa, PhD
St. Louis Zoo, St. Louis, MO 63110, USA

Lourens J.D. Zaneveld, DVM, PhD
Rush Presbyterian St. Luke Medical Center, Chicago, IL 60612, USA

Linda Munson, DVM, PhD
College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901, USA

Margaret Callahan, BS
Wildlife Science Center, Forest Lake, MN 55025, USA

Ann P. Byers, PhD
CBSG, Apple Valley, MN 55124, USA

Because of growing concern regarding the safety of progestin-based contraceptives in carnivores,\textsuperscript{1,7,8} development of alternative methods has become critical. The bisdiamine WIN 18,446 (Sterling Winthrop, Rensselaer, NY) has been found to block spermatogenesis without interfering with the production and secretion of testosterone in humans\textsuperscript{6} and a variety of laboratory species.\textsuperscript{1,2,3,4,5} The only deleterious finding reported in males has been an antabuse-like effect when alcohol is ingested,\textsuperscript{6} which does not preclude its use in captive wildlife.

A trial of the efficacy and safety of this bisdiamine was conducted using 10 male gray wolves (6 treated, 4 control) maintained outdoors at a research facility in Minnesota. The treated wolves, body weight 33 to 65 kg, were fed 9 g of bisdiamine in approximately 0.25 kg ground meat daily, from the first of November 1992 (thought to be prior to onset of spermatogenesis) through the first of March 1993. Once monthly, wolves were anesthetized with ketamine HCl (Ketaset, Fort Dodge Laboratory, Inc., Fort Dodge, KS 50501), xylazine HCl (Rompun, Miles, Inc., Shawnee Mission, KS 66201) and diazepam (Valium, Roche Products, Manati, PR) for electroejaculation (P-T Electronics, Boring, OR 97009) and blood collection for chemistry and hematology between November 1992 and May 1993. Testicular biopsies were then taken for histology.

Sperm first appeared in the semen of control wolves in early December and continued through April, with maximum concentrations per wolf ranging from 51 to 336 million cells/ml. Sperm were found in the semen of 5 of the 6 treated wolves in maximum concentrations of 1-15 million cells/ml, with the highest concentrations occurring in males weighing more than 45 kg. All treated males were housed with females, none of which gave birth. Hematology and serum chemistries did not differ between treatment and control groups nor from the normal range for domestic dogs.
During the 1993-94 breeding season, 2 of the previous control males were given bisdiamine starting in January, after sperm were first detected in semen, to determine whether the 9-g dose was sufficient to suppress ongoing spermatogenesis. In addition, reversibility was evaluated in the 6 previously treated males by monthly semen collection. Sperm were found in semen samples of all the males, including those being given bisdiamine.

These results demonstrate that the bisdiamine WIN 18,446, at a dose of at least 190 g/kg, can interfere with sperm production in the gray wolf if administration begins prior to the onset of spermatogenesis. Even wolves receiving approximately 150 mg/kg were oligospermic and probably infertile, since mating did not result in the birth of pups. However, even 250 mg/kg was not sufficient to interrupt spermatogenesis during the breeding season. Thus, for effective contraception, treatment must be started prior to the breeding season or a higher dose must be used. The reversibility and apparent absence of deleterious effects of this compound make it a promising candidate for contraception of carnivores and perhaps other taxa as well.

ACKNOWLEDGEMENTS

The authors thank Drs. Bill Sadler and Dan Hopkins of Purina Mills for advice, financial support and donation of feed; Sterling Drug Co. for donation of the bisdiamine; Patty Hagburg for sample preparation; Mark Beckel for animal care and handling; and Dr. Terry Kreeger for advice and assistance in project implementation.

LITERATURE CITED

UPDATE ON DISEASES ASSOCIATED WITH CONTRACEPTIVE USE IN ZOO ANIMALS

Linda Munson, DVM, PhD•, Lisa M. Harrenstien, DVM, Carol A. Haslem, BS, and Jennifer E. Stokes, DVM Dept. of Pathology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901, USA

Contraceptives are widely used in zoos to manage valuable breeding populations and limit reproduction of surplus animals when permanent sterilization is not an option. Few types of contraceptives are available and affordable to zoos, and these contraceptives have not been tested for safety and efficacy in zoo species. In response to the need for information on contraceptive safety in zoo species, we have been surveying zoo populations for adverse effects of contraceptives under the auspices of the AZA Contraceptive Advisory Group and with support from the AZA Conservation Endowment Fund/Ralston Purina Big Cat Survival Fund. We currently have information on 262 felids, 16 canids, and 61 primates. We also have initiated clinical trials to test new contraceptives on small populations of surplus animals. This report summarizes our research findings since our summary report in the 1993 Proceedings of the American Association of Zoo Veterinarians (Munson; pp 284-289).

Progestin contraceptives

Progestins, such as melengestrol acetate (MGA), megestrol acetate (Ovaban®), medroxyprogesterone (Depo-Provera®) and levonorgestrel (Norplant®), continue to be the most widely used contraceptives in zoos because of their efficacy and low cost. In zoo felids, there is a clear association with earlier development of endometrial hyperplasia, and hyperplasia of greater severity than in uncontracepted felids. Several cases of pyometra also were noted in MGA-contracepted felids. Through our surveillance program, we have identified 17 cases of uterine cancer, a condition that is considered extremely rare in carnivores. Sixteen of these 17 cases were exposed to progestin contraceptives for prolonged periods, an association that is highly significant. We also have 30 confirmed cases of mammary cancer in zoo felids, and 28 of these 30 were on progestin contraceptives. Histologic analysis of the ovaries from 31 MGA-contracepted felids revealed that approximately 84% of these felids had tertiary follicles, suggesting that folliculogenesis was not suppressed at currently used doses. These findings also suggest that most MGA-contracepted felids have endogenous estrogens, which may enhance the carcinogenic effects of MGA. Evaluation of ovarian lesions in both progestin-contracepted and uncontracepted (N = 69) felids determined that cystic rete ovarii, which can result in pressure atrophy of the cortex, were more prevalent in MGA-contracepted felids than uncontracepted felids. In this survey, primary ovarian cancer only occurred in jaguars regardless of MGA exposure, and all other cancers noted in ovaries were metastatic sites from mammary or uterine cancer. Because mammary and uterine cancer are aggressive, fatal diseases, prolonged use of progestins in felids is discouraged.
Canids and other carnivores also are susceptible to progestin-induced proliferative and inflammatory diseases of the reproductive tract (Asa CS and Porton I, Proc. Amer. Assoc. Zoo Vet. pp.298-303, 1991). All cases of cystic endometrial hyperplasia, pyometra, adenomyosis, mucometra, and mammary cancer in gray wolves in our survey were in progestin-contracepted animals. Severe endometrial hyperplasia and mucometra were noted in MGA-contracepted meerkats; endometrial hyperplasia and a case of endometrial carcinoma were observed in MGA-contracepted coati.

Some prosimians on MGA contraceptives developed endometrial hyperplasia and adenomyosis, and one case of endometrial carcinoma was noted. Progestin contraceptives in primates also have been associated with decidualization of the endometrial stroma, a change that is reversible after drug withdrawal. In Callimicos, this decidual response is particularly aggressive (Raverty S. et al., Proc. Amer Assoc Zoo Vet. pp.244-245, 1994). Most higher primates develop endometrial atrophy after prolonged progestin exposure, but this change also is expected to be reversible.

Porcine zona pellucida (PZP) vaccination

Immunocloaception through vaccination with heterologous zona pellucida vaccines has been successful in many ungulates and equids (Kirkpatrick, et al. Proc. Amer. Assoc. Zoo Vet., pp.290-292). However, the effects of vaccination on ovarian function and other health parameters have not been studied in depth. Because ovarian lesions have occurred in dogs and rabbits vaccinated with PZP, prospective trials were designed to assess if PZP had adverse effects on felids. Ten domestic cats and 22 captive wild felids are included in this study to date, and the study is still in progress, so that final results are pending. However, we have had severe local and systemic responses to Freund's adjuvants in this trial. Three of 10 domestic cats developed marked hypercalcemia that resulted in a 50% loss of renal function in one cat. Many wild felids and the domestic cats developed chronic myositis at the site of injection, and some lions subsequently developed fistulous tracts (C. Miller, personal communication). Because these reactions are likely caused by Freund's adjuvants, we recommend that PZP only be used with other adjuvants, such as carbopel. However, the immunostimulatory effects of other adjuvants may be less than those of Freund's adjuvants, so that contraception may not be as successful. New clinical trials in are progress to determine if these new adjuvants are safe and can stimulate adequate titers to achieve immunocloaception.

Vas deferens plugs

In situ formation of occlusive plugs in the vas deferens has been attempted by injecting soft silicone. In preliminary trials, inflammatory reactions including sperm granulomas were noted near the site of insertion and surrounding the vas; the epithelium lining the vas was compressed, but not inflamed. Although none of these lesions appeared to compromise vas patency, fertility was not restored after plug removal.
AZA Contraceptive Advisory Group Adverse Reaction reporting system

The AZA Contraceptive Advisory Group continues to request information on any adverse reactions in contracepted animals. Any reproductive problems or unexplained diseases in contracepted animals can be reported to the Group by completing the enclosed form. Each contribution to this database increases our collective understanding of the risks of using certain contraceptive methods. Information from our Adverse Reaction databank will be reported annually at the AZA meeting.

ACKNOWLEDGMENTS

This research was supported by grants from the AZA CEF/Ralston Purina Big Cat Survival Fund and was made possible through the collaboration of the following zoos:

Akron Zoo, Zoo Atlanta, Audubon Park Zoo, John Ball Zoo, Baltimore Zoo, Bergen County Zoo, Binder Park Zoo, Birmingham Zoo, Brandywine Zoo, Brookfield Zoo, Burnet Park Zoo, Caldwell Zoo, Calgary Zoo, Central Florida Zoo, Chaffee Zoological Gardens of Fresno, Cheetah Conservation Fund, Cheyenne Mountain Zoo, Cincinnati Zoo, Columbus Zoo, Dallas Zoo, Denver Zoo, Detroit Zoo, Dickerson Park Zoo, Exotic Feline Breeding Center, El Paso Zoo, Fossil Rim Wildlife Center, Franklin Park Zoo, Henry Doorly Zoo, Hogle Zoo, Honolulu Zoo, Houston Zoo, Jackson Zoo, Jacksonville Zoo, Kansas City Zoo, Kings Island, Knoxville Zoo, Lincoln Park Zoo, The Living Desert, Los Angeles Zoo, Louisville Zoo, Lowry Park Zoo, Memphis Zoo, Metro Washington Park Zoo, Miami Metrozoo, Micke Grove Zoo, Miller Park Zoo, Minnesota Zoo, National Zoo, NYZS/Wildlife Conservation Society, Oklahoma City Zoo, Dr. James Peddie, Philadelphia Zoo, Phoenix Zoo, Pittsburgh Zoo, Point Defiance Zoo, Gladys Porter Zoo, Potawatomi Zoo, Racine Zoo, Reid Park Zoo, Rio Grande Zoo, Riverbanks Zoo, Ross Park Zoo, Sacramento Zoo, San Antonio Zoo, San Diego Zoo, San Francisco Zoo, Santa Ana Zoo, Santa Barbara Zoo, St. Louis Zoo, Seneca Park Zoo, Sunset Zoo, Toledo Zoo, Tulsa Zoo, Utica Zoo, White Oak Conservation Center, Wildlife Safari, Wildlife Waystation, Roger Williams Park Zoo, and Woodland Park Zoo.
REPORT OF ADVERSE REACTIONS IN CONTRACEPTED ANIMALS

REPORTING INSTITUTION: ____________________________________________

REPORTING VETERINARIAN/CURATOR: ________________________________

ANIMAL SPECIES: ___________________________________________________

ANIMAL ID: STUDBOOK # _____________ ISIS # _________________________

<table>
<thead>
<tr>
<th>Contraceptive type and dose</th>
<th>Animal weight</th>
<th>Inclusive dates of contraceptive use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ADVERSE REACTIONS: (PLEASE INCLUDE MEDICAL RECORDS)

  MAMMARY GLAND CANCER: ____________________________________________
  UTERINE CANCER: ________________________________________________
  UTERINE INFECTION: _____________________________________________
  ENDOMETRIAL HYPERPLASIA: _______________________________________
  DIABETES MELLITUS: ________________________________
  SKIN DISEASE: _________________________________________________
  BEHAVIORAL CHANGES: __________________________________________
  OTHER DISEASES: ________________________________________________

PLEASE MAIL TO:  DR. LINDA MUNSON
                DEPT. PATHOLOGY
                CVM/UNIVERSITY OF TENNESSEE
                P.O. BOX 1071
                KNOXVILLE, TN 37901
                615-974-8215 (PHONE) / 615-974-5616 (FAX)
THE IMPACT OF ZOO BASED CONSERVATION RESEARCH ON WILDLIFE CONSERVATION

Steven D. Thompson, PhD
Lincoln Park Zoological Gardens, Chicago, IL 60614, USA

Historically, zoo research was largely descriptive and aimed at morphology, animal behavior, and some aspects of basic husbandry. Goals and objectives were diverse but seldom extended beyond the bounds of a single discipline or institution. During the past seven years there has been a proliferation of formal conservation and research programs at American Zoo and Aquarium Association (AZA) member institutions. These new programs emphasize complex, cooperative projects that typically address topics common to many institutions. Projects are often rigorously designed with adequate sample sizes, manipulations, and control groups. Although some of what is called research is really development, basic and applied research projects are common. Physiology, nutrition, genetics, applied animal behavior, veterinary medicine, demography, and population genetics, are the more prominent disciplines now represented in zoo research programs. Projects are now focused on improving management of captive and wild populations. Problems identified in the wild are often addressed in captivity and vice versa. Many projects involving, for example, reproductive technology, population genetics, and demography have impacts well beyond the zoo and conservation communities. Research and development of small population management techniques in zoos and aquariums are now the cornerstones of reintroduction projects, translocation projects, and the population viability analyses that are essential to prioritization of conservation actions. Behavioral studies in zoos offer important insight into management of problem animals in small, wild populations. It seems inevitable that the wildlife in most "natural areas" will eventually be intensively managed. Zoo research and conservation programs will grow in scope and importance as management practices of zoo and wild populations converge.
DO ZOOLOGICAL INSTITUTIONS REALLY SUPPORT CONSERVATION PROJECTS?

Michael K. Stoskopf, DVM, PhD, Dipl. ACZM
Environmental Medicine Consortium, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606, USA

The "knee-jerk" answer to the question posed by the title of this presentation is "Yes, definitely!". My thesis today, however, will be that "No" or "Perhaps to a limited extent" would be a more honest assessments. I do not intend to belittle the efforts of the many dedicated individuals that I am proud to consider colleagues, and who hold a dedication to the principles of wildlife conservation as part of their core values. I do intend to assess the strategies and resource commitments to conservation which are currently in place in the majority of zoological institutions today for their potential to produce significant long term conservation impacts and relate strategies I see as having the most potential for lasting impacts. To be fair, it might have been equally informative to assess the same question for governmental wildlife agencies in the U.S.A. whose strategies, while distinctly different from zoos, often fall short of perfection. I hope that everyone in the audience will be at least stimulated to think hard about their own and their institution's role in wildlife conservation.

Others before me have suggested that the efforts of zoological institutions would be better described as Preservation rather than Conservation. Certainly, it is important to look at that criticism carefully. There is room here for some confusion. Webster's dictionary, for example, uses the words nearly interchangeably, and in definitions of each other. Conservation, as a term in biological sciences seems to have fallen to the wayside. The word is not defined in Henderson's Dictionary of Biological Terms or the Dictionary of Life Sciences. Nevertheless, it is possible and appropriate to separate the concepts of preservation and conservation. Preservation is used in many contexts in biology, not the least common of which is to describe a process also called fixation when referring to individual organisms or tissues. Its connotation includes stasis, and retention of characteristics without change. Conservation has been defined as "The planning and management of resources so as to secure their wise use and continuity of supply while maintaining and enhancing their quality, value and diversity." Its connotations include the dynamic process of enhancement, and speaks to the question of value. It is important to realize that neither term is limited to biological systems and can be applied to natural and/or man made resources.

When asked about their conservation efforts, most zoological institutions are quick to point out their participation in AZA SSP and TAXA groups, and the number of endangered species on display in their facility. In the larger institutions, the technical efforts of their reproductive research and off exhibit breeding programs are brought up with obvious and deserving pride. Even larger institutions can trot out an impressive array of biological scientists pursuing field research, often, but not always focused on the "charismatic mega-vertebrates". These efforts have value, but I want to strongly state that species survival plans are not, in and of themselves, conservation. Nor will the very expensive and resource consuming technically augmented captive breeding programs so popular in today's zoological
institutions, alone, provide solutions to the pressing conservation issues facing the world. Effective natural resources conservation requires that we look beyond individual species preservation and work at the level of the ecosystem and above, to find ways to ensure that the habitats and biodiversity critical to the future quality of life on this planet are managed to enhance their quality and value. How can zoological institutions better use their resources to accomplish this?

Zoos today are well aware of limitations in their resources. To make a lasting impact on conservation, it will be imperative for individual zoological institutions to seek out, create and nurture partnerships with other institutions. Certainly this includes other zoos, but also government wildlife agencies, corporations, and educational institutions. Perhaps I could admonish zoological institutions with the same advice I have given veterinarians and veterinary students for years. It is important to work on teams, to realize that each team member brings unique perspectives and expertise to the mission. It is also important to realize that the "Zoo" does not always have to, and in many cases would not be the appropriate institution to lead the team. In our need for this advice, some veterinarians and some zoological institutions share the handicap of poor team skills. There is more than enough glory to go around and much more work than glory.

Zoos have tremendous potential in the arena of Conservation Education. To optimize the impact of this potential, it is important that the concepts of conservation pervade all levels of a zoo's exhibition plans, including but not limited to the choice of species to exhibit, how they are exhibited and how adjunct delivery of the message is accomplished. Most zoos would say they already do this. It is, however, very important that the message they deliver include all of the dynamic and resource utilization implications of conservation. Zoo visitors need to understand the economic importance of indigenous wildlife and its management as a sustained resource as well as understand the value of biodiversity not as an academic principle but as a basis for supporting human existence, today, and in the future. Then the question arises, why is this need limited to the zoo visitor? I could argue that delivery of the message to people who never visit the zoo may have even more importance to the overall conservation of our natural resources. How much commitment is there to educational outreach that does not bring visitors through the gate? How many institutions are seriously involved in conservation education of peoples outside of their local area, in the United States, or third world countries? The current effectiveness of zoos in conservation education is an inarguable question, because the documentation of impact of these efforts is rare, but zoological institutions need to question themselves and examine their allocation of resources in this arena. They need to find ways to increase their impact, and to document the value of that impact in order to make better decisions allocating their educational and outreach resources.

Major zoological institutions are not usually content to focus their entire resources on conservation education. This is good for those of us who make our contribution through scientific research and/or delivery of health care. But here, it is even more critical that zoos establish long term comprehensive plans that allow them to focus their use of resources and develop long term partnerships. The future success of natural resources conservation resides
in the challenge of effecting local stewardship and buy-in to the value of comprehensive conservation plans, not in single species recovery plans. Community based conservation is the foundation of wise global stewardship of biodiversity. It is important that zoological institutional research programs promote the economic value of local natural resources, including responsible and well managed consumptive uses of renewable stocks. Sustainable agricultural methods integrated with careful attention to habitat and biodiversity conservation must be developed and implemented. To do this more information (ie: research) will be needed, but that work will need to fit into a carefully designed plan to achieve an integrated conservation management program. More people are alive today than have died in the history of mankind, and at current rates, we can expect to have doubled our population within 40 years. Zoological institutions, and indeed all conservation institutions, must not ignore the fact that humans are an inescapable component of the resource equations of our biosphere. We cannot succeed if we do not consider and plan for the social, cultural and economic foundations of human activity and their impact on biodiversity.

LITERATURE CITED

Despite innovative efforts by individuals such as Dr. Tony Harthoom in the 1960-1970's in developing immobilizing techniques for a wide variety of wild animals, many decision makers and wildlife managers/researchers have not yet accepted veterinarians as part of a resource management team.

Historically, in Africa, there has been occasional antagonism between veterinarians and wildlife managers/ecologists. This stems from the fact that the veterinarian's traditional role has been the prevention of disease in domestic livestock, thus maintaining, in countries like Zimbabwe and Botswana, a lucrative overseas market in beef, and protection of local markets. One of the diseases in livestock that would have an adverse effect on marketing beef overseas is foot-and-mouth disease (FMD). Wildlife has been blamed for many devastating outbreaks. Consequently, veterinarians have been associated with unnecessary slaughter of a variety of wildlife species in the name of disease prevention/eradication, specifically buffalo (Syncerus caffer) with FMD and tsetse fly eradication programs. Therefore, wildlife in general has been considered a threat to the livestock industry, but this has been based on prejudice and inadequate research data as to the roles of wildlife, domestic livestock and disease. Indeed, these prejudices have been encountered historically with Directors of Veterinary Services actively discouraging veterinary involvement in wildlife.

Within the last decade this perception of veterinarians and their role in the wildlife field has changed. This has been due, in part, to a recognition of the need for increasing professionalism in the wildlife management field, as well as to significant efforts by veterinarians to bridge the gaps, eliminate antagonism and improve dialogue between veterinarians, wildlife managers and ecologists. Veterinarians have been regarded by some as only clinicians, disease specialists, and/or scientists who work in laboratories developing new drugs for immobilization in order that field managers may use these drugs to capture and relocate wild animals. Many wildlife managers have considered their work to be an "art" with little consideration for applied science. There are several historic and current examples in Africa of a lack of vision and understanding of the value of veterinary training in providing "professional" input into wildlife capture and relocation.

Despite this lack of vision by some, many progressive wildlife departments have recognized the value of veterinarians as part of the multi-disciplinary team tasked to manage precious and valuable wildlife resources. For example, veterinarians lead capture units in National Parks Board (NPB), Kruger and NPB, Southern Parks in South Africa, in Namibia, and in Kenya. Indeed, the Director of Wildlife in Mozambique is a veterinarian. Natal Parks Board recently lost an experienced veterinarian due to inter-departmental tensions. In
Zimbabwe, a Veterinary Unit has been established with funding from the European Union to the tune of Z$18,000,000 (US$2,117,650) but despite the skills available, problems have been encountered with traditional perceptions of game capture. Involvement of veterinarians has become increasingly necessary not only because of the skills obtained through professional training but because of concern for animal welfare. Veterinarians are trained to problem solve and through appropriate experience should be able to adopt a more holistic approach to wildlife management problems, not only with wild animal capture but with health and ecological problems and imbalances.

Traditionally, many wildlife departments have had a wildlife management branch separated from a research branch. These departments have often been at odds with regards inputs into resource management. Antagonisms have existed due to a perception by management of research being staffed by degree individuals whereas management individuals have field skills but are often not well educated. This has significantly constrained wildlife management practices historically. Veterinarians have a unique contribution to make in being able to bridge the gap between science and field management. Veterinarians are trained to carry out research and are equally skilled in managing field operations.

The "existing" order that has dominated wildlife management for decades needs to be challenged. There is also a need to recognize professionalism and a broader vision of a team approach. Attitudes are changing, but must continue to advance, not only in Africa, but elsewhere in the world if we are to see wildlife management enter the next millennium as a modern, balanced and vibrant profession.
INTEGRATING WILDLIFE HEALTH INTO ARGENTINEAN CONSERVATION ACTIVITIES AND A COASTAL MANAGEMENT PLAN

William B. Karesh, DVM,* Robert A. Cook, VMD
Wildlife Health Sciences, Wildlife Conservation Society, 185th and Southern Blvd., Bronx, NY 10460, USA

Marcela M. Uhart, DVM
Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, (7000) Tandil, Pa'a Bs. Ar., Argentina

Mirtha N. Lewis, DVM
Laboratorio Mammíferos Marinos, Centro Nacional Patagonico, (9120) Puerto Madryn, Chubut, Argentina

Introduction

The Wildlife Conservation Society's (WCS) field programs in Patagonia have been ongoing for 30 years. The main taxonomic focus has been on marine mammals and aquatic birds, and the geographic region involves close to 2000 Km of coastline in the southern half of Argentina. The Society's Field Veterinary Program (FVP) has developed a role in WCS efforts there during the last three years. Veterinary efforts focus on augmenting the Society's conservation projects by assisting field biologists with anesthesia and health care of selected study animals, and defining the types and levels of disease in a variety of populations via wildlife health assessments. This veterinary component has been developed to take advantage of ongoing conservation efforts and in-country expertise.

Veterinary involvement began by responding to requests from field staff for medical supplies in 1992 and has evolved into an integrated part of the conservation and management "team" working in the Patagonia region of Argentina. Our original field veterinary activities in 1994 were centered on assisting with the immobilization of South American sea lions (Otaria byronia) for the attachment of instrumentation (time-depth recorders and satellite transmitters). This provided the opportunity to meet individuals working in the region, evaluate the possibilities for expanded veterinary activities, and demonstrate some of the techniques we were interested in using. During the first field season, we were able to examine and collect blood from Magellanic Penguins (Spheniscus magellanicus) at three geographically isolated colonies, Imperial Cormorants (Phalacrocorax atriceps) and Kelp Gulls (Larus dominicanus) at another, and from the sea lions and southern elephant seals (Mirounga leonina) being tagged by the field biologists. Funds for the work were provided by WCS. Following the work in 1994, a basic plan of approach for subsequent years was developed to meet mutually agreed upon needs. This plan explained to interested parties the rational behind activities and was then used to guide activities in 1995. The plan is provided below with updates to indicate status as of February, 1995. The work from both years has been integrated into a project funded by the World Bank Global Environment Facility and implemented by an Argentinean conservation group, Fundacion Patagonia Natural, established by our key field staff to develop a coastal management plan for Patagonia. The planning process is dependent upon obtaining and evaluating high quality
biological information, and also on maintaining excellent lines of communication and collaboration between the variety of organizations and government agencies involved. Likewise, any outside veterinary involvement needs to provide critical services and information, and be closely attached to the organizations and agencies involved. Preparation of local counterparts for the future is a high priority and therefore, part of our efforts are always directed at training. A key component is also the demonstration of the essential nature of a team approach to problem solving and project implementation to avoid interdisciplinary segregation or conflicts that can plague wildlife management and conservation programs in other countries.

Work Plan and Status report:

I. Wildlife Health Services

Objective: To enhance ongoing conservation activities in Patagonia by the provision of wildlife health services. Initially, expertise will be provided by the Wildlife Conservation Society (WCS). The goal over time is to create a team of trained Argentinean veterinarians to handle all aspects of the work. WCS will continue to provide advisory services or help in the development of new initiatives. Areas of veterinary involvement are:

A. Animal Handling and Sample Collection.

1. Consulting and training in the area of anesthesia and restraint for marine mammals is geared towards helping ongoing conservation and biological research activities of pinnipeds. Traditionally, the anesthesia and restraint of some pinnipeds has been problematic. New developments in anesthetic drugs, monitoring techniques, and emergency procedures is helping to reduce the risks. Implementation in Patagonia to date has eliminated mortalities.

2. Proper sample collection, handling, and analysis are now being integrated into ongoing projects which involve the handling of animals for other purposes. This allows more information to be gathered on the health of wildlife populations without increasing the frequency of animal disturbance. Local scientists and students who are already involved in approved projects are being trained in proper techniques to augment their capabilities.

B. Emergency Services

The capability to respond to wildlife emergencies is an important component to conservation efforts. The colonial nature of key species in Patagonia put them at high risk for catastrophic events including disease outbreaks. The ability to identify the source of a problem and implement a management strategy is essential for proper conservation of these populations and prevention of similar losses in the future. Additionally, the public and government look to conservation scientists in times of emergencies and expect results.
1. Mass die-offs of wildlife occur frequently around the world. In recent times, reports of large losses of Black-necked Swans (Cygnus melanocoryphus) and cormorants in Patagonia have occurred. A response team is needed to effectively evaluate a wildlife population die-off, collect proper samples and determine the cause of the problem. This service could A) be provided by an external group that is available on demand, B) be established in various areas of Patagonia to respond to local problems, or C) could be a combination of these two approaches depending on the emergency situation.

2. Oil spills provide an example of a catastrophic event in the management of wildlife. In this situation, huge numbers of individuals are exposed to a potentially lethal agent resulting in large numbers of dead animals as well as animals that are sick and require treatment. Implementing an adequate response to this emergency is challenging and expensive but has been demonstrated by oil spill response programs in other parts of the world. Similar programs need to be adapted to address the biologic, geographic, political, and economic situations in Patagonia.

3. Caring for an illness in a high profile wild animal can be mandated by public opinion and governmental urging. The quality of the response to such situations affects all professionals and agencies involved. It can have positive effects on the conservation of wildlife by building public trust and gaining support for other issues. This is independent of the conservation value of the individual animal and frequently results in the investment of resources that appear disproportionately high. Having the organization, skills and equipment ready to deal with these situations facilitates a rapid and professional response. This capability needs to be developed in the region.

C. Misc. Services Available

1. Due to lack of in-country production along with restrictive import regulations, it is frequently difficult to acquire equipment needed for wildlife study and care. WCS health staff is currently helping in the identification of sources for needed materials and providing assistance in their acquisition to facilitate conservation activities (veterinary as well as handling, marking, telemetry, laboratory, etc.)

2. Information on new techniques and technologies: Field-based conservation efforts can be hampered by a lack of current knowledge on advancements in technology. As a result of global networking, the WCS staff are exposed to many new techniques. The FVP distributes to the field staff information in the areas of animal health, animal handling, laboratory techniques, biotelemetry and radio-telemetry, animal identification, etc.

3. Protocol review: We routinely review hundreds of procedures annually which involve animal handling and sampling, telemetry applications, and laboratory techniques to improve cost effectiveness, ensure safety, and improve efficiency. Evaluation of techniques and protocols is available at any phase of a project, from planning to field implementation.
4. Providing guidance in areas of specialization. WCS health science staff can consult on areas of expertise in wildlife medicine, pathology, nutrition, reproduction, and biotelemetry. Through a large network of consultants, we can facilitate the acquisition of additional expertise for field staff or government agencies with particular needs.

II. Wildlife Health Assessment & Monitoring

Monitoring the health of animal populations helps to identify specific causes of wildlife population declines. This data augments the information that has been gathered and evaluated by WCS/Argentinean scientists over the previous decades regarding wildlife population dynamics. This team approach to monitoring provides a unique opportunity to integrate information regarding health factors into the determination of viable populations. The development of areas in Patagonia for agriculture, industry, and tourism is providing economic benefits for human populations. Monitoring the impact of this change on the health of wildlife populations adds valuable information to other conservation studies. Areas of concern include:

1) the relationship of diseases of domestic animals and wildlife,
2) effects of industrial, agricultural, and urban pollution on wildlife,
3) impact of tourism on wildlife,
4) diseases spread among various wildlife species

There is little health information available on wildlife populations in Argentina. As animal populations change, evaluation of causes is limited by a lack of information regarding the present and historical occurrence pathogens, parasites, and toxins. Without this knowledge, conclusions regarding causality may be incomplete.

In order to establish a significant baseline of information on wildlife populations, the following species have been identified:

Kelp gulls: This species is widespread in distribution and abundant in the region. The adaptability of these animals allows them to take advantage of a wide range of resources. They can also act as hosts or carriers of pathogens and toxins. Health evaluations are being conducted to establish a profile on this species and gain insight into the potential impact on less adaptable species in close association with the Kelp Gulls such as terns, Dolphin Gulls (Larus scoresbii) and cormorants.

Dolphin Gulls and Olrog’s Gulls (Larus atlanticus): These two species are rare, are found in small colonies with restricted ranges and have specialized needs. Their vulnerability to localized extinction is high. This presents a paradox where the need to insure the health of the remaining populations is extremely important but the need to avoid disturbance of the colonies is equally important. Monitoring of sympatric species such as Kelp Gulls provides one mechanism for assessment. Another mechanism would be to expand on the information gathered when these birds are being routinely handled for banding by performing physical
examinations and collection of blood and feces. Proper post-mortem examination and collection protocols need to be provided and implemented to take advantage of the occasional opportunity of dead birds being found by researchers working around the colonies.

Magellanic Penguins: While penguin numbers are high, reductions in population size have been noted. The limited number of large colonies place this species at greater risk to catastrophic environmental or disease events. A limited sampling was conducted in 1994 at three sites in Chubut. Six sites, including the two largest colonies in Argentina, 1000 Km apart, were sampled in 1995. Plans to broaden this monitoring program are being developed.

Rockhopper Penguins (*Eudyptes chrysolophus*): A colony of Rockhopper Penguins is now on a small island off the coast of Patagonia. This population had doubled to 450 in the early 90's but has not increased in the last 3 years. Monitoring of Rockhopper Penguins began in 1995, providing the first information on their health status in Argentina.

Cormorants: Red-legged (*Phalacrocorax gaimardi*), Imperial, Rock (*P. magellanicus*), and Guanay (*P. bougainvillii*) Cormorants are found in Patagonia, but not in the high numbers seen in other areas of the world. Colony numbers and sensitivity to disturbance vary widely between the populations so a great deal of care needs to be taken in the selection of sites for evaluation. The somewhat mixed species nature of colonies does allow some flexibility in choice of individuals for sampling to reduce disturbance. Sampling at one site in 1994 and 1995 has been conducted, but the identification of more safe sampling sites is still needed.

Guanaco (*Lama guanaco*): Guanaco have been thought to be abundant in areas where there is not heavy hunting pressure. Preliminary surveys conducted recently indicate that numbers are lower than reported. The overlap in habitat utilization of guanaco and domestic sheep (*Ovis aries*) indicates a need for sampling both of these species to establish the degree of shared diseases. A pilot project was conducted in 1995 to work out immobilization techniques and demonstrate the feasibility to officials. Twenty guanaco and 40 domestic sheep were sampled at one reserve.

Mara (*Dolichotis patagonum*) and European Hare (*Lepus capensis*): It has been noted that mara populations have declined in areas where the introduced European hare have become well established. This is assumed to be due to competition for resources, though no work has been conducted to compare infectious diseases or parasites of either species. Health evaluations of both species need to be conducted.

Grey Foxes (*Pseudalopex griseus*), Culpeo Foxes (*Pseudalopex culpaeus*), domestic dogs (*Canis familiaris*): It is well established that wild canids are susceptible to many of the same infectious diseases and parasites as domestic dogs. Little work has been conducted in Patagonia to evaluate the level of disease transmission between domestic and wild populations. Additionally, parasite life cycles between domestic dogs and domestic ungulates are well established and similar cycles are recognized in their wild counterparts in North
America and Europe. The disease interactions between sheep, dogs, guanacos, and foxes needs to be defined to establish levels of risk and areas of potential problems.

Sea Lions and Elephant Seals: Like many of the sea birds, sea lions and elephant seals can serve as valuable monitors of toxins and pathogens of the marine environment. Current handling for ecological research projects is providing the opportunity to collect samples for health evaluation. Post-mortem examination and sample collection has been initiated to take advantage of carcasses found on beaches.

Conclusion

The development of veterinary activities in conjunction with other conservation work in Patagonia is still at an early stage. In a relatively short period of time, the FVP has become involved in a conservation programming activities as well as invited to participate in developing the form of future efforts. Working closely with biologists, administrators, and government officials has resulted in the development of a program that meets their concerns and has enhanced their understanding of the role veterinarians can play. As a result of this year's activities, officials have asked for expanded activities next year. Integrating Argentinean expertise into the planning process has reinforced the long-term conservation effort in the region as well as ensuring that the veterinary projects are relevant to and timely for the issues at hand. Our success in this region will be measured by our ability to educate decision makers about relevant issues, provide access to all relevant data and findings, develop health status profiles of key wildlife populations, and enhance local capabilities.
THE REINTRODUCTION OF GRAY WOLVES, Canis lupus, INTO CENTRAL IDAHO AND YELLOWSTONE NATIONAL PARK: A MULTIDIMENSIONAL INTERNATIONAL CONSERVATION EFFORT

Steve Fritts, MS, PhD
U.S. Fish & Wildlife Service, Ecological Services, Helena, MT 59601, USA

Mark R. Johnson*, MS, DVM
Center for Resources, National Park Service, Yellowstone National Park, WY 82190, USA

David L. Hunter, DVM
Idaho Department of Fish and Game, Caldwell, ID 83605 USA

Teny J. Kreeger, MS, DVM, PhD
International Wildlife Veterinary Services, Inc., Cedar, MN 55011, USA

The wolf was common in the northern Rocky Mountain states prior to 1870, but persecution associated with increased human settlement greatly reduced their numbers. Wolf populations disappeared from the western U.S. by 1930 and in 1973, the Endangered Species Act classified wolves as endangered. Recovery plans for the wolf in the northern Rocky Mountains were completed in 1987 and in 1991, Congress directed the U.S. Fish & Wildlife Service (USFWS) to prepare a draft Environmental Impact Statement (EIS) for the reintroduction of the wolf into Yellowstone National Park (YNP) and central Idaho. On June 15, 1994, the Record of Decision was signed directing the reintroduction. Lawsuits delayed the capture operation for several weeks. Nonetheless, on January 4, 1995, the injunctions were denied and the process was allowed to continue. During the latter stages of the EIS, USFWS and National Park Service personnel gathered information and solicited opinions on all the technical aspects of a reintroduction program. A working document was prepared addressing technical subjects such as previous wolf reintroductions, capture methods, handling, transport, confinement and release, disease aspects, legal aspects, and animal welfare issues. It was decided that the wolves should come from populations sharing similar habitat and prey base as would the reintroduced wolves. The area around Hinton, Alberta was subsequently chosen as the capture site. The plan called for the capture of 30 wolves, 15 each to go to central Idaho and YNP. The wolves targeted for Idaho would be adults and animals of prime dispersal age. These animals would be 'hard' released; they would not be held for any time, but upon arrival at an appropriate wilderness site, be immediately released. In YNP, the emphasis was on capturing packs. The wolves would be held for 8-12 weeks and then released. It was thought that this method of 'soft' release held the highest probability of the wolves remaining in the area. Helicopter darting was chosen as the primary means of capture. Captured wolves were kept in temporary holding facilities located in Switzer Provincial Park outside of Hinton. Wolves were given a thorough physical examination and blood and tissue samples were taken for disease screening, health evaluation, and genetics. On January 11, 12 wolves were transported to the U.S. - 4 to Idaho and 8 to YNP. While en route, an injunction was imposed on the release of the wolves. The stay was lifted the following day and the 8 wolves in YNP were
immediately released from their transport cages into a 1-acre holding facility. The wolves bound for Idaho were not released until January 14 due to several weather-related delays. A week later, an additional 17 wolves were again airlifted to the U.S. with 6 scheduled for release in YNP and 11 for release in Idaho. All the wolves have since been released into the wild. The reintroduction of the wolf into Idaho and YNP was a major international success story, the repercussions of which still reverberate in state and federal courts, legislative bodies, the media, and ranches throughout the West.
Introduction

Yumka, the Center of Interpretation and Coexistence with Nature, represents the main means of promoting and implementing conservation strategies in the protected areas of Tabasco and the southeast of Mexico. The park is an ample, protected and effectively managed area, permitting the development of natural resource preservation investigations which can be observed and witnessed by a wide public, creating an ideal recreational and ecological learning experience. The total objective is to change the general public's attitude towards a harmonious coexistence with nature.

The site comprises 101 hectares (349.5 acres), declared as a protected area in 1984, as a Center of Interpretation and Coexistence with Nature. It is located within a buffered area of 1613 hectares (4,032.5 acres) in which human settlements and both public and private works have been prohibited.

Due to its composition, the reserve integrates three ecosystems: jungle, savanna and lagoons. A management program was designed in which education plays a fundamental role in favor of changing the general public's attitude towards the natural resources that still remain, and which tries to guarantee the conservation of the natural balance.

Considering the objectives of conservation, preservation, environmental education, investigation, ecotourism and recreation, a master plan for conservation and management of the Center of Interpretation and Coexistence with Nature was designed, which permits the conservation of the natural resources of the site and defines the profile of the human resources necessary for the accurate management of the Center. Tours through Yumka are closely controlled to avoid to the maximum the deterioration of the place and so that the visitor takes the conservation message that is given.

THE OBJECTIVES OF "YUMKA" ARE:

1). Effective conservation and preservation, as well as use and management of natural resources, by means of protective actions.
2). Environmental education designed to modify visitor's attitudes towards the environment - from changing their perception and understanding of the natural elements, to the use that is made of them, thus initiating a change in their view towards and ecological culture.
3). Investigations to generate knowledge that is useful to the state and the country in a global context, thus allowing adequate decisions to be made on the basis of technical and scientific facts.
4). Ecotourism designed to capture the tourist trade that enjoys visiting nature reserves, with minimum damage and contamination, to study, admire and enjoy the landscape, flora and fauna, as well as the remains of ancient cultures that are to be found in these areas.

5). Recreation to stimulate and encourage open air activities to improve and strengthen family bonds.

The fundamental necessity of conserving large areas in zones of high animal and botanical diversity in conjunction with the good results obtained by Yumka in the first 18 months of operations led to the initiation and implementation of seven complimentary projects as the next phase of the Yumka development. The proposed projects are the following: Ecotourism in Tabasco, Botanical Gardens, The Tabascan Typical Orchard, Visits to Yumka via the Grijalva River from the city of Culture, and Environmental Monitoring Station, Recuperation of the ecosystems neighboring Yumka and Enlargement of the Protected Natural Area of Yumka.

The Center of Interpretation and Coexistence with Nature Yumka in general has completed the first phase. It has been demonstrated that under a conservationist scheme a protected natural area can generate sufficient resources for its sustenance. In Yumka it has also been demonstrated the plausibility of the ecotourism strategy as an alternative to conservation. Being that educating and protecting is a profitable activity and at mid-term becomes self-sufficient to guarantee its permanency and development. Of the 70 full time employees, 20 are professionals from different areas of Mexico and the remaining are local farmers or fishermen who have been trained in the areas in which they work.

As an acknowledgment to the protected area of Yumka and the environment education programs, the Federal Government of Mexico has awarded the 1994 National Forestry and Wildlife Merit Award, in the wildlife education category to the Center of Interpretation and Coexistence with Nature Yumka.
Evaluation of immune function in non-domestic species has been hindered by lack of interpretable databases on normal immunologic parameters. Too often an assessment of impaired immune function is based on post-mortem findings of depleted lymphoid tissue and opportunistic organisms. Invariably, "stressors" are cited as predisposing factors. These include inbreeding, co-existing infections, and environmental factors ranging from inadequate diet and adverse weather to anthropogenic effects such as pollution and habitat loss. Prospective studies on immune function are limited by availability of suitable reagents and inappropriate numbers of samples for making meaningful conclusions. Too often assays are performed because they can be done rather than because they are appropriate. A mitogen lymphoproliferative (LP) assay is a good example. The test can be performed with lymphocytes from any species but results can be extremely variable making interpretation difficult. Furthermore, an individual can be severely immunocompromised before LP responses to mitogens are significantly depressed.  

Assessing compromised immune function is difficult. During the past decade, the National Toxicology Program (NTP) has been addressing this problem in order to determine if a chemical agent is immunotoxic for mice and concluded that only two to three immune tests were needed to predict whether a compound was immunotoxic. Histopathology and weights of lymphoid tissue as well as LP responses were not good predictors of immunotoxicity, whereas splenic cell antibody plaque formation and T lymphocyte cell surface marker analysis were. These latter two tests reflect humoral immunity (HI) and cell-mediated immunity (CMI), respectively. Clearly, harvesting spleens from non-domestic species to perform plaque forming assays is neither feasible nor desirable. Although some T lymphocyte cell surface marker analysis has been performed in non-domestic species, the ability to conduct such studies is dependent upon the cross-reactivity of antibodies that recognize characterized cell determinants in other species.

One approach for evaluating immune function that is currently receiving considerable attention is the measurement of cytokine production by cells of the immune system. Cytokine is a general designation which includes monokines (cytokines secreted by monocytes), lymphokines (cytokines secreted by lymphocytes), interleukins (IL), interferons (IFN), tumor necrosis factors, transforming growth factors, macrophage activating factors, and migration inhibition factors. Cytokines are regulatory proteins and glycoproteins that mediate cellular interactions in an immune response. They are typically not produced constitutively, production being regulated at the level of transcription or translation, and act in an autocrine or paracrine fashion.
The complex interaction between cytokines has recently become better understood with the recognition that certain cytokines promote cellular immunity, while others promote humoral immunity.\(^1\) Cell-mediated responses, defined as type 1 responses, are associated with normal or increased levels of IL-2, IL-12, and IFN-γ. Humoral immune functions, defined as type 2 responses, are associated with an increase in IL-4, IL-5, IL-6, IL-10, and/or IL-13. The outcome of an immune response to a given stimulus is currently believed to be dependent upon the predominant cytokine profile. For instance in humans, progressive lesions in cutaneous *Mycobacterium leprae* are associated with type 2 cytokines, IL-4 and IL-10, while resolving lesions are associated with the expression of type 1 cytokines, IL-2 and IFN-γ.\(^8\)

As cytokines play a key role in immune responses, it is not surprising that there should be evidence for their existence (or functionally equivalent analogues) in phylogenetically diverse species. Cytokine activity can be measured by bioassays using human and murine systems. Although species cross-reactivities occur for some cytokines, results are often confusing and difficult to interpret. Cytokine protein levels can be measured by ELISA, but the monoclonal antibodies developed for humans and mice have minimal species cross-reactivity. Finally, cytokine mRNA can be measured as a reflection of cytokine protein production.

Following this premise, consensus nucleic acid sequence primers for type 1 and type 2 cytokines were made for use in reverse transcription quantitative competitive polymerase chain reactions (RT-qPCR).\(^6,7\) Briefly, total RNA is extracted from unstimulated and stimulated lymphocytes. The RNA is reverse transcribed to yield cDNA. A consistent amount of cDNA is added to serial dilutions of a competitive DNA fragment that consists of a linear array of the consensus sequence primers for cytokine genes upstream and their complementary sequences downstream. This reaction mixture is used for PCR to amplify the amount of cDNA specific for each cytokine mRNA. A housekeeping gene (G3PDH) which is expressed at a relatively constant rate independent of the activation state of a cell is included on the competitive fragment to control for sample to sample variation in both the RT reaction and the PCR reaction. This allows comparison of relative mRNA expression levels (cytokine:G3PDH) between samples.

Following this protocol, we have begun to look at the relative amounts of cytokine mRNA's from a variety of domestic (cat, dog, horse, cow, and pig)\(^7\) and non-domestic (cheetah, harbor seal, and hybrid striped bass) species. Consensus sequence primers for IL-2, IL-4, IL-6, IL-10, IL-12, and IFN-γ have been successful in higher vertebrates while the primers for IL-10, IL-12, and IFN-γ show promise for a variety of fish species. The ability to utilize one technique to measure cytokine expression in a variety of species is a potentially powerful tool for evaluating the impact of limited genetic diversity, infectious diseases, and environmental contaminants on animal populations.
LITERATURE CITED

MANAGEMENT OF BLACK (*Diceros bicornis*) AND WHITE (*Ceratotherium simum*) RHINOCEROS IN ZIMBABWE: VETERINARY IMPLICATIONS

*Veterinary Unit, Department of National Parks and Wildlife Management, PO Box CY140, Causeway, Harare, Zimbabwe*

In Zimbabwe, during the period 1991 to 1994, 586 immobilizations of both white (*Ceratotherium simum*) (n=179) and black (*Diceros bicornis*) (n=407) rhinoceroses were carried out. Of these, more than 400 animals were dehorned as part of a management program to reduce the incentive to illegal hunters entering Zimbabwe from Zambia. Further attempts to protect the rhino have been made by the establishment of Intensive Protection Zones (IPZs) and the translocation of rhino into them, from vulnerable areas in the country.

In the past three years, immobilization and monitoring techniques and standards of boma confinement have been refined and improved considerably. Modifications in drugs dosages and combinations have resulted in faster and smoother induction times with a reduction in stress and capture related complications. Capture related mortality in black rhino is <1%. Black rhinos being transported or confined in a boma are routinely administered long acting neuroleptics (LAN) such as Clopoxol-acuphase. Naltrexone is used in preference to diprenorphine for the antagonism of etorphine. An Intensive Management Center (IMC) for rhino and other wild animal species has been established in Zimbabwe and research into nutrition and immobilizing and therapeutic drug pharmacokinetics involving black rhinos is continuing.

Advances have been made in white rhino immobilization with new drug combinations providing exceptional quality of anesthesia. A protocol has been developed for the optimum reversal of these drug combinations in anesthetized animals. Data collected during the 1994 reimmobilizations have provided much information on the reproductive status of both black and white rhino populations as well as information on horn regrowth, home ranges and movement and dispersal.

Calf survivorship for dehorned rhino, even in areas of high predator density, appears to be high. Of seven black rhino cow/calf pairs (calves <6 months old) immobilized in Hwange National Park in 1992, five were again located in 1994. Two pairs were not located and are presumed to have been poached. This represents a calf survival rate of up to 100%. A further 10 cow/calf pairs (calves 6 months to 3 years old when immobilized in 1992) from the same area, showed a >70% calf survival rate.

During the 1994 operation, four new calves were located. One calf was born approximately one week after its mother was immobilized and radio collared. The ages of these cow/calf combinations varied from 1 week to 3 months. Most mothers have radio-collars fitted and will be followed closely to determine calf survival.
Dehorning is an effective conservation strategy, with little or no effect on the health and behavior of the rhinos. Evidence from the various dehorning operations in IPZ’s in Zimbabwe suggest that the impacts on adult rhino are minimal, calf losses are sustainable and the overall effects of dehorning may be significantly position. However, in order for it to be successful it needs to be supported by aggressive law-enforcement. It is a controversial management option but the dehorning operations have allowed considerable data to be accumulated on its deterrent effect, rhino demography, immobilizations, horn size, horn regrowth and veterinary-related problems.
HEALTH IMPLICATIONS OF TRANSLOCATIONS OF ENDANGERED SPECIES IN AFRICA: TRYPANOSOMIASIS IN RHINOCEROS

Steve Mihok, PhD
International Centre of Insect Physiology and Ecology, P.O. Box 30772, Nairobi, Kenya

Richard Kock, MRCVS
Kenya Wildlife Service, P.O. Box 40241, Nairobi, Kenya

Rachel Masake, PhD
International Livestock Research Institute, P.O. Box 30709, Nairobi, Kenya

Areas such as Tsavo National Park in Kenya that once harboured many black rhinoceros are now being restocked with surplus animals from a variety of sanctuaries established in the 1980's. Many of the roughly 400 black rhinoceros remaining in Kenya are presently living in sanctuaries in the highlands where tsetse flies are not present; in contrast, areas receiving rhinos are mostly in the lowlands where tsetse flies and trypanosomiasis are prevalent. The disease implications of these movements have been studied since 1989 following concerns resulting from the death of a translocated rhino at Ngulia in Tsavo West National Park. The rhino may have died as a result of complications arising from the acquisition of a Trypanosoma brucei infection. This case prompted detailed studies of tsetse and trypanosome epizootiology at Ngulia in conjunction with health monitoring of translocated rhino. Initial epidemiological surveys indicated this animal was moved to an area with a high tsetse density at a time of the year when both density and infection rates in flies were at a peak. Subsequent introductions were therefore planned to avoid tsetse challenge as much as possible by relocating the bomas to a more appropriate area. Within logistical constraints relating to rainfall and availability of green vegetation for feeding animals during confinement, subsequent translocations have been undertaken to minimise disease risks. This strategy has resulted in the acquisition of very few patent infections and no further deaths. To date rhinos have survived infections of T. congolense, T. vivax and a new genotype of Nannomonas parasite without the need for chemotherapy.

Although serious problems have not occurred, hematological data have revealed indications of stress in terms of moderate anemia, depressed lymphocyte and platelet counts and elevated neutrophil counts a few weeks after translocation. These effects have occurred mostly in animals moved long distances in highland to lowland translocations. Changes in blood parameters have also been pronounced in the few animals that acquired trypanosome infections, but it has been difficult to attribute causes to any single factor. Diagnostic information from antigen-ELISA tests for trypanosome antigens have also complicated a simple interpretation of conventional parasitological data. These results have suggested most animals harbour cryptic infections, particularly of T. brucei, and hence current routine diagnostic techniques only pick up parasites during an acute phase of parasitaemia.

Recently, we have also started to monitor trypanosomiasis in white rhinoceros. A small number of animals were moved from South Africa to Kenya in the early 1970's to two areas...
(Solio Ranch - without tsetse) and Meru National Park (with tsetse). The Meru animals did poorly. The Solio animals thrived. To test the effects of trypanosome infection on white rhino, two Kenyan-born animals were moved from an area without tsetse to the Maasai Mara (with tsetse) in 1992. Challenge was minimised on introduction by reducing tsetse numbers by about 80% with odour-baited traps near the bomas where the animals were confined for a few months. The rhinos were then allowed to roam freely. At six months, they were both diagnosed with chronic *T. brucei* infections accompanied by mild anemia. After two years, they were still alive, apparently healthy, and had self-cured any infections. After this initial positive result, ten white rhinos were introduced directly to the Mara from South Africa in September 1994. Unfortunately, tsetse densities increased dramatically shortly after introduction due to heavy rainfall. A few months later nearly all animals were diagnosed with active or chronic *T. brucei* infections. One animal died after a few months and another had to be treated with trypanocides. Other animals moved at the same time to Nakuru National Park (without tsetse) fared well. Although the interpretation and outcomes of these two introductions from South Africa are not final, it is clear that the white rhino is susceptible to trypanosomiasis at the time of translocation. Also, it is clear that the white rhino is a particularly good wildlife host for *T. brucei*. These results have serious implications for management plans for moving white rhinos into and out of areas with human sleeping sickness (such as the Serengeti-Mara ecosystem). Since we know very little about the efficacy of trypanocides in both black and white rhino, there are real dangers of inadvertently introducing human-infective forms of *T. brucei* to new areas when animals are moved.
THE CONTROL OF AN ANTHRAX OUTBREAK IN THE KRUGER NATIONAL PARK

Jacobus P. Raath, BVSc
Kruger National Park, Private Bag X 402, Skukuza, 1350 South Africa

The Kruger National Park (KNP) has an endemic anthrax area in the far northern Pafuri area. This came about when the flow pattern of the Luvuvhu river was altered, leading to the establishment of a pan into which the surrounding areas drain. The pan is no longer washed clean by annual floods, hence anthrax spores that drain down into the pan from the higher lying areas remain and increase in number. Towards the end of the dry season, the pan dries and the last bit of water, highly contaminated with spores, is utilized by the game. This results in their death and subsequent recontamination of the pan.

In the conservation of undisturbed ecosystems it is essential to retain intact functional and dynamic processes. Indigenous parasites and diseases contribute to biological diversity and represent an integral part of the biotic community of the KNP. In a healthy natural ecosystem such as the KNP, a dynamic balance is maintained between the environment, the hosts and the parasites. Disease normally manifests itself when one or more of these components are changed, i.e. a new host or parasite is introduced, or a drought changes the environment. With this as background the control of anthrax in a balanced ecosystem such as the KNP can be questioned. However, considering the socioeconomic and zoo-sanitary interests beyond the borders of the KNP we abide by all prescribed procedures to contain notifiable diseases including anthrax.

The KNP has implemented an early warning system whereby rangers make blood smears of all carcasses encountered in the veld. This is, together with an info pamphlet, sent down weekly to the laboratory at Skukuza where all smears are scanned for anthrax.

Once an outbreak has been detected, there is a fivefold strategy for monitoring and controlling the disease.

Firstly, our neighboring communities are informed through our state veterinary offices and our local forums. This allows farmers ample time to vaccinate livestock and game and to inform people not to utilize any fresh carcasses found.

A mobile laboratory is set up in the area of the outbreak, complete with microscopes, freezing facilities and large scale maps of the area. Four to five teams are activated as field personnel. Daily searches for carcasses are instituted using vultures as indicators. The helicopter is used in cases of severe outbreaks. A blood smear and a culture sample is taken from all carcasses and the carcasses are burned using fivefold and wood. The smears are investigated every night and all positive cases are plotted on the map.

All artificial waterholes in the area are sampled and drained at regular intervals. Special attention is given to the sludge at the bottom, as anthrax spores have a fast sedimentation
time. A 40% formalin solution is thoroughly mixed into the sludge before it is pumped out. The watertroughs are cleaned and washed with a 10% solution of formalin and left to dry for three days before being refilled.

Vaccination of the rarer species such as roan antelope takes place using a freeze dried Stem vaccine. The vaccine is pelleted in Skukuza and loaded into the cellulose capsules of the biobullets of the Ballistivet System. Vaccination is done by two marksmen sitting in the back of a Bell Jet Ranger helicopter.

Once completed, all site information is fed into our GIS program to allow complete analyses and plotting of data.

Future developments include the use of GPS systems in carcass plotting, the use of infrared cameras from helicopters to detect carcasses, phasing out specific artificial water points, and investigations into the development of an oral vaccine to be dissolved in water.
ANTIBODY RESPONSES OF RED WOLVES TO CANINE DISTEMPER VIRUS AND CANINE PARVOVIRUS VACCINATION

Lisa Harrenstien, DVM,* Linda Munson, DVM, PhD
Department of Pathology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901, USA

Christopher F. Luesch, BS
Red Wolf Recovery Project, U. S. Fish and Wildlife Service, Townsend, TN 37882, USA

Edward C. Ramsay, DVM, Stephen A. Kania, MS, PhD, Leon N. D. Potgieter, BVSc, MS, PhD
Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901, USA

Canine distemper virus (CDV) and canine parvovirus type-2 (CPV2) have caused disease and death in many species of carnivores, including the red wolf (Canis rufus). Protection of non-domestic carnivores by vaccination against these viruses has been problematic, however, due to variable serologic responses to vaccines, vaccine-induced disease (most often associated with use of modified-live-virus [MLV] CDV products of canine cell line origin), and suspicion of transient immunosuppression after use of multivalent MLV vaccine products. This study was developed in order to assess whether substantial CDV and CPV2 titers could be produced in red wolf pups and adults using (1) MLV CDV vaccine of avian cell line origin and MLV CPV2 vaccine of mammalian cell line origin, administered concurrently, (2) MLV CDV vaccine of avian cell line origin and MLV CPV2 vaccine of mammalian cell line origin, administered alternating weeks, and (3) multivalent MLV vaccine of mammalian cell line origin.

Twenty captive red wolves, including 16 housed by the Red Wolf Reintroduction Program of the U. S. Fish and Wildlife Service, Great Smoky Mountains National Park, Cades Cove, TN, and 4 housed at the Knoxville Zoological Gardens, Inc., Knoxville, TN, were included in this study. Wolves were vaccinated with MLV CDV and CPV2 (FrommDR CDV vaccine, Solvay Animal Health, Mendota, MN, and DuramuneR KF-11 CPV2 vaccine, Fort Dodge Laboratories, Fort Dodge, IA); adult wolves had been previously vaccinated with multivalent MLV vaccine (DuramuneR DAzP+PV+Leptospira, Fort Dodge Laboratories, Fort Dodge, IA). Sera were collected, and immunofluorescent staining (IFA) was performed for determination of CDV IgM, CDV IgG, and CPV2 IgG titers. A capture ELISA was performed for validation purposes, to confirm the reactivity of our standard diagnostic reagents with red wolf serum.

This study demonstrates that red wolves are capable of producing an antibody response after vaccination with commercial canine products in all vaccination schedules of this study, and that this response can be assayed using tests developed for domestic dogs. Low level exposure to wild type virus (due to contact with feral mammals) cannot be excluded, however, as a possible additional stimulus for antibody production. Titers varied widely among individual wolves. No consistent correlation could be detected in individual wolves for their degree of response to CDV versus CPV2 vaccination. Mean post-vaccination titers for CDV IgG were above those generally considered "protective" for domestic dogs, even
when measured more than one year after vaccination. Mean post-vaccination titers for CPV2 IgG were lower than CDV titers, however, and were lower than the level generally considered protective for domestic dogs. Further studies are needed to assess the protection afforded by measured titers, and to determine the effects of concurrent CDV/CPV2 vaccination on immune function in red wolves.
MORTALITY IN THE ENDANGERED EASTERN BARRED BANDICOOT (Perameles gunnii) FROM 1990-1994

Rosemary Booth, BVSc*
Currumbin Sanctuary, Tomewin St, Currumbin, Queensland, 4223, Australia

Helen McCracken, BVSc, BSc(Vet), MVSc
Melbourne Zoo, PO Box 74, Parkville, Melbourne, Victoria, 3052, Australia

John Seebeck, BSc
Arthur Rylah Institute, Department of Conservation and Natural Resources, 123 Brown St, Heidelberg, Victoria, 3084, Australia

As part of the Management Plan for the endangered Eastern Barred Bandicoot, Perameles gunnii, a review of the causes of mortality for the species in the wild and in captivity has been carried out. Post mortem records from zoological institutions and from veterinarians receiving wild P. gunnii cadavers were collated and summarized according to aetiology.

Trauma was found to be the most significant cause of mortality in P. gunnii, accounting for 66% of recorded deaths. Trapping trauma during population monitoring exercises caused a 0.6% mortality rate of the 3758 bandicoots trapped and contributed to 25% of the deaths in this study. Trauma due to predation contributed to 21% of the recorded mortality with Red Foxes (Vulpes vulpes) being implicated as the most significant predator. Miscellaneous trauma and car trauma contributed (16%) and (4%) respectively, to the recorded mortality.

Infectious diseases, most commonly bacterial septicemias, accounted for 13% of mortality. Toxoplasmosis was the primary cause of death in 3% of the animals autopsied. The significance of this disease to the wild population is being further investigated.

Management options for reducing mortality in wild and captive populations include implementation of strategic predator control programs, modification of trapping protocols and emphasis on maintaining experienced staff.
DESIGN AND APPLICATIONS OF A "FREE-STALL" CHUTE FOR PASSIVE RESTRAINT OF THE NON-SEDATED WHITE RHINOCEROS (*Ceratotherium simum simum*)

Robin W. Radcliffe, DVM•, Steven A. Osofsky, DVM, and Adam I. Eyres, BA
Animal Health Services, Fossil Rim Wildlife Center, P.O. Box 2189, Glen Rose, Texas 76043, USA

In most instances, the large size and unpredictable nature of the rhinoceros mandates some form of chemical restraint to permit hands-on evaluation of the animal for medical or research purposes. Risks are always present whenever an animal undergoes sedation or anesthesia, and the risks of serial immobilizations for research purposes are obviously to be avoided. A variety of chute styles have been designed to facilitate handling and restraint of rhinoceros species, but uncooperative animals can be difficult to evaluate even in these enclosed structures.

A novel approach to the safe restraint of untranquilized white rhinoceros has been implemented at the Fossil Rim Wildlife Center. The system employed incorporates a "free-stall" chute design analogous to that used in dairy barns, with some modifications for use in these large, extremely strong mammals. The purpose of this chute design is to allow the rhinoceros to *choose* its own response to a situation, an option not available when utilizing more conventional restraint via a closed chute or chemical immobilization. While this design by nature introduces new challenges for personnel, the benefits of a nonstressful restraint situation far outweigh the lack of total restraint a free-stall by definition provides.

The need to develop a *nonstressful* method for the reproductive evaluation of the female white rhinoceros was the driving force behind the design of this chute. The idea stemmed from early work with transrectal ultrasonography of the female white rhino in a closed chute situation in which three of four females were successfully scanned, although stress was a factor in repeatability of the procedure in most animals. The rectal exam itself went practically unnoticed on most days. The four-wall closed chute restraint, however, was a common source of subject anxiety. The fourth female would enter the chute, but would react aggressively when enclosed completely despite the reward of sweet feed. Multiple attempts at conditioning this female to the closed chute design failed. Thus, with the realization that the rectal exam was tolerated far better than the act of restraint, the free-stall design concept was developed.

The results obtained to date have proven remarkable considering the fact that this female was, prior to the application of the free-stall, an uncooperative subject for the reproductive ultrasound research undertaken at Fossil Rim. In our attempts to elucidate the normal reproductive biology of these large Perissodactylids, this design has allowed for thorough daily to every-other-day evaluations of the female rhinoceros. Over the course of several weeks, the formerly intractable female became accustomed to feeding in the free-stall, allowing successful application of daily transrectal ultrasonography. The free-stall chute is located within the holding pen at the rhino barn as illustrated in Figure 1. The rhinos are kept out of the chute via a swinging gate when not in use. When the gate is opened, the
female rhino can enter the chute and eat a mixture of sweet feed and alfalfa hay, with additional feed being given as needed during a procedure. The design incorporates a 24 inch wide, 7 foot tall section of vertical pipe that extends into the chute from the right-hand side and allows the researcher safe access to the caudal end of the rhinoceros as well as a safe exit outside the pen if the rhino backs out of the chute (see Figure 2 and Figure 3). This wall provides the examiner with a "safe" area from which he/she can perform rectal palpation and ultrasonography. This design also readily facilitates routine medical examinations and minor procedures such as blood collection.

If used properly, the potential disadvantages of this type of restraint in the rhinoceros are minimal. Obviously, safety of personnel is of prime concern. The fact that the veterinarian is literally in the same enclosure with these large and sometimes unpredictable mammals adds some element of risk not associated with other methods; the design described here attempts to minimize that risk. The ability of the rhino to back out of the chute at any time suggests that the application of moderately invasive techniques such as transrectal ultrasonography would prove difficult, if not impossible. However, the adaptation of this method of restraint to transrectal ultrasonography in the white rhinoceros actually has proven beneficial. We have found that a rhinoceros that can choose between entering or leaving the chute soon becomes confident with this arrangement and allows more intensive manipulation. The absence of complete confinement makes for a calmer research subject, with less likelihood of self-inflicted trauma.

The elimination of stress as a variable in the behavior of the rhinos is beneficial in at least two important ways. Firstly, stress with its associated release of corticosteroids can adversely affect the steroid hormone profile and thus interfere with a thorough evaluation of basic reproductive biology in these species. Secondly, only when the entire handling protocol enhances overall efforts at positive reinforcement will the rhinoceros subjects continue to return to the chute. This aspect of conditioning is crucial to projects such as transrectal reproductive ultrasound where serial monitoring over time is fundamental to understanding normal biology.

The ability to monitor large, nondomestic species like the rhinoceros in a nonstressful manner has proven valuable in the consistent daily to every-other-day evaluation of reproductive events. The free-stall approach, although not ideal for all situations, can provide a unique alternative to more conventional chute restraint or chemical immobilization in a variety of contexts.

ACKNOWLEDGMENTS

The authors would like to thank the following individuals for their contributions. Christine Juryzkowski and Jim Jackson for support and encouragement in efforts to learn more about the rhinoceros. Bruce Williams for input into chute design and his enthusiasm for this research. Rodney Marsh and Kelley Snodgrass for help with animal handling and ultrasound control manipulation. Meg Bommarito for all of her help as Fossil Rim’s first rhino intern, and all of the interns that have contributed to this project.
Figure 1. Female holding pens in white rhino barn, Fossil Rim Wildlife Center.

Outdoor Rhino Pen (50' x 32')

Indoor Rhino Pen (17' x 44')

Free-stall Chute

Exit

See Figures 2 & 3 for details of the Free-stall Chute

Rhino Barn

Alley to pasture

Lane to 10 acre pasture

(40' x 54')

(17' x 44')

(50' x 32')

Drawing not to scale
Figure 2.  
*Free-Stall Chute Design* 

*top view*

- Scale: 1" = 2'
- Vertical pipes at corners = 4 & 1/2"
- Horizontal pipes = 2 & 7/8"
- Vertical pipes = 2 & 7/8"
- Total height = 7'/2"
Figure 3.

- Free-Stall Chute Design -
  right side view

(rhino has access to this 11' area)
LAPAROSCOPIC VASECTOMY: A SIMPLE TECHNIQUE FOR STERILIZATION OF THE MALE CHEETAH (*Acinonyx jubatus*)

Robin W. Radcliffe, DVM* and Steven A. Osofsky, DVM
Animal Health Services, Fossil Rim Wildlife Center, P.O. Box 2189, Glen Rose, Texas 76043, USA

Douglas Decker, MD
Texas Primary Care Associates, 1325 Pennsylvania Avenue, Fort Worth, Texas 76104, USA

Barbara A. Wolfe, DVM, PhD and Mitchell Bush, DVM
National Zoological Park, Center for Research and Conservation, 1500 Remount Road, Front Royal, Virginia 22630, USA

The Fossil Rim Wildlife Center has been fortunate in its development of one of the most successful cheetah breeding programs in North America. Sterilization of a male cheetah would not have been contemplated a mere decade ago when most zoological facilities were faced with the basic problems surrounding the establishment and maintenance of viable groups of captive cheetah. The fact that laparoscopic vasectomies were performed at all is a compliment to the cheetah SSP as well as to the management approaches succeeding at a variety of institutions.

A simple laparoscopic technique to vasectomize male cheetah is described here: this procedure is applicable in any species of felid for permanent sterilization. The decision to permanently sterilize an individual will of course depend on consultation with SSP advisors and the development of an appropriate set of criteria to evaluate the animal's mean kinship relationships. It may also be appropriate to cryopreserve sperm from the animal for genome banking prior to the surgery.

The use of laparoscopic surgery in humans has become commonplace, with laparoscopic techniques frequently replacing conventional approaches previously requiring major abdominal incisions. In zoological medicine, laparoscopy has been utilized as a diagnostic, surgical, management, and research tool in a variety of species for over a decade. The laparoscopic vasectomy technique has been used sporadically in lieu of open surgical vasectomy in several domestic and non-domestic species.1,3

The laparoscopic procedure is relatively simple. The patient is anesthetized, intubated, and maintained on an inhalation anesthetic. The animal is placed in dorsal recumbency with its caudal end elevated approximately 30 degrees via a tilt table. The patient is closely monitored using physical observations of respiratory rate and depth, pulse rate and quality, and mucous membrane color. Additional monitoring should include pulse oximetry, indirect blood pressure measurement, as well as rectal temperature monitoring. The ventral abdomen is clipped, prepared, and draped for sterile surgery. A 10 cm Verres needle is inserted through the abdominal wall, approximately 10 cm cranial to the pelvic brim along the ventral midline, and the animal is insufflated via the Verres needle with CO₂ at conventional pressure. A 1 to 2 cm skin incision is made on the ventral midline
approximately 2 to 4 cm caudal to the umbilicus, allowing for introduction of the laparoscope-cannula assembly. The inguinal ring is identified. The Verres needle is useful in elevating and isolating the vas deferens from the spermatic artery and vein (located laterally, descending into the inguinal canal). The vas is identified by observing it emerging from the internal inguinal ring. It has two small vessels (the deferent artery and vein) associated with it. It is important not to confuse the ureter for the vas, the ureter being thin-walled and parallel to the long axis of the body. A secondary trocar-cannula assembly is similarly inserted, approximately 10 cm caudolateral to the laparoscope, allowing for insertion of instruments needed for the various procedures.

Once the vas has been isolated and identified there are several methods available for the vasectomy which include:

1) Transection of the vas: this technique should be combined with cautery and/or some form of ligation to prevent sperm leakage and the formation of an intra-abdominal sperm granuloma.

2) Cautery of the vas: this may be adequate, but it may be more prone to complications such as adhesion formation and/or recanalization of the vas.

3) Double ligation of the vas: this procedure can be performed with surgical ligatures or metal clips. This technique can be followed by transection and/or cautery of the vas between the ligation sites, although this may be unnecessary.

For this project, vasectomies were performed on five cheetahs via the following four procedures, combinations of several of the above techniques:

- Procedure 1 -- cautery of the vas with no transection.
- Procedure 2 -- ligation, cautery, and transection.
- Procedure 3 -- ligation and cautery of the vas.
- Procedure 4 -- two metal clips on the vas.

Seven weeks post-operatively, two cheetahs were reevaluated by electroejaculation and laparoscopic visualization. The first cheetah had one vas ligated and cauterized (procedure 3) and the other vas transected, ligated and cauterized (procedure 2). Ligation prior to transection is preferable to minimize the chances of losing either end of the vas as it retracts. Equipment failure in this case had led to transection prior to ligation, but both ends of the vas had been successfully located. This cat produced no sperm by electroejaculation and the surgical sites demonstrated only minimal tissue reaction and minor adhesions to the mesoductus. This cat was deemed sterile. The second cheetah had both vasa double ligated with metal clips only (procedure 4). Less than 1,000 dead sperm were collected by electroejaculation, and these were considered to be residual. On laparoscopic examination, this cheetah had no significant tissue reaction or changes around the surgical sites. This cat was also deemed sterile.
At present, we consider the method of choice to be a double ligation of the vas using metal clips, and there is probably no indication for additionally transecting or cauterizing the vas. Recanalization seems unlikely. The tool used for this procedure was the Ethicon Ligaclip ERCA with med/large titanium staples (Ethicon Ltd, 1421 Lansdowne St. W, Peterborough, Ontario, Canada K9J 7B9).

The advantages of this laparoscopic technique are:

1) Reliable permanent sterilization;
2) No skin incision around the scrotal area which could become irritated and inflamed by licking;
3) The opportunity to examine and, if necessary, biopsy other internal organs of interest such as the liver and kidney during the same anesthetic episode;
4) A rapid procedure with low morbidity.

LITERATURE CITED

ASSISTED BREEDING AT TARONGA AND WESTERN PLAINS ZOOS

LG. Russ, BVSc*, BAgr, MACVSc, L. Vogelnest, BVSc, MVS, F. Hulst, BVSc, MVS
Taronga Zoo, PO Box 20, Mosman, New South Wales, 2088, Australia

Western Plains Zoo, Obley Road, Dubbo, New South Wales, 2830, Australia

Introduction

The preservation of endangered wildlife is a critical function of modern Zoos. Taronga Zoo (TZ) and Western Plains Zoo (WPZ) have developed an Assisted Breeding (AB) Program for the preservation of many endangered species such as Black Rhino Diceros bicornis, Clouded Leopard Pardofelis nebulosa, Sumatran Tiger Panthera tigris sumatrae, Komodo Dragon Varanus komodoensis, Malleefowl Leipoa ocellata, Yellow-footed Rock Wallaby Petrogale x xanthopus, Short-beaked Echidna Tachyglossus aculeatus and Platypus Ornithorhynchus anatinus.

A collaborative team dedicated to AB involves experts from TZ and WPZ, Monash University, University of Sydney, University of Queensland, USA, South Africa and veterinary specialists.

Case Report

Introduction

Clouded Leopards Pardofelis nebulosa have a low reproductive success rate both in the wild and captivity, often as low as 25%. TZ has a male and female Clouded Leopard, both aged five years. They have been housed together for the past four years without achieving a viable pregnancy. This report discusses the reproductive investigation and procedures adopted with assisted breeding in the Clouded Leopard.

Background

In January 1994 the male and female Clouded Leopard were transferred to Taronga Zoo from Henry Doorly Zoo. The animals were housed together and had no significant medical problems. In May 1994 both animals underwent full health checks which did not reveal any significant abnormalities. The male was electroejaculated producing 0.75ml of semen with a high percentage of live sperm, but also many abnormal sperm.

Eight months later the female was given another full examination. The haemogram, biochemistry panel and urinalysis were unremarkable. However, free T4 was 5.2nmol/l which was considered low. Thyroxine 200mcg twice daily therapy was given orally. Vaginal cytology and serum oestrogen levels were consistent with oestrus and remained unchanged for a further six weeks.
Two months later the female was given 500 IU of Chorulon intramuscularly. The following day the male and female were anaesthetised with Zoletil and maintained with Isoflurane and oxygen. The male was electroejaculated producing semen that had 50% motility and 30% abnormals. The semen was extended in Hams F10 media. The female was given a further 500 IU of Chorulon intramuscularly and artificially inseminated using the transvaginal route. Progesterone assays were performed on saliva and were consistently greater than 25mmol/l over the next month.

Two months post-insemination the female was anaesthetised and underwent an ultrasound examination which revealed that she was not pregnant. Saliva progesterone assay was 26mmol/l. Subsequent hormone assays revealed a saliva progesterone of 24mmol/l, serum progesterone of 4mmol/l and a free T4 of 32.3nmol/l.

Three months later the female was separated from the male and she was given 500 IU of Chorulon intramuscularly. 48 hours later the male and female were anaesthetised. The male was electroejaculated and the female given a further 500 IU of Chorulon. Laparoscopy was performed through the ventral midline with 0.1ml of diluted semen being injected into the proximal one-third of each uterine horn.

Discussion

The failure to achieve a viable pregnancy in the Clouded Leopard may be attributed to a number of factors. The female has a nervous temperament which makes natural mating unlikely to occur. In the wild they are usually solitary animals and it is debatable as to whether housing them together influences reproduction. The possible hypothyroid condition of the female may also impact on fertility. The persistence of oestrus for six weeks may indicate ovarian follicle abnormalities.

The discrepancy between serum and saliva progesterone assays will be the subject of further studies. It is desirable to refine saliva, faecal or urine progesterone assays for convenience of sampling.

The transvaginal AI was not successful and the subsequent AI was performed intrauterine via laparoscopy, which is generally considered to be the more successful technique.

Conclusion

Establishing a viable pregnancy in Clouded Leopards is difficult. Further studies are being undertaken on oestrus detection, semen storage, hormone assays, behaviour, AI techniques and timing, and IVF to ensure the preservation of Clouded Leopards and other endangered species.
Outline of Assisted Breeding Program

The AB program at TZ and WPZ has a clearly defined action plan. As a basis for each species an assessment of individual biology, blood and urine parameters, nutrition, behaviour and genetics will often be undertaken. Hormone assays are performed and may include GnRH, Thyroxine, Cortisol, Prolactian, Progesterone, and Oestrogen.

The collection and storage of sperm and assessment of semen quality are the priorities in males. The collection of sperm using techniques such as electroejaculation, fine needle aspirate of the epididymis, and retrieval from testes of dead animals are all begin further developed and refined. In these techniques human technology is being used as the model.

Sperm storage is refined in many domestic animals whereas in many zoo animals there is scope for further development. Cryobanking and recovery of viable sperm is critical. The program will pursue cryobanking, sperm membrane biology and sperm cryosurvival, seasonal and hormonal effects on sperm production, and the optimal time for sperm collection, and the storage of testes of dead animals.

The ability to determine and characterise the oestrus cycle of each female accurately is vital to ensure optimal timing for mating or insemination. Oestrus detection utilising behavioural assessment, hormonal assays, vaginal cytology, and ultrasound examinations are regularly undertaken. In the Black Rhino Diceros bicornis, close monitoring has ensured accurate determination of their oestrus cycle. Black Rhino have been trained to accept ultrasound examination as a routine procedure.

Hormone assays are being refined for many species. In Black Rhino and the Felids assays are being performed on blood, urine, faeces and saliva which can detect very low levels of hormones. The technique of using faeces, saliva or urine avoids the need for venipuncture and provides a simple means of frequent assaying.

The collection of oocytes (from live or dead animals) and their storage is an integral function of the work. The harvesting and storage of embryos and their transfer to a recipient will enhance reproductive success, reduce the need to transport animals around the world and lessen the risk of disease transfer from one country to another.

Artificial Insemination (AI) techniques and timing are quite variable for individual animals. The precise site for intra-uterine placement of sperm in AI varies with each species. The Black Rhino may be inseminated using the transvaginal route, whereas many Felids achieve a higher success rate with intrauterine insemination performed by laparoscope. Marsupials require further detailed investigation as to the most desirable location within the uterus and the appropriate timing for AI.

The in-vitro fertilisation (IVF) of oocytes particularly in Black Rhino is being developed using techniques adapted from humans. This technique which is used successfully in humans
will ensure Black Rhino are given every possible chance of survival. The IVF team at Monash University will lead this unique program on Black Rhino and other species.

To ensure the dissemination of information a database for stored reproduction material will be established on ISIS and MEDARKS.

Animal Gene Storage Resource Centre of Australia

The AGSRCA is a unique joint initiative of the Zoological Parks Board of NSW and the Institute of Reproduction and Development of Monash University. The Gene Storage Centre will collect, store and preserve genetic resources of many endangered Australian native and exotic species.

The AGSRCA will serve the following functions:

- a reduction in the number of animals in breeding colonies while preserving the high level of genetic diversity
- the preservation of endangered species
- oocyte collection, IVM, IVF, AI and ET
- obtain genetic resources from wild populations whilst allowing the animals to remain in their natural habitat
- reducing the cost and risk of transporting animals around the world
- reducing the risk of transfer of disease.

Proposed List of Animal Species for AB

1. Native marsupials and monotreme
   Koala Phascolarctos c. cinereus
   Short-beaked Echidna Tachyglossus a. aculeatus
   Yellow-footed Rock Wallaby Petrogale x. xanthopus
   Northern Hairy-nosed Wombat Lasiorhinus kreffitii
   Platypus Ornithorhynchus anatinus
   Bilby Macrotis lagotis
   Rufous Hare-Wallaby Lagorchestes h. hirsutus

2. Exotics
   Black Rhino Diceros bicornis
   Clouded Leopard Pardofelis nebulosa
   Snow Leopard Panthera uncia
   Sumatran Tiger Panthera tigris sumatrae
   Cheetah Acinonyx jubatus
   Golden Lion Tamarin Leontopithecus rosalia rosalia
3. **Birds**
   - Malleefowl *Leipoa ocellata*
   - Glossy Black Cockatoo *Calyptorhynchus banksii*
   - Gouldian Finch *Erithrura gouldiae*

4. **Reptiles**
   - Komodo Dragon *Varanus komodoensis*
   - Fijian Crested Iguana *Brachylophus vitiensis*

**Conclusion**

The ultimate success of the AGSRCA and the AB program will be measured by viable pregnancies and from the collection, fertilisation, storage and transfer of genetic material to recipient donors and the successful return of animals to their native habitat.

**ACKNOWLEDGMENTS**

The authors thank the many dedicated people who collaborate in this program.
HEMOLYTIC ANEMIA IN A PRONGHORN (Antilocapra americana)

Edward J. Gentz, MS, DVM
Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

Lisa A. Harrenstien, DVM
Department of Pathobiology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901, USA

Jeff Baker, DVM
Denver Zoological Gardens, City Park, Denver, CO 80205, USA

James W. Carpenter, MS, DVM, Dipl ACZM
Exotic Animal, Wildlife, and Zoo Animal Medicine Service, Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66502, USA

Introduction

Hemolytic anemia is characterized by an accelerated rate of erythrocyte destruction. The disease processes and pathophysiologic mechanisms associated with hemolytic anemia vary considerably. Likewise, the clinical manifestations of hemolytic anemia may vary according to the degree of anemia, rate of erythrocyte destruction, and causative disease or disease agent. This case report documents the successful treatment of what may be the first reported case of hemolytic anemia in a pronghorn.

Presentation

A four-year old, 34 kg, female pronghorn (Antilocapra americana) was hospitalized after she was noted to be recumbent in the exhibit she shared with another pronghorn, two white-tailed deer, and two wild turkeys. Physical examination revealed lethargy, depression, moderate dehydration, grade II systolic murmur, prolonged capillary refill time, red-colored urine, and marked icterus of the sclera, gingiva, and vulvar mucous membranes. Hematology revealed a significant anemia (PCV = 22%, normal = 38.3-50.5). Blood serum chemistry analysis revealed hyponatremia (136 mmoVL, normal = 155), hypochloremia (97 mmoVL, normal = 111), hypokalemia (3.9 mmoVL, normal = 5.5), hypocalcemia (7.9 mg/dl, normal = 9.6), and azotemia (urea nitrogen 127 mg/dl, normal = 29-45). Venous blood gas analysis showed a metabolic acidosis (pH 7.33 with decreased pCO₂ @ 34.4 mm Hg and decreased HCO₃ @ 18.2 mmol/L).

A catheter was placed in the right jugular vein and fluids started at a rate of 120 ml/hr (1.5x maintenance). After an initial liter of 5% dextrose, these were changed to lactated Ringer's solution. After 24 hr of fluid therapy, the serum sodium and chloride returned to normal range. However, the serum calcium (6.7 mmol/L) and potassium (3.1 mmol/L) worsened, due to hemodilution. The PCV also decreased to 18% after 24 hr. Subsequently, the fluid rate was decreased to maintenance and 20 meq/L of potassium chloride (KCl) were added daily to the fluid therapy until the serum potassium returned to normal range. Additionally,
25 ml of 23% calcium gluconate were added to the fluid therapy, after which the serum calcium also returned to normal range. Urinalysis of the red-colored urine at the time of presentation revealed 2+ bilirubin, 3+ protein, and 3+ occult blood, but no erythrocytes, indicating the problem was hemoglobinuria, or less likely, myoglobinuria, as the muscles seemed to be normal, rather than hematuria. After 24 hr of fluid therapy, the urine color was a hazy yellow, however, repeat urinalysis still revealed 2+ protein and 2+ occult blood. The initial venous blood gas abnormalities were normalized on subsequent blood gas analysis (pH 7.37, pCO₂ 44.9 mm Hg, HCO₃ 25.6 mmol/L).

The combination of presenting signs of anemia, icterus, and hemoglobinuria were indicative of hemolytic anemia.

**Differential Diagnoses**

Initial differential diagnoses for the hemolytic hematuria in this pronghorn included intraerythrocytic parasitism (anaplasmosis, babesiosis, eperythrozoonosis, or hemobartonellosis), bacterial infection (leptospirosis, or bacillary hemoglobinuria caused by *Clostridium hemolyticum*), autoimmune hemolytic anemia, or the ingestion of toxic plants causing Heinz body hemolytic anemia (wild onion or red maple leaf poisoning). Other differential diagnoses considered less likely included water intoxication (salt poisoning) seen in calves and copper toxicosis seen in lambs. No potential source of copper toxicity was apparent, and excessive water consumption had not been noted.

**Diagnostics**

The presence of hemoglobinuria ruled out anaplasmosis as a differential diagnosis, as this disease is characterized by yellow urine and an absence of either hematuria or hemoglobinuria.¹ No other red blood cell parasites were seen on repeated blood smear examinations, ruling out babesiosis, eperythrozoonosis, and hemobartonellosis. Paired leptospirosis titers, utilizing banked serum from the previous year's annual physical exam and stored in an ultra-cold freezer, were negative. Phase contrast microscopy also failed to reveal any leptospires in the urine. Bacillary hemoglobinuria caused by *Clostridium hemolyticum* was considered unlikely, because the pronghorn was currently vaccinated with a multivalent clostridial vaccine, including tetanus. A Coombs' test for autoimmune hemolytic anemia, using bovine reagent, was negative at dilutions 1:2 through 1:128. Additionally, specific examination of repeated blood smears for the presence of spherocytes was negative. Careful inspection of the pronghorn's exhibit found no wild onions, red maple leaves, or other toxic plants capable of causing Heinz body hemolytic anemia.

**Therapeutics**

In the absence of a definitive diagnosis, it was decided to initiate a broad-spectrum treatment regime. Oxytetracycline (LA 200) was initiated at a dose of 20 mg/kg SQ q48hr to treat for suspected red blood cell parasitism as well as leptospirosis. Additionally, procaine penicillin G was administered (22,000 IU/kg IM BID), as this would be the
treatment of choice for Clostridium hemolyticum, although generally penicillin and tetracycline are not administered simultaneously. Concurrently, the pronghorn received dexamethasone (12 mg q24hr IV) for suspected autoimmune disease. Intravenous fluid therapy was continued both for general supportive therapy and specifically for possible plant toxicity.

Despite these treatments, the pronghorn's condition continued to deteriorate. When, after 5 days, the PCV reached a nadir of 11%, it was decided to attempt a blood transfusion utilizing her unaffected sibling from the same exhibit. A cross-match between donor and recipient revealed neither major nor minor agglutination. The donor pronghorn was sedated at the zoo with 0.1 mg/kg detomidine and 1.5 units (750 ml) whole blood removed for transfusion. Post-transfusion, the anemic pronghorn's PCV was 21% and she appeared clinically improved. The PCV remained stable for two days, when a CBC revealed a WBC of 26,600, with 24,000 neutrophils. The azotemia resolved at this time (urea nitrogen = 33 mg/dl), indicating hemolysis had ceased. Urinalysis at this time showed no blood or bilirubin in the urine, and only trace protein. Intravenous fluids were discontinued, the dexamethasone was slowly tapered off, the penicillin continued, and the oxytetracycline replaced with 1.1 mg/kg ceftiofur (Naxcel) IM q24hr. Over the next 10 days, the CBC returned to normal and the PCV slowly rose to 31%, when the pronghorn was returned to exhibit at the zoo. The systolic murmur, suspected to be due to hypovolemia, was no longer present at the time of discharge from the hospital.

Discussion

Hemolytic anemia has, apparently, not been previously reported in pronghorn (E. S. Williams, pers. comm. 1994). Blood samples from hundreds of pronghorns from Texas, Wyoming, and Oregon analyzed for leptospirosis and anaplasmosis were negative. Anemia in pronghorn fawns secondary to copper deficiency was seen at the Los Angeles Zoo (J. Boehm, pers. comm., 1995).

Wild ruminants such as deer, elk, and bison rarely have clinical anaplasmosis but can be asymptomatic carriers; in California, native deer are considered to be a major source of infection for cattle.\(^1\) Wild ruminants known to be susceptible to bovine babesiosis include white-tailed deer, bison, reindeer, water buffalo, and African buffalo.\(^2\) Two sable antelope imported from a zoo in West Germany to South Africa died of babesiosis after displaying hemoglobinuria and anemia.\(^4\) Bacillary hemoglobinuria caused by *Clostridium hemolyticum* may affect wild ruminants.\(^5\) Leptospirosis has been reported in white-tailed deer, but may be self-limiting in exotic ruminants.\(^6\) Hemobartonellosis (eperythrozoonosis), caused by *Eperythrozoon ovis*, has been reported in white-tailed deer, mule deer, elk, eland, and blesbok.\(^6\) Hemolytic anemia in the black rhinoceros is currently under intense investigation; some, but not all, cases are linked to leptospirosis. Additionally, decreased serum vitamin E and low ATP levels in black rhino erythrocytes may decrease red blood cell membrane stability and predispose them to lysis.\(^7\)
None of the above diseases was able to be documented in the pronghorn of this report. Additionally, a Coombs' test for autoimmune hemolytic anemia was negative. However, the Coombs' test is not always positive in affected animals. The Coombs' test is also based on species-specific reagents; the bovine reagent used may not be appropriate for use in pronghorn. The Coombs' test was also performed following several corticosteroid treatments, which may inhibit antibody production and lead to a false negative result. It is also possible that despite a recent vaccination, the hemolytic anemia of this pronghorn was caused by Clostridium hemolyticum. A blood culture may have been useful in determining if this was the case. Serum vitamin E and copper levels also may have been useful.

**Conclusions**

Hemolytic anemia can occur in the pronghorn. In the absence of a definitive diagnosis, the judicious use of broad-spectrum therapeutics, supportive care, and conspecific blood transfusions can contribute to successful case management.

**LITERATURE CITED**

FECAL STEROID ANALYSIS: VALIDATION OF EXTRACTION AND RADIOIMMUNOASSAY FOR ESTRADIOL AND PROGESTAGENS IN AFRICAN ELEPHANTS (Loxodonta africana) AND ANALYSIS OF FECAL SAMPLES UTILIZING A VALIDATED METHOD

Sophie Papageorge, V'96*
Tufts University School of Veterinary Medicine, 200 Westboro Road, N. Grafton, MA 01536, USA

Samuel K. Wasser, Ph.D
Center for Wildlife Conservation, c/o Veterinary Hospital, Woodland Park Zoo, 5500 Phinney Avenue North, Seattle, WA 98103, USA

Charles Foley
Princeton University, c/o Serengeti Select Safari, P.O. Box 2708, Arusha, Tanzania

Janine Brown, Ph.D
National Zoo Conservation & Research Center, 1500 Remount Road, Front Royal, VA 22060, USA

Blood, urine and vaginal smears are difficult to collect from wild elephants because of hazards inherent in immobilizing this species. Fecal samples are easier and safer to collect and offer a practical method for non-invasive assessment of the reproductive status of wild African elephants. The objective of the initial phase of this project is to validate a method for extracting estrogens and progestagens from feces in a captive African elephant herd and to analyze samples collected from these animals. Five African elephants from the Indianapolis Zoo ranging in ages 11 to 26 years were used. Daily fecal samples were collected from mid-June 1992 to mid-January 1993. Blood was collected weekly from ear veins, without anesthesia, during the same time period. Fecal samples were dried (Savant instruments Speedvac Rotary Evaporator, Forma Scientific, Inc., Marietta, OH) and pulverized. Triplicate ~0.2 g aliquots of well-mixed powder were extracted as follows: 1) boiled in 100% ethanol for 20 minutes, centrifuged, the pellet re-extracted in ethanol and the supernatants combined; 2) boiled in ethanol and the resulting supernatant extracted twice with dichloromethane; or 3) extracted with diethyl ether. There were no differences (P>0.05) in steroid recovery between samples extracted with or without dichloromethane, and only 20% conjugates were in the water fraction of the ether extraction. All samples from the captive elephant study were extracted with 100% ethanol. Estradiol and progestagen metabolites in extracted fecal samples, matched to weekly blood samples collected, were analyzed by radioimmunoassay (RIA). The estradiol RIA used an antibody raised in rabbits (provided by Dr. Samuel Wasser, Center for Wildlife Conservation, Seattle, WA) against estradiol-17β 6-o-carboxymethyloxime:BSA11,3, H-labeled estradiol tracer, and estradiol standards. The progesterone RIA used a monoclonal P4 antibody produced against 4-P-11-ol-3,20-dione hemisuccinate:BSA (provided by Dr. Jan Roser, University of California, Davis, CA). On the basis of serum progesterone analysis, four of the five Indianapolis elephants indicated cycles for estrogen and progestagen. The results indicate that the ethanol extraction and radioimmunoassay methods are valid for measuring estradiol and progestagens in the feces of African elephant females.
A metastatic cholangiocarcinoma was diagnosed in an adult red-tailed hawk (*Buteo jamaicensis*) maintained in captivity for 13 years. Antemortem radiography revealed an extensive periosteal reaction and medullary opacification of the left femur and moderate hepatomegaly. Laboratory sampling over a five week period revealed a persistent leukocytosis and elevations in aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, and creatinine phosphokinase. On postmortem, unencapsulated masses consisting of epithelial lined tubule-like structures were observed in the liver, adrenal gland, lung and left femur. The neoplastic epithelial cells contained eosinophilic cytoplasm and large, round to ovoid nuclei with single large or multiple nucleoli, and exhibited extensive nuclear pleomorphism and variably abundant mitotic figures. This is the first report of bile duct neoplasia in a bird of prey.
FECAL SHEDDING OF SALMONELLA IN EXOTIC FELIDS

Victoria L. Clyde, DVM, Ed Ramsay, DVM, and David Bemis, PhD
Department of Comparative Medicine, University of Tennessee, Knoxville, TN 37901-1071, USA

A private collection of exotic felids was screened for Salmonella by fecal culture after an index case of systemic salmonellosis in an aged serval was confirmed by blood culture. A single fecal culture was obtained from each pen by placing approximately 1 gm from the center of a fecal mass directly into selenite broth. Ninety-three percent (26/28) of the cats were Salmonella positive on a single fecal culture. The collection consisted of cougars, leopards, snow leopards, caracals and servals. The only sample negative for Salmonella came from a pen of two servals. All Salmonella isolates were serotyped by the National Veterinary Services Laboratories in Ames, Iowa. Salmonella serotypes isolated included S. typhimurium (10), S. panama (8), S. bredeney (3), S. johannesburg (2), S. enteritidis (1) and S. dublin (1). The diet in this collection consisted of a commercial frozen horsemeat-based loaf diet and raw chicken. Two successive selenite broth cultures of the horsemeat diet grew S. typhimurium and one of two cultures of the chicken was positive for S. kentucky.

A comparison study was conducted on felids from the Knoxville Zoo. Feces from cats were cultured as described above. Ninety-five percent (19/20) of the samples were positive for Salmonella on a single selenite culture. Positive cultures were obtained from cheetahs, cougars, lynx, snow leopards, tigers and lions. The only negative culture was obtained from a lone male lion. A large variety of salmonella serotypes were present in this collection. The predominant serotype was S. typhimurium (copenhagen) with seven isolates. Other serotypes included S. hadar (2) and S. muenchen (2) with single isolates of S. typhimurium, S. meleagridis, S. dublin, S. cerro, S. muenster, S. dublin, S. poona, S. montevideo, S. kentucky and one untypeable Salmonella (3, 10:L, W-monophasic). Two varieties of commercial frozen horsemeat-based loaf diet were fed at this collection. A culture of one diet was negative for Salmonella; the other diet was positive for S. dublin.

From these preliminary data, >90% of healthy exotic felids fed a raw horsemeat or poultry-based diet are positive for Salmonella on a single fecal culture. None of these cats appeared ill or showed evidence of diarrhea at the time of fecal collection nor in the subsequent year. Apparently, exotic felids can harbor Salmonella as part of the normal, nonpathogenic bacteria of their gastrointestinal tract, which may be a physiological adaptation to carnivory. This high rate of fecal shedding of Salmonella precludes the use of fecal culture as a single diagnostic test in an ill or diarrheic felid. Ideally, clinical salmonellosis should be confirmed by positive biopsy or blood culture. All zoo employees having contact with cat feces or raw diets should exercise good hygienic precautions due to their occupational exposure to Salmonella.
TRANSMISSION OF FELINE IMMUNODEFICIENCY VIRUS IN DOMESTIC CAT SEMEN BY ARTIFICIAL INSEMINATION: MODEL FOR SEMINAL TRANSMISSION OF FIV IN NONDOMESTIC FELIDS

JoGayle Howard, DVM, PhD* and David Wildt, PhD
National Zoological Park, Washington, DC 20008, USA

Holly Jordan, DVM, PhD, Suzanne Kennedy-Stoskopf, DVM, PhD, and Wayne Tompkins, PhD
North Carolina State University, College of Veterinary Medicine, Raleigh, NC 27606, USA

Numerous species of wild felids have been found to be seropositive for feline immunodeficiency virus (FIV), a lentivirus in the family Retroviridae. Species affected include lions, cheetahs, leopards, pumas, bobcats, Pallas' cats and snow leopards, however, seroprevalence varies among species and geographic location. For example, FIV seroprevalence in captive African lions ranges from 12-14%, while free-ranging lions in East and South Africa have a high seroprevalence exceeding 80%, and lions in West Africa are seronegative. The clinical impact of the virus on infected wild felids is not known, however, neurological changes, retinal lesions, reduced CD4+:CD8+ lymphocyte ratios and high globulin levels have been detected in FIV seropositive lions. In domestic felids, FIV infects 2-5% of domestic cats, causing an acquired immunodeficiency syndrome-like disease similar to that caused by human immunodeficiency virus type 1 (HIV-1). FIV has been isolated from saliva, blood, cerebrospinal fluid and milk in domestic cats. Like the human virus, FIV can be experimentally transmitted horizontally via parenteral (intravenous, intraperitoneal), rectal or vaginal exposure, and vertically through milk. Little information is available on the sexual transmission of FIV. Using co-culture and polymerase chain reaction (PCR) amplification, we recently determined that infectious FIV is present in whole semen and cell-free seminal plasma in asymptomatic, chronically infected domestic cats. Objectives of this study were to: 1) determine the effect of semen processing (washing versus swim-up separation) on FIV expression; and 2) assess FIV transmission after artificial insemination (AI). Electroejaculates from 7 asymptomatic FIV positive domestic cats (infected with FIV/NCSU1 at birth) and 4 FIV negative control cats were centrifuged and split into 3 aliquots: 1) seminal plasma (SP); 2) non-swim-up (NS) semen cells (sperm + non-sperm cells); and 3) swim-up sperm (SU). After recovery of SP, SU sperm were allowed to migrate into fresh Ham's F10 for 1 h, and NS aliquots were resuspended in fresh medium. NS and SU aliquots were analyzed for the presence of the FIVgag segment by polymerase chain reaction (PCR) using nested primers. SP and NS aliquots were co-cultured with FCD4E cells (a feline CD4+ T lymphocyte cell line developed at NCSU). Co-cultured cells were assessed for FIVgag by PCR. For AI, 5 FIV negative females were given 100 IU eCG and 75 IU hCG and inseminated in utero laparoscopically using fresh (n=3 females) or frozen-thawed (n=2 females) FIV positive NS aliquots. Females were monitored biweekly for anti-FIV antibody and FIVgag provirus. Peripheral blood leukocytes from all cats were subjected to PCR. FIV was detected in at least one seminal component from each of the 7 infected males. Five of 7 SP and 4 of 7 NS co-cultures from infected cats were positive for FIV by PCR. Virus also was demonstrated in 3 of 7 NS and 4 of 7 SU samples using nested PCR. By 8 weeks post-AI, one female seroconverted and expressed FIV provirus.
The other 4 females remained FIV negative as of 12 weeks post-AI. Two females inseminated with fresh sperm became pregnant. All blood samples from infected cats were FIV positive by PCR. None of the blood or semen samples from the 4 uninfected control cats were FIV positive. Results demonstrate that FIV is present in seminal plasma, washed semen cells and swim-up sperm in the domestic cat. Like HIV, FIV can be transmitted by intrauterine AI. These data suggest that the domestic cat may be a useful model of FIV in nondomestic felids. (Funded by USPHS grant #1RO1A132310-02 and grants from the U.S. Fish & Wildlife Service and the Smithsonian Institution Scholarly Studies Program)
Case Report

At the Montgomery Zoo, during September 1994, a six-week old female sika deer (Cerous nippon taiwanus) was reported to have diarrhea. The deer was housed off-exhibit with its dam, and another female sika deer with its single offspring.

Off-exhibit housing consisted of concrete floor with wooden walls, 16' x 50' x 8' high chain link fence outside run with limestone, gravel and sand mix for solid footing.

Diet for the sika deer consisted of alfalfa-based pellets (ADF-25, MGA Zoo) and a good-quality alfalfa hay.

Visual exam of the animals in the enclosure was unremarkable except for the slight brown soiling of the perineal region of the affected sika deer. Normal volume, of otherwise abnormal color (green) and consistency (watery), stool was noted from the one animal, with all others being unaffected. Fecal direct and flotations of normal stool and diarrhea were negative for parasites. Stool culture was negative for bacterial pathogens. Stool samples were negative for Giardia antigen and Cryptosporidium by IFA.

Under manual restraint and a blindfold covering the eyes, physical exam and blood collection for CBC/SMAC were performed. The physical exam was normal, i.e., the animal was bright and alert; normal body weight and strength; and normal vital signs. Results of the CBC were normal. Significant finds on the SMAC included: hyperglycemia (182, X 115 +/- 52); azotemia (BUN 75, X 28 +/- 8); hyperphosphatemia (11.6, X 6.7 +/- 2.3); and hypokalemia (2.9, 4.8 +/- 0.8). These results were interpreted to be due to stress, as well as prerenal azotemia and electrolyte loss due to diarrhea.

Virology of the feces was negative by ELISA for rotovirus but coronavirus-like particles were seen by electron microscopy. Nevertheless, corona virus was not clinically thought to be the major factor in the case, due to the explosive, often bloody nature of the epizootic that affected only mature animals at the Montgomery Zoo eight months before. Also, by this time, the diarrhea had been present for approximately ten days, whereas affected animals with winter dysentery showed clinical signs for 3-5 days before resolution. Due to the bright alert nature of the animal, as well as good hair coat and body weight, pica or ingestion of material other than normal diet was suspected, especially since the animal was at an age to begin ingesting solid food or objects. No signs of chewing on wooden stalls or galvanized
fencing were found, and no such activities were observed. Recommendations were made to isolate the young deer and its dam inside the wooden stalls to feed a controlled diet, but this was not done. Various symptomatic treatments were tried but did not alter the course of the diarrhea. Between two and three weeks after clinical onset, the young deer was found dead.

Gross necropsy was unremarkable except the abomasum appeared to be full of the limestone/gravel outside flooring material. Superficial erosions were present in several locations and were circumscribed one to three centimeter diameter. At the time this was ascribed to the mechanical affect of a large amount of coarse material. Some plant material, apparently hay, was present in the rumen, as well as various levels throughout the intestines. No other gross lesions were seen, and abundant body fat was observed. Based on gross findings, partial impaction, abomasal ulceration, and diarrhea secondary to pica was diagnosed.

Tissues were sent to Veterinary Diagnostic Laboratory in Auburn, AL for histopathology and toxicology. Microscopic findings included multifocal areas of hemorrhage and hyperemia within the mucosa of the abomasum. Submucosal hemorrhage was present within a section of the ileum. The mucosa of the large intestine had focal areas of hemorrhage and neutrophilic infiltrate. Sections of liver, lung, kidney, and spleen were hyperemic. Other organs submitted contained no significant microscopic lesions.

Rumen contents submitted for toxicology revealed no significant concentrations of copper, lead or zinc. Insecticide screen was negative for toxins. Concentrations of iron in the contents was high (2,000 ppm) but probably not indicative of toxicosis. The concentration of arsenic (9.2 ppm, wet weight) was high, and along with the lesions observed within the gastrointestinal tract were consistent with arsenic toxicosis.

Outside crushed rock/gravel flooring was removed and replaced with fresh material analyzed for various heavy metals. Several different shipments of this outdoor footing material had been used in several barn areas, so samples from all outside stalls in the adjacent African barn were checked also. These areas all proved to be negative for a variety of heavy metals, including arsenic.

Discussion

Arsenic is fairly ubiquitous in nature and exists in different forms that exert different species-specific effects. Inorganic arsenicals, such as arsenic trioxide or lead arsenate, are used as insecticides. Other forms are used as herbicides and defoliants. None of these sources were found or had been used at the Montgomery Zoo in the past. Copper-chromium-arsenic-(CCA)treated wood is used in areas bordering animal exhibits and barns but is no hazard to animals unless it is burned.

Acute arsenic toxicosis is associated with severe abdominal pain, weakness, and diarrhea. This progresses rapidly to prostration, renal failure, and death in most exposed animals.
Subacute arsenic toxicity is associated more with clinical signs of chronic renal failure, i.e., depression, anorexia, and first polyuria, then oliguria. Weakness, tremors, and death are seen in the final stages. Chronic arsenic toxicity consists of a general loss of condition and a rough haircoat. Relating the present case to a distinct toxicosis was difficult due to lack of any clinical signs other than a mild diarrhea.

Postmortem signs of arsenic toxicity exhibit gastrointestinal hyperemia and large amount of foul-smelling bloody gut contents. Histologic gut sections have extensive areas of edema, necrosis and sloughing. Glomerular sclerosis and renal tubular necrosis are seen in more chronic cases. In this case, several gross and microscopic features consistent with arsenic toxicity were present but could have been attributed to the partial impaction of coarse material without toxicological testing. Profuse bloody lumenal contents were not seen in this case as are often described in the literature.

Toxicology may reveal liver and kidney arsenic concentrations greater than 8 ppm wet basis. Since arsenic is rapidly excreted by the body tissues, levels may decline to normal background levels over several days. Thus, prompt testing of tissues, as well as possible sources of exposure, is necessary in subacute or chronic cases. In this case report, arsenic levels in the liver or kidney may have been normal but this was not done. Fortunately, the source of exposure was evident at necropsy.

Additional laboratory testing in suspected cases should include other toxicoses such as organophosphate or lead poisoning, and infectious causes such a cryptosporidiosis. Positive results, such as the coronavirus found in the stool in this case report, must be judged in light of the total clinical picture as well as other laboratory results.

Treatment of arsenic toxicity is often unrewarding. Gastric lavage along with oral and intravenous sodium thiosulfate and intravenous fluids are used in acute cases. Chelation therapy with BAL in the United States can be used also. Although expensive, its use may be justified in valuable animals.

Finally, this case report illustrates the intermittent, but serious danger of various fill material or flooring substrate used in holding areas or new zoo exhibits. Such material may come from a wide variety of sources with little history available as to previous industrial site use or contamination present. With many areas of the U.S. now evidencing environmental toxins of a wide variety, analysis of water sources or substrate to be used in new exhibits before their incorporation is essential. Technical help for such surveys is readily available through state diagnostic laboratories or state environmental management agencies, often at no cost. As seen in this case report, toxins in environmental substrate may not present a practical danger unless abnormal feeding habits or pica are present. This etiology should be considered in vague or obscure clinical cases especially when animals are in a transitional state; i.e. weaning from milk to solids; even if the pica is not observed or found on inspection of the holding area.
LITERATURE CITED


ACKNOWLEDGMENTS

The author wishes to acknowledge the help of the Alabama Department of Environmental Management (ADEM) in toxicological analysis of outdoor holding substrate samples.
COPPER DEFICIENCY IN A GEMSBOK (Oryx gazella)

Don Gillespie, DVM*
Montgomery Zoo, Montgomery, AL 36110, USA

George D’Andrea, DVM and Susan Lockaby, DVM
C.S. Roberts Veterinary Diagnostic Laboratory, P.O. box 2209, Gilmer-Turnham Building, Auburn, AL 36831, USA

Case Report

On January 19, 1994 a three-year old female gemsbok (Oryx gazella) was reported to have depression, partial anorexia, diarrhea, and slight rear paresis with muscle spasms present. Previous medical history was unremarkable except strongyles and Trichuris sp. parasites for which quarterly fecal flotations and treatment as needed was done. Samples of the diarrhea were taken for parasitology, virology, and microbiology. Fecal flotation revealed strongyles and Trichuris sp. eggs and the animal was treated with fenbendazole (Panacur, Hoechst-Roussel) at 10mg/kg orally (PO) for three days. Stool culture was negative for bacterial pathogens. Virology was negative for rotavirus by ELISA testing but positive for coronavirus-like particles. Additional treatment included flunixin (Banamine, Schering-Plough) at 1.1 mg/kg intramuscularly (IM) q24h X 3d and oxytetracycline (LA-200, Pfizer Co.) at 20 mg/kg IM once and lincomycin/spectinomycin (LS-50, Upjohn Co.) in the drinking water for three days. By day three, the stool was 80% of normal, and paresis was significantly reduced. By day five, the animal was clinically normal and returned to normal activity on exhibit.

Five days after return to the exhibit the gemsbok was found at the entrance aisle of the night holding areas, recumbent and unable to use the rear legs. After carefully blindfolding the animal, manual restraint was used for physical exam, treatment and blood collection. Physical exam was unremarkable with a body temperature of 102.5 degrees F, heart rate 85/minute, and respiratory rate of 20/minute. Additional findings included normal intestinal mobility and rumen contractions at two per minute. Treatment included flunixin (Banamine, Schering-Plough) at 1.1 mg/kg intravenously (IV), ceftiofur sodium (Naxcel, Upjohn) at 4.4 mg/kg IV, dexamethasone sodium phosphate (Dex-A-Vet, Anthony) at 2 mg/kg IV, and procaine penicillin G (Pen G, Phoenix) at 10,000 I.U./kg IM. Blood was drawn for a complete blood count (CBC) and serum chemistries (SMAC). CBC results indicated a white blood cell count (WBC) of 5,000 by automated count, and 1,000 by manual count. A moderate microcytic, hypochromic anemia was also present (PVC 18.4 X 14.1 +/- 10.1; Hb 5.4 X 14.1 +/- 2.8). SMAC results were normal except for hypoglycemia (60, X 142 +/- 57) and hypoalbuminemia (1.8, X 3.8 +/- 1.1). Tentative diagnosis was generalized weakness secondary to hypoglycemia associated with sepsis along with chronic blood loss anemia due to parasitism. The animal could move all parts of the body normally during the physical exam except the rear legs which had some movement but would not bear weight.

The next day the gemsbok was alert but still recumbent. Under blindfold and manual restraint, a 14 gauge intravenous jugular catheter (Cathlon IV, Johnson & Johnson) was
placed, and 15 liters of Lactated Ringer’s solution (Lactated Ringer’s Solution, Baxter)/5% Dextrose (5% Dextrose in Water, Baxter) was given over the course of the day.

Intravenous flunixin and ceftiofur were repeated with the ceftiofur being given twice daily (BID). Additional treatments included five ml iron dextran IM (Ferrodex 100, Agrilabs), 2500 I.U. vitamin E (Rocavit E, Roche) IM, and 3,000,000 I.U. procaine penicillin G IM (Pen G, Phoenix). Through the course of the day the animal became severely depressed and body temperature dropped to 98 degrees F and then 96 degrees F. A CBC was repeated and the differential remained the same as the previous day although the WBC had increased to 12,000. This was interpreted as both a stress leukogram and rebound in the face of infection.

By January 28, 1994 the gemsbok was comatose but alive. Body temperature remained at 96 degrees F. Treatment was repeated as on January 27. A SMAC was drawn and abnormal results included a mild hypochloremia (96, X 102 +/- 4) and metabolic alkalosis (39, X 20-31). Intravenous fluid therapy was changed to 0.9% saline (Normal Saline, Baxter)/5% Dextrose (5% Dextrose in Water, Baxter). Nevertheless, the animal died at the end of the day.

A complete necropsy was conducted at the Charles S. Roberts Veterinary Diagnostic Laboratory in Auburn, Alabama. Gross findings included mild edema of the ventral body wall, pericardial sac, mesentery, and abomasal wall. Cecal contents included some Trichuris sp. nematodes, but minor mucosal damage was observed. The lungs had 0.5 cm disseminated red foci which bled on sectioning. The uterus contained a fetus of approximately 120 days gestation. Histologic sections were normal from numerous organs examined except the lungs which revealed isolated small foci of alveolar and bronchiolar hemorrhage. Sections of lung, liver, and kidney from the fetus were normal. Gross pathology findings were interpreted as edema secondary to hypoproteinemia and recumbency in the face of intensive fluid therapy. Parasitism effects were minimal. Pulmonary hemorrhage was minimal also.

Routine bacterial culture of the lung, liver, spleen, and small intestine revealed no pathogenic isolates. Significant toxic concentrations of zinc, arsenic, lead, carbamate, organophosphate, and chlorinated hydrocarbons were not found in liver, kidney, or rumen contents. Serology for leptospirosis, brucellosis, bluetongue, bovine leukemia virus, infectious bovine rhinotracheitis, bovine virus diarrhea, and bovine respiratory syncytial virus were negative.

Significant results were found on plasma trace mineral analysis. Selenium was 0.23 ppm wet weight; copper was 0.34 ppm wet weight; and zinc was 0.43 ppm wet weight. Results were interpreted as normal background selenium, whereas copper and zinc were in a moderate deficient range for livestock in this area of the country.

These results correlated with a very low liver copper content of 18 ppm dry weight (normal for most livestock 200-450 ppm dry weight). Also, iron in the liver was higher than expected
at 900 ppm dry weight. Final interpretation included primary copper deficiency anemia along with early enzootic ataxia and immune system depression, resulting in terminal septicemia.

Systematic survey of plasma and/or liver copper levels was not possible in the other 1.1 gemsbok or other artiodactylids in the collection. Therefore, opportunistic sampling during other procedures, or at necropsy, was undertaken as well nutritional analyses of hay, pasture and alfalfa-base commercial pellets (ADF-25, MGA Zoo) for trace mineral levels and total sulfate levels. Over the next several months five species of antelope and five species of cervids, as well as other artiodactylids, i.e., bighorn sheep (*Ovis canadensis*) and pronghorn (*Antilocapra americana*) were opportunistically surveyed for plasma zinc and copper levels. These samples taken over several months revealed no overt or marginal deficiencies except for one 0.1 pronghorn and a group of 2.4 Bighorn sheep which previously had been documented with this problem.

Analysis of feed samples revealed the apparent problem. Alfalfa hay at the Montgomery Zoo had adequate amounts of copper at 20 ppm, and the alfalfa-based commercial pellets (ADF-25, MGA Zoo) also contained adequate amounts at 25 ppm dry weight. The commercial sweet feed (Fortified All Grain, Nutrena) was low to marginal at 5 ppm dry weight. Coastal Bermuda hay, as well as Bermuda grass exhibit pasture in all areas, consistently yielded 5 ppm copper level dry weight. Molybdenum (Mo) did not appear to contribute to the problem as the highest level seen in pelletal feeds or pasture was 1.27 ppm dry weight. Thus in all cases the copper/molybdenum ratio was above the critical 2:1 level for safety. Sulfates were not found in significant levels. However, iron levels in coastal Bermuda grass, as well as exhibit pastures were considered high (500-550 ppm dry weight). Considering that in the climate of South Central Alabama the exhibit pasture is effectively used all year, low to marginal copper levels with absorption antagonism by iron was considered to be the main problem. Total feed intakes were not possible with cost and large groups of animals having individual variation in pasture consumption. Conservative copper supplementation was undertaken due to the following: (1) continuously updated copper status not available due to speed of the opportunistic sampling program; (2) danger of inducing copper toxicosis in the face of multi-species exhibits and incomplete information on all species in each exhibit; and, (3) low number of total species in all exhibits documented with the problem.

Since all species, with very few exceptions, are brought into wooden stall/chain link holding areas at the Montgomery Zoo, trace mineral blocks (Rotomin Horse and Cattle Salt Licks, Roto Salt Co.) and/or trace mineral salt (Rotomin Trace Salt, Roto Salt Co.) were placed in all holding areas as indicated by individual consumption. Consumption was monitored on daily keeper and veterinary rounds. Changes made, such as adding molasses to certain salt blocks or offering free salt form instead of the blocks, were done until all animals on exhibit pastures were being effectively supplemented. The bulk of opportunistic plasma copper samples represent a supplemented status.
Other changes have included using part or all alfalfa hay instead of the coastal Bermuda hay for the bighorn sheep and gemsbok. This makes the hay component of the diet a high normal adequate source of copper without being a potent source of the absorption antagonist, iron. Gemsbok plasma samples taken since these management changes have shown low normal values. Bighorn sheep continue to be erratic between low normal and deficient levels, although no signs of clinical deficiency, such as anemia or impaired reproductive performance, have been observed. The next level of supplementation would probably involve some form of copper sulfate which is more bioavailable than the cupric oxide form used in trace mineral salt and blocks. Another alternative would be use of an injectable copper product, but currently none are on the market in the United States.

Conclusions

Copper deficiency is a complex clinical phenomenon in ruminants, resulting in unthriftiness, diarrhea, neurologic signs, and anemia. Copper may be deficient in the soil primarily, or a number of other factors can result in secondary deficiencies. Copper and molybdenum occur in an inverse balance where high levels of one impair the absorption of the other. This is also true for other minerals such as zinc, iron, lead, and selenium. Dietary sulfate levels can also potentiate the effect of molybdenum. Signs vary with age (younger animals generally are more susceptible) as well as breed differences in sheep and cattle. Copper is better absorbed and available in diets low in fiber i.e., cereals or stored feeds such as hay. Thus fresh forage has a higher chance of inducing deficiency and also being a seasonal problem depending on climate and grazing practices. In this case report, pasture was a year-round factor in development of the problem. Pasture with 3 - 5 ppm dry matter of copper are at marginal levels, and 7 - 12 ppm dry weight is usually safe unless other factors complicate absorption.

Copper is incorporated into ceruloplasmin, an enzyme which plays an important role in tissue oxidation processes. Depending on the organ system, the following deficiency signs may include: (1) loss of normal quality haircoat including color; (2) poor overall body condition; (3) diarrhea due to unknown mechanisms; (4) anemia due to loss of iron reutilization after normal breakdown of hemoglobin; (5) defective myelination usually seen in young animals resulting in coordination problems; (6) immune system problems, including lack of normal neutrophil killing function; and, (7) miscellaneous effects on the heart, blood vessels, connective tissue, and bone.

In this case report the gemsbok showed signs of copper deficiency related to the hematopoietic, nervous, and possibly the immune system. Neurologic involvement includes classic forms, such as swayback or enzootic ataxia, in young sheep. However, enzootic ataxia in red deer (Cervus elaphus) occurs in young adults and adults as seen in this case. None of the histopathological changes such as demyelination and midbrain neuronal degeneration seen in the textbook cases were found in the gemsbok. As in enzootic ataxia of lambs, there was no true paralysis as the animal was able to use its legs somewhat even while recumbent.
The use of clinical pathology as plasma or liver copper values in diagnosis must be used with some caution. Levels have been established as guidelines for marginal deficiency and functional deficiency, but these are only guidelines and must be correlated with clinical signs. A state of depletion may exist where normal blood values are maintained while the storage site in the body, such as the liver, is losing copper. This may be the case currently under investigation with the group of slender-horned gazelle (Gazella leptoceros) at the Montgomery Zoo. Blood values for all individuals and multiple values on some individuals have all been in normal to high normal range over the last year (range 0.9 - 1.3 ppm wet weight) but recent analysis of one animal revealed a liver copper level of 14 ppm dry weight which could be considered deficient by cattle or sheep standards. No clinical signs, as seen in the gemsbok, have been noted. Possibly poor haircoat and poor reproductive performance can be traced to the problem but this has not been verified.

Blood copper levels may measure low at the start of a marginal deficiency, but only when concentrations of copper-containing tissue enzymes become low will actual clinical signs of deficiency develop with considerable lag time between individuals. Copper supplementation in problem areas must be done carefully to avoid changing the deficiency into a poisoning. Cupric oxide is the major ingredient in most commercial trace mineral mixes, but copper sulfate is more bioavailable if needed in a supplementation program. Oral dosing, mineral mixtures and annual top dressing of pasture are all possible. Parenteral copper injections which avoid antagonists in the GI tract give good results but are currently unavailable in the U.S.

LITERATURE CITED

A 50 kg, 6-year-old male alpaca was presented to the MSU veterinary teaching hospital showing opisthotonos and paddling of all limbs. A blood analysis revealed a mature neutrophilia and lymphopenia with no toxic changes. CSF tap revealed numerous neutrophils. Radiographs of the cervical and thoraco-lumbar spine revealed no trauma, only a slight increase in intervertebral disk space between C2-C3. The owner decided to euthanize the animal due to the poor prognosis.

The necropsy revealed that this animal was in poor nutritional condition and moderately dehydrated. It had a mucoid discharge from both nares. There were petechial hemorrhages in the cranial sternum and subcutaneous edema. There was about 200 ml of serosanguinous fluid in the abdomen. Very little visceral fat was observed and it had a gelatinous appearance. There were fibrin tags over the large intestine that had a clockwise 360 degree volvulus. The affected loops of large intestine were dilated and dark red. The mucosa of the large intestine was dark red and thickened and the lumen contents were fluid and red. The spleen was enlarged (25 x 20 cm) and occupied part of the left and ventral aspects of the abdominal cavity. It had a nodular yellow-whitish surface. On cut section, the spleen had numerous coalescing caseous areas and very little grossly normal splenic tissue was observed. The splenic lymph node was enlarged and wet on cut section. There was about 50 ml of straw colored fluid in the pleural cavity. The lungs were diffusely red and heavy and on cut section oozed red frothy fluid.

Bacteriologic examination gave more than 1,000 colonies of E. coli from the small and large intestine. Clostridium glycolicum was isolated from the large intestine. The spleen gave a mixed culture of Pseudomonas species and Actinomyces species. No significant isolates were obtained from the brain, liver, lungs and lymph nodes. Virologic study of the brain and lymph nodes gave negative results for equine herpes virus, bovine viral diarrhea virus and pseudorabies antigens upon fluorescent antibody testing. Mineral analysis from the liver was within normal range.

Multiple tissues including the brain, spinal cord and the spleen and intestines were fixed in formalin for microscopic examination. Some vessels in the brain had numerous eosinophils and others mononuclear leukocytes in their lumen. There was a well circumscribed nodule in the brain stem consisting of a central necrotic area, partially calcified, surrounded by a band of connective tissue and an external layer containing macrophages, multinucleated giant cells, lymphocytes and plasma cells as well as some connective tissue fibers. The surrounding neuropil had mild vacuolar changes. Gram stain did not reveal any microorganisms. There were multifocal accumulations of degenerate neutrophils in the leptomeninges as well as
neutrophils and eosinophils in the choroidal plexus. The aqueduct was filled with
degenerated leukocytes (probably neutrophils) but no inflammatory reaction was observed
in the adjacent nervous tissue. The normal architecture of the spleen was almost completely
effaced by multifocal to coalescing areas of caseous necrosis with supuration. There were
large bacillary to filamentous bacterial colonies in some foci as revealed with Steiner silver
stain. Acid fast stains (including peanut oil) did not reveal organisms. The non necrotic areas
contained a mixed population of leukocytes with increased numbers of neutrophils,
eosinophils and megakaryocytes. There were numerous hemosiderin laden macrophages. The
capsule was thickened by a granulation tissue of variable appearance depending on the zone
and foci of eosinophils. There were numerous neutrophils occupying both cortical and
medullary areas of several abdominal lymph nodes. The adrenal gland had multiple areas
of coagulative necrosis and hemorrhage surrounded by a mixed inflammatory infiltrate with
numerous neutrophils. There were gram negative cocci or coccobacilli in the affected areas
in the cortex. The liver had portal infiltrates of neutrophils and eosinophils as well as plasma
cells, lymphocytes and hematopoietic cells including megakaryocytes. There was bile ductular
proliferation that in some areas was very extensive. There was portal bridging fibrosis and
random accumulations of neutrophils as well as sinusoidal congestion and leukocytosis.
There was full thickness necrosis and hemorrhage of the colon and cecum with vascular
thrombosis, leukocytoclastic and suppurative vasculitis and a suppurative infiltrate in the
submucosa and muscularis. The adjacent mesentery had similar lesions. There were
numerous gram negative cocci filamentous rods in the intestinal mucosa. Multiple sections
of the spinal cord (cervical, thoracic and lumbar) did not reveal significant changes.

This animal had several unrelated problems. The terminal state of this animal was most
likely due to the intestinal lesions producing a severe vascular compromise. The splenic
lesion was granulomatous and most likely produced by bacteria. The cerebral lesions
(meningitis, ventriculitis and granuloma) might be related to infestation with the meningeal
worm, *Pneumostrongylus (Parelaphostrongylus) tenuis* or other parasites although parasites
were not found in multiple sections of the brain and spinal cord examined. Probably the
splenic and brain lesions caused a wasting disease as it was demonstrated by the state
emaciation of this animal. Intestinal volvulus is not uncommon in camelids and usually the
cause is undetermined. We postulate that the splenic mass occupying a space in the
abdomen might have altered the peristalsis of the large intestine and contributed to the
volvulus.
A RETROSPECTIVE STUDY OF HISTOLOGIC LESIONS DIAGNOSED IN REPTILES AT THE UNIVERSITY OF GEORGIA (JANUARY, 1989 TO JANUARY, 1995)

Christopher R. Gregory, DVM*, Kenneth S. Latimer, DVM, PhD, Elizabeth W. Howeth, DVM, PhD, Barry G. Harmon, DVM, PhD
University of Georgia College of Veterinary Medicine, Department of Pathology, Athens, Georgia 30602, USA

Pauline M. Rakich, DVM, PhD
Athens Diagnostic Laboratory, University of Georgia, Athens, Georgia 30602, USA

Histopathology reports of reptilian tissues from January, 1989 to January, 1995 were obtained from the document archives of the University of Georgia Department of Pathology and the Athens Diagnostic Laboratory. Individual cases were categorized by gender, approximate age, source (zoo or other including: the UGA Veterinary Teaching Hospital, private veterinary practice, and privately-owned zoological collections), common name, Linnaean classification (order, suborder, genus, species), and histological diagnoses. Histological lesions were classified further as to organ(s) affected and type of lesion (degenerative, inflammatory, infectious, necrotic, metabolic, traumatic, neoplastic, miscellaneous, none observed). The types of lesions were subsequently subdivided (for example, infectious: bacterial, mycotic, etc.) for a more detailed classification of the disease process or etiology.

Tissues from 483 reptiles were submitted over a 5 year period. Three hundred (62%) of the tissues were submitted by 2 large metropolitan zoological parks. Two hundred nine (43.3%) of these reptiles were adult (greater than one year of age), 84 (17.4%) were less than one-year-old, and the remaining 190 (39.3%) were of unknown age. Males and females were equally distributed (145 and 146, respectively, approximately 30% each). The remaining 192 (40%) were of unknown gender. [Table 1]

Reptiles from 4 of the 5 major groups of the class Reptilia were represented and included 4 from the order Crocodylia (alligators, crocodiles, caimans), 48 from the order Chelonia (turtles, tortoises, terrapins), and 431 from the order Squamata. The latter order included 204 reptiles from the suborder Sauria (lizards), and 227 reptiles from the suborder Serpentes (snakes). [Table 2]

Overall tissue submissions (zoo and other) from the order Crocodylia were equally represented by the American alligator (Alligator mississippiensis) and Morelet's crocodile (Crocodylus moreleti). The most common reptile from the suborder Sauria was the common iguana (Iguana) and composed 39/204 (19%) of the total number of saurians represented. The Ball python (Python regius) was the most common representative of the suborder Serpentes and totalled 20/227 (9%) of the suborder. The loggerhead sea turtle (Caretta) was the most common chelonian (9/48 or 19%). [Table 2]

The most common reptiles from the large metropolitan zoological parks were: Gray's monitor lizard (Varanus olivaceous), gaboon viper (Bitis gabonica), and the loggerhead sea
turtle. The most frequently represented reptiles from other sources were: the common iguana, and ball python. There was no predominant chelonian. [Table 2]

Eight hundred and eighty-nine histological lesions were described. Degenerative lesions were observed in 43/889 (5%) instances. Thirteen of the degenerative lesions (13/43 or 30%) were comprised of skeletal myofiber degeneration. Hepatocellular vacuolar degeneration occurred in 14 (33%) instances. [Table 3]

Infectious agents accounted for 281 of the 889 lesions (32%). Eighty-one lesions (81/281 or 58%) were due to parasites and included protozoa (18/81 or 22%), helminths (54/81 or 67%), and mites (2/81 or 2%). One hundred sixteen of the 281 organisms (41% of infectious agents or 13% of total lesions) were bacterial rods. Both gram negative and gram positive rods were observed; however, gram staining was not performed in the majority of the cases. The remaining infectious agents consisted of mixed organisms or rare cellular changes suggestive of viral infection. [Table 3]

One hundred eighty-six lesions (21%) of the 889 observed were classified as inflammatory with no evidence of infectious agents. Infiltrates were composed of heterophils, lymphocytes, plasma cells, and/or macrophages. [Table 3]

Lesions associated with metabolic diseases comprised 194/889 (27%) of the histologic diagnoses. Hepatic lipidosis accounted for 69/194 (36%) of the metabolic changes described. Forty lesions (21%) were soft tissue mineralization. Both hemosiderosis (35/194 or 18%) and soft tissue uric acid deposition (gout) (29/194 or 15%) were observed and accounted for a total of 64 of the lesions seen (33%). [Table 3]

Sixty-five lesions (7% of the total observed) encompassed a broad range of microscopic changes and were classified as miscellaneous diagnoses. The major organs contained such lesions were the liver (11/65 or 17%) and lung (7/65 or 12%). The remaining 47/65 (72%) of the lesions observed, in descending order of occurrence, were in the gastrointestinal tract, vasculature, kidney, adrenal gland, cartilage, spleen, unknown origin, multiple (systemic) tissues, ovary, pancreas, yolk sac, skeletal muscle, heart, brain, trachea, air sac, and parathyroid. [Table 3]

Thirty-four of the 889 lesions observed (4%) were purely necrotic with no inflammatory infiltrate(s) observed. [Table 3]

Twenty-nine neoplasms (3% of total lesions) were observed. Tumors of epithelial (14) and mesenchymal (10) origin accounted for 83% of the neoplasms observed. Seventeen of the 29 tumors (59%) were malignant. Fibrosarcoma occurred in 4 of 29 instances and represents the predominant malignant neoplasm (14%). The remaining 5 tumors were of hematopoietic, lymphoid, and adipose origin. [Table 3]

Traumatic lesions were observed infrequently (2 or 0.2% of total lesions observed). Both of these lesions involved fracture of bone. [Table 3]
Fifty-five accessions (6%) were devoid of microscopic lesions. [Table 3]

The majority of histologic lesions were seen in the liver (157/889 or 18%). The major lesion observed was hepatic lipidosis (69/157 or 44%), followed by infectious diseases (17/157 or 11%). Sixteen percent (139/889) of the lesions were observed in the gastrointestinal tract (includes oral cavity, esophagus, stomach, intestines, cloaca, peritoneum). The small intestine (39/157 or 25%) and the stomach (32/157 or 20%) were the major organs affected. Infectious agents were the primary causes of lesions in the GI tract. Inflammation (26/98) and mineralization (50/98) were the main causes of lesions in the kidney (98/889 or 11%). Skin lesions were seen in 74/889 instances (8%). The majority of lesions (64% or 47/74) in the skin were infectious. Lung lesions also were observed approximately 9% of the time. Lesions were approximately evenly distributed between infectious and inflammatory causes. The remaining tissues individually represented 5% or less of the observed lesions. [Table 4]

The data suggest that common lesions seen in reptiles are metabolic changes associated with inadequate or improper diet (hepatic lipidosis, soft tissue mineralization), infectious diseases, particularly bacterial, and inflammation of unknown etiologies. The most common areas affected are the liver (mainly due to lipidosis), gastrointestinal tract, especially infectious diseases, the kidneys, and the skin (infectious dermatitis). Reptiles require specific dietary requirements, as well as adequate hydration and antimicrobial, particularly parasitic management. The major lesions observed may reflect the need for proper husbandry.
Table 1. Distribution of reptiles by order (suborder), gender, and age.

Number of cases: 483

<table>
<thead>
<tr>
<th>Order</th>
<th>Total (Gender)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order Crocodylia (Alligators, crocodiles, etc.):</td>
<td>4</td>
</tr>
<tr>
<td>Order Chelonia (Turtles, etc):</td>
<td>48</td>
</tr>
<tr>
<td>Order Squamata/suborder Sauria (Lizards):</td>
<td>204</td>
</tr>
<tr>
<td>Order Squamata/suborder Serpentes (Snakes):</td>
<td>227</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crocodylia</th>
<th>Adult</th>
<th>Juvenile</th>
<th>Unknown age</th>
<th>Total (Gender)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males:</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Females:</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unknown:</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chelonia</th>
<th>Adult</th>
<th>Juvenile</th>
<th>Unknown age</th>
<th>Total (Gender)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males:</td>
<td>8</td>
<td>0</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Females:</td>
<td>5</td>
<td>0</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Unknown:</td>
<td>4</td>
<td>9</td>
<td>12</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sauria</th>
<th>Adult</th>
<th>Juvenile</th>
<th>Unknown age</th>
<th>Total (Gender)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males:</td>
<td>35</td>
<td>3</td>
<td>27</td>
<td>65</td>
</tr>
<tr>
<td>Females:</td>
<td>38</td>
<td>5</td>
<td>15</td>
<td>58</td>
</tr>
<tr>
<td>Unknown:</td>
<td>15</td>
<td>27</td>
<td>39</td>
<td>81</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serpentes</th>
<th>Adult</th>
<th>Juvenile</th>
<th>Unknown age</th>
<th>Total (Gender)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males:</td>
<td>45</td>
<td>3</td>
<td>17</td>
<td>65</td>
</tr>
<tr>
<td>Females:</td>
<td>38</td>
<td>10</td>
<td>28</td>
<td>76</td>
</tr>
<tr>
<td>Unknown:</td>
<td>20</td>
<td>26</td>
<td>40</td>
<td>86</td>
</tr>
</tbody>
</table>

Total (age): 209 84 190 483
Table 2. Representative species from tissue submissions from 2 major metropolitan zoological parks and private sources.

(Zoo) Number:

**Crocodylia**
- Morelet’s crocodile (*Crocodylus moreletii*) 2
- American alligator (*Alligator mississippiensis*) 1

**Chelonia**
- Loggerhead sea turtle (*Caretta caretta*) 9
- Mata mata turtle (*Chelys fimbriata*) 3
- Green sea turtle (*Chelonia mydas*) 2
- Amazon river turtle (*Dermatemys mawii*) 2
- Murray river turtle (*Emydura macquarrii*) 2
- Cape tortoise (*Homopus signatus*) 2
- Pancake tortoise (*Malacochersus turcicus*) 2
- Red-footed tortoise (*Geochelone carbonaria*) 1
- Spengler’s Vietnamese wood turtle (*Geomyda spengleri*) 1
- Hingedback tortoise (*Kinixys sp.*) 1
- Striped mud turtle (*Kinosternon bauri*) 1
- Big headed turtle (*Platemyys megacephalum*) 1
- Arrau river turtle (*Podocnemis expansa*) 1
- Radiated tortoise (*Testudo radiata*) 1
- Hispanolian slider turtle (*Trachemys decurtata*) 1
- Eastern slider turtle (*Trachemys scripta*) 1

**Squamata/Sauria**
- Gray’s monitor lizard (*Varanus olivaceous*) 14
- Ornate spiny tailed lizard (*Uromastix sp.*) 11
- Leaf-tailed gecko (*Phelsuma serraticauda*) 10
- Girdled lizard (*Cordylus sp.*) 9
- Prehensile-tail skink (*Corucia zebrata*) 9
- Parson’s chameleon (*Chamaeleo parsoni*) 7
- Veiled chameleon (*Chamaeleo sp.*) 7
- Bearded lizard (*Amphibolurus barbatus*) 6
- Granite spiny lizard (*Sceloporus olivaceus*) 6
- Desert iguana (*Dipsosaurus dorsalis*) 5
- Leopard gecko (*Eublepharis macularius*) 4
<table>
<thead>
<tr>
<th>Animal Type</th>
<th>Species/Species</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chuckwalla</td>
<td>Sauromalus obesus</td>
<td>4</td>
</tr>
<tr>
<td>Green tree monitor lizard</td>
<td>Varanus prasinus</td>
<td>4</td>
</tr>
<tr>
<td>Basilisk lizard</td>
<td>Basiliscus sp.</td>
<td>3</td>
</tr>
<tr>
<td>Sungazer</td>
<td>Cordylus giganteus</td>
<td>3</td>
</tr>
<tr>
<td>Broad headed skink</td>
<td>Eumeces laticeps</td>
<td>3</td>
</tr>
<tr>
<td>Black tree monitor</td>
<td>Varanus beccari</td>
<td>3</td>
</tr>
<tr>
<td>Sandfish skink</td>
<td>Scincus scincus</td>
<td>2</td>
</tr>
<tr>
<td>Water monitor lizard</td>
<td>Varanus salvator</td>
<td>2</td>
</tr>
<tr>
<td>Spiny-tailed agamid</td>
<td>Agama atricolliz</td>
<td>1</td>
</tr>
<tr>
<td>Chameleon</td>
<td>Chamaeleo sp.</td>
<td>1</td>
</tr>
<tr>
<td>False chameleon</td>
<td>Chamaeleolis camaleontides</td>
<td>1</td>
</tr>
<tr>
<td>Collared lizard</td>
<td>Crotaphytus collaris</td>
<td>1</td>
</tr>
<tr>
<td>Gecko</td>
<td>Sauria</td>
<td>1</td>
</tr>
<tr>
<td>Gliding gecko</td>
<td>Sauria</td>
<td>1</td>
</tr>
<tr>
<td>Beaded lizard</td>
<td>Heloderma horridum</td>
<td>1</td>
</tr>
<tr>
<td>Gila monster</td>
<td>Heloderma suspectum</td>
<td>1</td>
</tr>
<tr>
<td>Lizard</td>
<td>Sauria</td>
<td>1</td>
</tr>
<tr>
<td>Glass lizard</td>
<td>Ophiosaurus varitalis</td>
<td>1</td>
</tr>
<tr>
<td>Blue-tailed skink</td>
<td>Tiliqua scincoides</td>
<td>1</td>
</tr>
<tr>
<td>Shingleback skink</td>
<td>Trachydosaurus rugosus</td>
<td>1</td>
</tr>
<tr>
<td>Mangrove monitor</td>
<td>Varanus indicus</td>
<td>1</td>
</tr>
<tr>
<td>Timor monitor lizard</td>
<td>Varanus timorensis</td>
<td>1</td>
</tr>
<tr>
<td>Squamata/Serpentes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gaboon viper</td>
<td>Bitis gabonica</td>
<td>12</td>
</tr>
<tr>
<td>Eyelash viper</td>
<td>Bothriechis sclegeli</td>
<td>11</td>
</tr>
<tr>
<td>Green tree python</td>
<td>Chondropython viridis</td>
<td>9</td>
</tr>
<tr>
<td>Eastern diamondback rattlesnake</td>
<td>Crotalus adamanteus</td>
<td>9</td>
</tr>
<tr>
<td>Diamond python</td>
<td>Python curtus</td>
<td>7</td>
</tr>
<tr>
<td>Timber rattlesnake</td>
<td>Crotalus horridus</td>
<td>6</td>
</tr>
<tr>
<td>Emerald tree boa</td>
<td>Corallus caninav</td>
<td>5</td>
</tr>
<tr>
<td>Cottonmouth moccasin</td>
<td>Agkistrodon piscivorus</td>
<td>3</td>
</tr>
<tr>
<td>Rattlesnake</td>
<td>Crotalus sp.</td>
<td>3</td>
</tr>
<tr>
<td>Eastern indigo snake</td>
<td>Drymarchon conis</td>
<td>3</td>
</tr>
<tr>
<td>King cobra</td>
<td>Ophiophagus hannah</td>
<td>3</td>
</tr>
<tr>
<td>Mangrove pit viper</td>
<td>Trimeresurus purpureomaculatus</td>
<td>3</td>
</tr>
<tr>
<td>Taylor's cantil viper</td>
<td>Agkistrodon bilineatus taylor</td>
<td>2</td>
</tr>
<tr>
<td>African shield-nosed cobra</td>
<td>Aspidelaps scutatus</td>
<td>2</td>
</tr>
<tr>
<td>Amazon tree boa</td>
<td>Boa corks</td>
<td>2</td>
</tr>
<tr>
<td>Urutu snake</td>
<td>Bothrops alternatus</td>
<td>2</td>
</tr>
<tr>
<td>Horned sand viper</td>
<td>Cerastes cerastes</td>
<td>2</td>
</tr>
<tr>
<td>Southwestern speckled rattlesnake</td>
<td>Crotalus michilli pyrrhus</td>
<td>2</td>
</tr>
<tr>
<td>Yucutan neotropical rattlesnake</td>
<td>Crotalus sp.</td>
<td>2</td>
</tr>
<tr>
<td>Green mamba</td>
<td>Dendroaspis angusticeps</td>
<td>2</td>
</tr>
<tr>
<td>Corn snake</td>
<td>Elaphe guttata</td>
<td>2</td>
</tr>
</tbody>
</table>
Annulated boa (*Epicrates* sp.)
Anaconda (*Eunectes murinus*)
Children's python (*Morelia childreni*)
Egyptian cobra (*Naja haje*)
Banded water snake (*Natrix* sp.)
Snake (Serpentes)
Mexican bush viper (*Vipera* sp.)
Death adder (*Acanthophis antarcticus*)
Southern copperhead moccasin (*Agkistrodon contortrix*)
Rhinoceros viper (*Bitis gabonica rhinoceros*)
Sand viper (*Cerastes vipera*)
Ornate sand viper (*Cerastes vipera* spp.)
Western shovelnose snake (*Chionactes* sp.)
Red racer (*Coluber* sp.)
Western diamondback rattlesnake (*Crotalus atrox*)
Black tailed rattlesnake (*Crotalus molossus*)
Mojave rattlesnake (*Crotalus scutulatus*)
Tiger rattlesnake (*Crotalus tigris*)
Aruba island rattlesnake (*Crotalus unicolor*)
Black mamba (*Dendroaspis polylepis*)
Black rat snake (*Elaphe obsoleta* spp.)
Yellow rat snake (*Elaphe* sp.)
Gray rat snake (*Elaphe* sp.)
Sand boa (*Eryx* sp.)
Yellow anaconda (*Eunectes* sp.)
Eastern hognose snake (*Heterodon platyrhinos*)
South American bushmaster (*Lachesis muta*)
Eastern kingsnake (*Lampropeltis getulus getulus*)
King snake (*Lampropeltis* sp.)
Coachwhip (*Masticophis flagellum*)
Red coachwhip (*Masticophis flagellum* spp.)
Bibron's side-stabbing snake (*Pelochelys bibroni*)
Leaf nosed snake (*Phyllorhynchus decurtatus*)
Florida pine snake (*Pituophis melanoleucus*)
Mexican jumping viper (*Porthidium mummifer mexicanum*)
Ball python (*Python regius*)
Andean milksnake (*Scoloporus poinsetti*)
Pygmy rattlesnake (*Sistrurus miliarius*)
Wagler's temple pit viper (*Trimeresurus wagleri*)
Hog island boa (*Ungaliophis* sp.)
Russell's viper (*Vipera russeli*)
Daglet's viper (*Vipera* sp.)
Eastern smooth earth snake (*Virginia virginia valeriae*)
### Crocodylia

American alligator (*Alligator mississippiensis*)

### Chelonia

<table>
<thead>
<tr>
<th>Species</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tortoise (Chelonia)</td>
<td>4</td>
</tr>
<tr>
<td>Turtle (Chelonia)</td>
<td>3</td>
</tr>
<tr>
<td>Ringed map turtle (<em>Graptemys oculifera</em>)</td>
<td>2</td>
</tr>
<tr>
<td>Midlands painted turtle (<em>Chrysemys picta</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Wood turtle (<em>Clemmys insculpta</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Hawksbill turtle (<em>Eretmochelys imbricata</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Desert tortoise (<em>Gopherus agassizi</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Map turtle hybrid (<em>Graptemys sp.</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Mud turtle (<em>Kinosternon subrubrum</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Pancake tortoise (<em>Malacochersus tormei</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Box turtle (<em>Terrapene carolina</em>)</td>
<td>1</td>
</tr>
</tbody>
</table>

### Squamata/Sauria

<table>
<thead>
<tr>
<th>Species</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common iguana (<em>Iguana iguana</em>)</td>
<td>39</td>
</tr>
<tr>
<td>Lizard (Sauria)</td>
<td>11</td>
</tr>
<tr>
<td>Water dragon lizard (<em>Physignathus sp.</em>)</td>
<td>4</td>
</tr>
<tr>
<td>Nile monitor lizard (<em>Varanus niloticus</em>)</td>
<td>3</td>
</tr>
<tr>
<td>Monitor lizard (<em>Varanus sp.</em>)</td>
<td>3</td>
</tr>
<tr>
<td>Chameleon (<em>Chamaeleo sp.</em>)</td>
<td>2</td>
</tr>
<tr>
<td>Prehensile-tail skink (<em>Corucia zebrata</em>)</td>
<td>2</td>
</tr>
<tr>
<td>Green lizard (<em>Lacerta viridis</em>)</td>
<td>2</td>
</tr>
<tr>
<td>Basilisk lizard (<em>Basiliscus sp.</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Senegal chameleon (<em>Chamaeleo sp.</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Rock iguana (<em>Cyclura sp.</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Gliding gecko (Sauria)</td>
<td>1</td>
</tr>
<tr>
<td>Gecko (Sauria)</td>
<td>1</td>
</tr>
<tr>
<td>Alligator lizard (<em>Gerrhonotus sp.</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Glass lizard (<em>Ophiosaurus ventralis</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Skink (Sauria)</td>
<td>1</td>
</tr>
<tr>
<td>Spiny tailed skink (<em>Scincus philbyi</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Shingleback skink (<em>Trachydosaurus rugosus</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Savannah monitor (<em>Varanus exanthematicus</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Timor monitor lizard (<em>Varanus timorensis</em>)</td>
<td>1</td>
</tr>
</tbody>
</table>

### Squamata/Serpentes

<table>
<thead>
<tr>
<th>Species</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ball python (<em>Python regius</em>)</td>
<td>19</td>
</tr>
<tr>
<td>Corn snake (<em>Elaphe guttata</em>)</td>
<td>9</td>
</tr>
<tr>
<td>Red tailed boa (<em>Boa sp.</em>)</td>
<td>7</td>
</tr>
<tr>
<td>Burmese python (<em>Python molurus bivittatus</em>)</td>
<td>6</td>
</tr>
</tbody>
</table>
Boa (Boa sp.)
Snake (Serpentes)
Python (Python sp.)
King snake (Lampropeltis sp.)
Red rat snake (Elaphe sp.)
Puff adder (Bitis arietans)
Green tree python (Chondropython viridis)
Emerald tree boa (Corallus canina)
Venezuelan rattlesnake (Crotalus durissus)
Red diamond rattlesnake (Crotalus ruber)
Black mamba (Dendroaspis polylepis)
Black rat snake (Elaphe obsoleta spp.)
Rat snake (Elaphe sp.)
Chinese corn snake (Elaphe sp.)
Mandarin rat snake (Elaphe sp.)
California king snake (Lampropeltis getulus californiae)
Albino king snake (Lampropeltis sp.)
British water snake (Natrix natrix)
Midland water snake (Natrix sipedon pleuralis)
Florida pine snake (Pituophis melanoleucus)
Forest cobra (Pseudohaje goldii)
Indian rock python (Python molurus)
Reticulated python (Python reticulatus)
Albino python (Python sp.)
Carolina pygmy rattlesnake (Sistrurus miliarius)
Tiger rat snake (Spilotes pullatus)
Albino garter snake (Thamnophis sp.)
Black tree snake (Thasops jacksoni)
Viper (Vipera sp.)

Table 3. Classification of diseases by type.

Degenerative (43)
Atherosclerosis 1
Hepatic atrophy 1
Hepatocellular vacuolar degeneration 14
Myocardial degeneration 2
Myofiber degeneration 13
Neuronal degeneration 2

1995 JOINT CONFERENCE AAZV / WDA / AAWV 471
Osteopenia 1
Parathyroid atrophy 1
Renal glomerulopathy 2
Renal tubular hemosiderosis 1
Renal tubular nephrosis and fibrosis 1
Testicular atrophy 3
Testicular lipofuscinosis 1

**Infectious (281)**
Parasitic Amoebic hepatitis 1
Bacterial and parasitic/Amoebic septic necrotizing hepatitis 1
Bacterial and parasitic/Amoebic septic ulcerative fibrinonecrotic enteritis 1
Bacterial rods/Bacillary septicemia 1
Bacterial and fungal/Bacterial and mycotic dermatitis 9
Bacterial and fungal/Bacterial and mycotic stomatitis 1
Bacterial rods/Bacterial dermatitis 25
Bacterial rods/Bacterial enteritis 10
Bacterial rods/Bacterial myositis 2
Bacterial rods/Bacterial septicemia 19
Parasitic/Biliary trematodiasis 1
Parasitic/Catarrhal and eosinophilic bronchitis with intrapulmonary mite 1
Bacterial (+)/Chronic active fibrosing septic purulent myositis (Clostridial) 2
Bacterial (+)/Clostridial cellulitis 1
Bacterial (+)/Clostridial colitis 1
Parasitic/Coccidial enteritis 1
Mycotic/Coccidiodes (granulomatous) pneumonia 1
Parasitic/Colonic balantidiasis 1
Parasitic/Cutaneous acariasis 1
Mycotic/Cutaneous candidiasis 1
Parasitic/Degenerative myositis with parasitic cysts 1
Bacterial rods/Disseminated mycobacteriosis 7
Bacterial/Disseminated mycobacteriosis 1
Mycotic/Disseminated mycosis 1
Parasitic/Enteric cryptosporidiosis 2
Parasitic/Enteritic nematodiasis 1
Parasitic/Gastric cryptosporidiosis 2
Parasitic/Gastric nematodiasis 9
Parasitic/Gastric physolopteriasis 1
Parasitic/Gastric trematodiasis and nematodiasis 1
Parasitic/Gastrointestinal cryptosporidiosis 1
Parasitic/Granulomatous gastroenteritis with metazoan parasites 1
Parasitic/Granulomatous verminous gastritis 1
Parasitic/Hemogregariniasis 4
Parasitic/Intestinal cestodiasis 3
Parasitic/Intestinal coccidiosis 2
<table>
<thead>
<tr>
<th>Disease</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitic/Intestinal cryptosporidiosis</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Intestinal metazoan and ciliate parasitism</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Intestinal nematodiasis</td>
<td>10</td>
</tr>
<tr>
<td>Parasitic/Intestinal parasitism</td>
<td>3</td>
</tr>
<tr>
<td>Parasitic/Mite</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial (mixed)/Mixed bacterial septicemia</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial/Mycobacterial granulomatous hepatitis</td>
<td>1</td>
</tr>
<tr>
<td>Myotic/Mycotic granulomatous hepatitis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial and fungal/Mycotic and mycobacterial mucopurulent enteritis</td>
<td>1</td>
</tr>
<tr>
<td>Myotic/Mycotic dermatitis</td>
<td>11</td>
</tr>
<tr>
<td>Myotic/Mycotic granuloma, submandibular</td>
<td>1</td>
</tr>
<tr>
<td>Myotic/Mycotic granulomas</td>
<td>3</td>
</tr>
<tr>
<td>Myotic/Mycotic granulomatous cellulitis</td>
<td>1</td>
</tr>
<tr>
<td>Myotic/Mycotic granulomatous cellulitis</td>
<td>1</td>
</tr>
<tr>
<td>Myotic/Mycotic granulomatous splenitis</td>
<td>1</td>
</tr>
<tr>
<td>Myotic/Mycotic granulomatous thymitis</td>
<td>1</td>
</tr>
<tr>
<td>Myotic/Mycotic myositis</td>
<td>1</td>
</tr>
<tr>
<td>Myotic/Mycotic osteomyelitis</td>
<td>1</td>
</tr>
<tr>
<td>Myotic/Mycotic pneumonia</td>
<td>1</td>
</tr>
<tr>
<td>Myotic/Mycotic pyogranulomatous cellulitis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial(mixed) and fungal/Mycotic septic myositis</td>
<td>1</td>
</tr>
<tr>
<td>Myotic/Mycotic septic yolk sac</td>
<td>1</td>
</tr>
<tr>
<td>Viral/Necrotizing hepatitis with intranuclear inclusion bodies</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Nematodiasis</td>
<td>3</td>
</tr>
<tr>
<td>Parasitic/Pancreatic duct nematodias</td>
<td>2</td>
</tr>
<tr>
<td>Viral/Paramyxovirus pneumonia</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Parasite</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Parasitic (Acanthocephalid) cysts, peritoneum</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Parasitic purulent enteritis</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Pentostomiasis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Perilaryngeal septic purulent cellulitis</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Peritoneal cestodias</td>
<td>2</td>
</tr>
<tr>
<td>Parasitic/Peritoneal pentostomias</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Pinworm ova</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Protozoal myositis</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Pulmonary acanthocephalidias</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Pulmonary larval migrants</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Pulmonary metazoan parasitism</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Pulmonary parasites</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Purulent protozoal pneumonia</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Pyogranulomatous pentostomias, trachea</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Pyogranulomatous verminous peritonitis and intestinal nematodias</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Renal flukes</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Renal trematodias</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Septic hepatitis</td>
<td>8</td>
</tr>
<tr>
<td>Condition</td>
<td>Count</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Bacterial and parasitic/Septic amoebic necrotizing cloacitis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial (+)/Septic fibrosing cellulitis (Clostridial)</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Septic foreign body granulomas, intestine</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial (+)/Septic granulomatous hepatitis</td>
<td>3</td>
</tr>
<tr>
<td>Bacterial rods/Septic heterophilic granulomas</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Septic heterophilic keratitis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Septic keratin cyst, spleen</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial/Septic mesenteric thrombosis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial (mixed) and fungal/Septic mycotic purulent inflammation</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial (+) and fungal/Septic necropurulent mycotic cellulitis (Clostridial and cocci)</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial (mixed)/Septic necrotic myocarditis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Septic necrotic nonsuppurative myocarditis</td>
<td>2</td>
</tr>
<tr>
<td>Bacterial (mixed)/Septic necrotic purulent tissue</td>
<td>2</td>
</tr>
<tr>
<td>Bacterial rods/Septic necrotizing colitis</td>
<td>4</td>
</tr>
<tr>
<td>Bacterial (mixed)/Septic necrotizing peritonitis</td>
<td>2</td>
</tr>
<tr>
<td>Bacterial rods/Septic necrotizing purulent pyelonephritis</td>
<td>2</td>
</tr>
<tr>
<td>Bacterial rods/Septic nonsuppurative necrotizing hemipenisitis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Septic obstructive purulent tracheitis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Septic osteomyelitis</td>
<td>3</td>
</tr>
<tr>
<td>Bacterial rods/Septic pneumonia</td>
<td>17</td>
</tr>
<tr>
<td>Bacterial rods/Septic purulent conjunctivitis</td>
<td>2</td>
</tr>
<tr>
<td>Bacterial rods/Septic purulent cystitis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial (+)/Septic purulent enteritis (gram + rods)</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial (+)/Septic purulent myositis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Septic purulent omphalitis</td>
<td>4</td>
</tr>
<tr>
<td>Bacterial rods/Septic purulent peritonitis</td>
<td>2</td>
</tr>
<tr>
<td>Bacterial (+)/Septic purulent pneumonia</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Septic purulent rhinitis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Septic purulent ulcerative salpingitis</td>
<td>2</td>
</tr>
<tr>
<td>Bacterial rods/Septic purulonecrotic cellulitis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Septic purulonecrotic cellulitis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial/Septic purulonecrotic pneumonia</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Septic splenitis</td>
<td>3</td>
</tr>
<tr>
<td>Bacterial rods/Septic ulcerative gastritis</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial rods/Septic ulcerative stomatitis</td>
<td>4</td>
</tr>
<tr>
<td>Bacterial rods/Septic yolk sac</td>
<td>2</td>
</tr>
<tr>
<td>Parasitic/Skeletal muscle sarcocystosis</td>
<td>2</td>
</tr>
<tr>
<td>Viral/Syncytial cell, lung</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Ulcerative enteric amebiasis</td>
<td>2</td>
</tr>
<tr>
<td>Parasitic/Verminous (nematode) pneumonia</td>
<td>1</td>
</tr>
<tr>
<td>Parasitic/Verminous pneumonia (pentostomidiasis)</td>
<td>2</td>
</tr>
<tr>
<td>Parasitic/Sarcocytosis</td>
<td>1</td>
</tr>
</tbody>
</table>

**Inflammatory (186)**

Aspiration pneumonia                                           2
Bilateral granulomatous keratoconjunctivitis 1
Catarrhal tracheitis 1
Cerebral edema 1
Colonic fibrosis 1
Dermatitis 12
Edematous nonsuppurative cloacitis 1
Egg yolk peritonitis 1
Encephalitis 1
Enteritis 18
Eosinophilic gastritis 3
Eosinophilic tracheitis with squamous metaplasia 1
Fibrinonecrotic cloacal cellulitis 1
Fibrinonecrotic colitis 1
Fibrosing pancreatitis 2
Gastritis 3
Glomerulonephritis 1
Granulomatous cellulitis 2
Granulomatous fibrosing epiduritis 1
Granulomatous hepatitis 5
Granulomatous inflammation 6
Granulomatous necrotizing splenitis 1
Granulomatous nephritis 2
Granulomatous nephritis 1
Hepatic amyloidosis 2
Hepatic fibrosis 2
Heterophilic granuloma 1
Heterophilic inflammation, corpora lutea 1
Heterophilic uveitis 1
Interstitial nephritis with urate deposition 1
Lymphocytic cellulitis 1
Lymphocytic cellulitis, renal hilus 1
Lymphocytic hepatitis 1
Lymphocytic meningoencephalitis 1
Lymphocytic pancreatitis 1
Lymphocytic tracheitis 1
Lymphoplasmacytic interstitial nephritis 1
Lymphoplasmacytic nephritis, pericholangitis, gastritis 1
Lymphoplasmacytic perivasculitis 1
Myocarditis 5
Myositis 2
Necrotizing granulomatous nephritis 1
Necrotizing granulomatous splenitis 1
Nonsuppurative encephalitis 1
Nonsuppurative interstitial nephritis 1
Nonsuppurative myocarditis 1
Nonsuppurative pneumonitis  1
Osteomyelitis  3
Ovarian miliary granuloma  1
Peritonitis  3
Pneumonia  26
Pulmonary edema  7
Purulent balantitis  1
Purulent cellulitis  1
Purulent conjunctivitis  1
Purulent granulation tissue  1
Purulent hepatic serosisitis  1
Purulent hepatitis  5
Purulent inflammation  1
Purulent interstitial nephritis  1
Purulent keratitis  1
Purulent pyelonephritis  1
Purulent serositis  1
Purulent splenitis  1
Purulent tubulointerstitial nephritis  6
Purulonecrotic adrenalitis  1
Purulonecrotic esophagitis  1
Purulonecrotic inflammation of yolk sack  1
Purulonecrotic omphalitis  1
Purulonecrotic stomatitis  1
Pyogranulomatous cellulitis  1
Pyogranulomatous thymitis  1
Renal amyloidosis  1
Renal edema  1
Renal fibrosis  1
Renal interstitial fibrosis with tubular regeneration  1
Renal pyogranulomatous fibrosis  1
Renal tubular nephrosis  2
Splemic histiocytosis  2
Splenetic lymphoid hyperplasia  1
Steatosis  1
Subcutaneous fibrosis  1
Submucosal edema, stomach and intestines  1
Tubulointerstitial nephritis  1
Ulcerative colitis  1
Ulcerative purulent cloacitis  1
Visceral granulomas  1

Metabolic (194)
Bile accumulation, renal tubular epithelium  1
<table>
<thead>
<tr>
<th>Condition</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colloid goiter</td>
<td>4</td>
</tr>
<tr>
<td>Dermal mineralization</td>
<td>1</td>
</tr>
<tr>
<td>Disseminated soft tissue mineralization</td>
<td>1</td>
</tr>
<tr>
<td>Fibrosis and mineralization of panniculus</td>
<td>1</td>
</tr>
<tr>
<td>Fibrous osteodystrophy</td>
<td>3</td>
</tr>
<tr>
<td>Gastric hemosiderosis</td>
<td>1</td>
</tr>
<tr>
<td>Gastric mineralization</td>
<td>4</td>
</tr>
<tr>
<td>Visceral gout</td>
<td>5</td>
</tr>
<tr>
<td>Hepatic hemosiderosis</td>
<td>17</td>
</tr>
<tr>
<td>Hepatic lipidosis</td>
<td>69</td>
</tr>
<tr>
<td>Hepatic melanosis and hemosiderosis</td>
<td>1</td>
</tr>
<tr>
<td>Medial mineralization, vein</td>
<td>1</td>
</tr>
<tr>
<td>Melanosis, oral and skin</td>
<td>1</td>
</tr>
<tr>
<td>Mesenteric cholesterol granulomas</td>
<td>1</td>
</tr>
<tr>
<td>Mesenteric melanosis</td>
<td>1</td>
</tr>
<tr>
<td>Mineralizing myopathy</td>
<td>1</td>
</tr>
<tr>
<td>Myocardial gout</td>
<td>1</td>
</tr>
<tr>
<td>Myocardial mineralization</td>
<td>5</td>
</tr>
<tr>
<td>Nephrocalcinosis</td>
<td>1</td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>1</td>
</tr>
<tr>
<td>Ovarian hemosiderosis and ceroid accumulation</td>
<td>1</td>
</tr>
<tr>
<td>Ovarian intimal mineralization</td>
<td>1</td>
</tr>
<tr>
<td>Pulmonary hemosiderosis</td>
<td>2</td>
</tr>
<tr>
<td>Pulmonary mineralization</td>
<td>2</td>
</tr>
<tr>
<td>Renal gout</td>
<td>24</td>
</tr>
<tr>
<td>Renal hemosiderosis</td>
<td>12</td>
</tr>
<tr>
<td>Renal lipidosis</td>
<td>1</td>
</tr>
<tr>
<td>Renal tubular mineralization</td>
<td>8</td>
</tr>
<tr>
<td>Skeletal muscle mineralization</td>
<td>1</td>
</tr>
<tr>
<td>Smooth muscle mineralization of colon</td>
<td>1</td>
</tr>
<tr>
<td>Smooth muscle mineralization of lung</td>
<td>1</td>
</tr>
<tr>
<td>Soft tissue mineralization</td>
<td>10</td>
</tr>
<tr>
<td>Splenic hemosiderosis</td>
<td>1</td>
</tr>
<tr>
<td>Splenic melanosis</td>
<td>1</td>
</tr>
<tr>
<td>Testicular fibrosis and atrophy</td>
<td>1</td>
</tr>
<tr>
<td>Urate nephropathy</td>
<td>1</td>
</tr>
<tr>
<td>Urolithiasis</td>
<td>2</td>
</tr>
<tr>
<td>Vascular mineralization</td>
<td>3</td>
</tr>
<tr>
<td>Miscellaneous (65)</td>
<td></td>
</tr>
<tr>
<td>Adrenal gland hyperplasia</td>
<td>2</td>
</tr>
<tr>
<td>Anemia</td>
<td>1</td>
</tr>
<tr>
<td>Bile duct hyperplasia</td>
<td>4</td>
</tr>
<tr>
<td>Cartilagenous metaplasia and fibroplasia</td>
<td>1</td>
</tr>
<tr>
<td>Chondroid hyperplasia</td>
<td>1</td>
</tr>
<tr>
<td>Condition</td>
<td>Count</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Colonic impaction</td>
<td>1</td>
</tr>
<tr>
<td>Corpora amylacea, trachea</td>
<td>1</td>
</tr>
<tr>
<td>Dermal chromophobe hyperplasia</td>
<td>1</td>
</tr>
<tr>
<td>Dystrophic cartilage with mineralization</td>
<td>1</td>
</tr>
<tr>
<td>Esophageal squamous metaplasia</td>
<td>1</td>
</tr>
<tr>
<td>Extramedullary hematopoiesis</td>
<td>1</td>
</tr>
<tr>
<td>Fibrosing gastropathy</td>
<td>1</td>
</tr>
<tr>
<td>Gastric epithelial inclusions</td>
<td>1</td>
</tr>
<tr>
<td>Gastric hyperplasia</td>
<td>3</td>
</tr>
<tr>
<td>Glomerular hyalinosis</td>
<td>1</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1</td>
</tr>
<tr>
<td>Hemorrhagic enteritis</td>
<td>1</td>
</tr>
<tr>
<td>Hepatic congestion</td>
<td>2</td>
</tr>
<tr>
<td>Hepatic melanomacrophage hyperplasia</td>
<td>1</td>
</tr>
<tr>
<td>Hepatic serosal hemorrhage</td>
<td>1</td>
</tr>
<tr>
<td>Hepatic telangiectasia</td>
<td>1</td>
</tr>
<tr>
<td>Hepatopathy</td>
<td>1</td>
</tr>
<tr>
<td>Hyperkeratosis</td>
<td>2</td>
</tr>
<tr>
<td>Inclusion bodies</td>
<td>1</td>
</tr>
<tr>
<td>Interstitial fibrosis and cysts, kidney</td>
<td>1</td>
</tr>
<tr>
<td>Intestinal hemorrhage</td>
<td>1</td>
</tr>
<tr>
<td>Kursteiner's cyst</td>
<td>1</td>
</tr>
<tr>
<td>Lack of osteoid formation</td>
<td>1</td>
</tr>
<tr>
<td>Luteal cysts</td>
<td>1</td>
</tr>
<tr>
<td>Melanosis</td>
<td>1</td>
</tr>
<tr>
<td>Myelopathy with gemistocytes and intranuclear inclusion bodies</td>
<td>1</td>
</tr>
<tr>
<td>Myocardial hemorrhage</td>
<td>1</td>
</tr>
<tr>
<td>Ovarian cyst</td>
<td>1</td>
</tr>
<tr>
<td>Pancreatic hyperplasia</td>
<td>2</td>
</tr>
<tr>
<td>Polycystic kidney</td>
<td>1</td>
</tr>
<tr>
<td>Polycystic liver</td>
<td>1</td>
</tr>
<tr>
<td>Proliferative enteropathy</td>
<td>1</td>
</tr>
<tr>
<td>Pulmonary and air sac congestion</td>
<td>1</td>
</tr>
<tr>
<td>Pulmonary and renal shock bodies</td>
<td>1</td>
</tr>
<tr>
<td>Pulmonary congestion</td>
<td>3</td>
</tr>
<tr>
<td>Pulmonary hemorrhage</td>
<td>3</td>
</tr>
<tr>
<td>Pulmonary osseus metaplasia</td>
<td>1</td>
</tr>
<tr>
<td>Renal tubular epithelial hyperplasia</td>
<td>1</td>
</tr>
<tr>
<td>Retained yolk sac</td>
<td>2</td>
</tr>
<tr>
<td>Splenic congestion</td>
<td>2</td>
</tr>
<tr>
<td>Splenic lymphoid depletion</td>
<td>1</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>3</td>
</tr>
<tr>
<td>Thrombosis, adrenal gland</td>
<td>1</td>
</tr>
</tbody>
</table>
Necrotic (34)
Dermal necrosis 1
Fat necrosis and mineralization 3
Hepatic necrosis 10
Ischemic necrosis 2
Necrotic bone 2
Necrotic tissue 1
Necrotizing gastritis 1
Necrotizing neuritis 1
Pancratic necrosis 2
Renal cortical necrosis and hemosiderosis 1
Renal tubular necrosis 5
Shell necrosis 1
Splanic necrosis 2
Toxic epidermal necrolysis 1
Uterine transmural necrosis 1

Neoplastic (29)
Epithelial Branchial cyst 1
Epithelial Cholangiocarcinoma 1
Mesenchymal Chondrosarcoma 1
Epithelial Cystic adenoma 1
Mesenchymal Disseminated malignant melanoma 1
Epithelial Epidermal cyst 3
Epithelial Epidermal inclusion cysts 1
Mesenchymal Fibrosarcoma 4
Blood Granulocytic myeloproliferative disease 2
Mesenchymal Hemangiosarcoma 1
Epithelial Hematocysts 1
Adipose Lipoma 1
Epithelial Malignant chromatophoroma 1
Lymphoid Malignant lymphoma 2
Mesenchymal Melanoma 1
Epithelial Mucinous colonic adenocarcinoma 1
Mesenchymal Osteochondroma 1
Epithelial Oviductal adenoma 1
Epithelial Renal carcinoma 1
Epithelial Renal tubular adenocarcinoma 1
Epithelial Renal tubular adenoma 1
Mesenchymal Undifferentiated sarcoma 1
Trauma (2)
Healing fractures 1
Vertebral and rib fractures with fibroplasia 1

No microscopic lesions (55)

Table 4. Organs and tissue systems with histologic lesions, classified by disease type.

<table>
<thead>
<tr>
<th>Adipose(4)</th>
<th>Inflammatory 1</th>
<th>Necrotic 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal(4)</td>
<td>Inflammatory 1</td>
<td>Miscellaneous 3</td>
</tr>
<tr>
<td>Air sac(1)</td>
<td>Miscellaneous 1</td>
<td></td>
</tr>
<tr>
<td>Bone(17)</td>
<td>Degenerative 1</td>
<td>Infectious 4</td>
</tr>
<tr>
<td></td>
<td>Infectious 4</td>
<td>Inflammatory 3</td>
</tr>
<tr>
<td></td>
<td>Metabolic 4</td>
<td>Miscellaneous 1</td>
</tr>
<tr>
<td></td>
<td>Necrotic 2</td>
<td>Neoplastic 1</td>
</tr>
<tr>
<td></td>
<td>Trauma 2</td>
<td></td>
</tr>
<tr>
<td>Brain(8)</td>
<td>Degenerative 2</td>
<td>Inflammatory 5</td>
</tr>
<tr>
<td></td>
<td>Miscellaneous 1</td>
<td></td>
</tr>
<tr>
<td>Cardiac(17)</td>
<td>Degenerative 2</td>
<td>Infectious 2</td>
</tr>
<tr>
<td></td>
<td>Infectious 2</td>
<td>Inflammatory 6</td>
</tr>
<tr>
<td></td>
<td>Metabolic 6</td>
<td>Miscellaneous 1</td>
</tr>
<tr>
<td>Cartilage(4)</td>
<td>Miscellaneous 3</td>
<td>Neoplastic 1</td>
</tr>
<tr>
<td>Cloaca(5)</td>
<td>Infectious 2</td>
<td>Inflammatory 3</td>
</tr>
<tr>
<td>Organ</td>
<td>Infectious</td>
<td>Inflammatory</td>
</tr>
<tr>
<td>----------------</td>
<td>------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Colon(11)</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Connective tissue(16)</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Esophagus(2)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Eye(7)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>General GI(4)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Intestine(26)</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>Kidney(98)</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Liver(156)</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Lung(80)</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td>Tissue</td>
<td>Infectious</td>
<td>Neoplastic</td>
</tr>
<tr>
<td>-----------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>Multiple</td>
<td>29</td>
<td>3</td>
</tr>
<tr>
<td>Muscle</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Nares</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Nerve</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No microscopic lesions</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Ovary</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Oviduct</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Parathyroid</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hemipenes</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Peritoneum</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>47</td>
<td>13</td>
</tr>
</tbody>
</table>

1995 PROCEEDINGS JOINT CONFERENCE AAZV / WDA / AAWV
<table>
<thead>
<tr>
<th>Organ</th>
<th>Infectious</th>
<th>Inflammatory</th>
<th>Metabolic</th>
<th>Miscellaneous</th>
<th>Necrotic</th>
<th>Neoplastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small intestine (39)</td>
<td>19</td>
<td>18</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen (17)</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach (32)</td>
<td>15</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testes (5)</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymus (2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid (4)</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Trachea (6)</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Umbilical (5)</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown tissue (32)</td>
<td>11</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Vascular (17)</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1995 JOINT CONFERENCE AAZV / WDA / AAWV
<table>
<thead>
<tr>
<th>Category</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic</td>
<td>4</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>4</td>
</tr>
<tr>
<td>Neoplastic</td>
<td>2</td>
</tr>
<tr>
<td>Infectious</td>
<td>3</td>
</tr>
<tr>
<td>Inflammatory</td>
<td>2</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>2</td>
</tr>
</tbody>
</table>
Ratites have a tendency to ingest a variety of foreign objects during routine grazing. Ingestion of metallic objects has been implicated in heavy metal toxicoses due to the absorption of compounds such as zinc, copper, lead, and iron.\textsuperscript{1,3} Common sources of zinc include galvanized fencing (98\% zinc), galvanized fence clips (96\% zinc), and pennies minted in the U.S.A. since 1983 (98\% zinc).\textsuperscript{2,4,6,8} There are few published reports of zinc levels in ratites, with normal values ranging from 22 to 130 parts per million (wet weight).\textsuperscript{3,5,7} This report presents liver zinc values from thirteen ratites (A through M), including 5 ostriches (\textit{Struthio camelus}), 5 emus (\textit{Dromaius novaehollandiae}), and 3 rheas (\textit{Rhea americana}), that were presented to the University of Georgia for necropsy or histopathology. Fresh or frozen unfixed liver and/or liver fixed in 10\% buffered formalin was used for analysis. Both unfixed and fixed liver samples were available for 3 birds (B,D,I). Zinc levels were determined using a wet ashing (oxidation procedure). Results from unfixed liver samples ranged from 37.1 to 208.8 ppm, while results from the 10\% formalin fixed liver samples ranged from 13.1 to 152.9 ppm (Table 1). Two of the five unfixed liver samples had a zinc value greater than the published high normal value (130 ppm), while two of the eleven fixed liver samples had a zinc level greater than 130 ppm. In the three cases where both fresh and formalin fixed tissue was available for analysis, values for the fixed tissues were 65, 28, and 11 percent lower than for the fresh tissues, in birds B, D, and I, respectively (Table 1). The reason for this difference is unknown; however, in all 3 cases the livers were frozen or in formalin for variable lengths of time prior to analysis (6,28, and 41 days). We postulate that dehydration may have occurred while in frozen storage, falsely elevating zinc levels in the fresh tissue. Another more likely possibility is that zinc leached out of the liver while in formalin, falsely lowering the zinc levels in fixed tissue. The pathologic findings for all birds are given in Table 2; zinc toxicosis was not considered to be the cause of death in any of these birds. However, it is interesting to note that five of the birds had histological lesions which have been associated with zinc toxicosis (e.g.: hemosiderosis and pancreatic necrosis).\textsuperscript{2} There were too few birds to analyze species, age, and sex differences. Normal zinc levels appear to be higher in ratites than other avian species (normal Anseriforme liver zinc levels are 34.9 ppm wet weight),\textsuperscript{8} and this may be a result of environmental conditions and diet. Freezing or formalin fixation, may falsely
elevate or lower zinc levels, respectively. Therefore, values need to be interpreted with care. Controlled studies on the effect of freezing and formalin fixation on liver zinc levels are in progress.

LITERATURE CITED


### TABLE 1. ZINC LEVELS IN RATITES

<table>
<thead>
<tr>
<th>BREED</th>
<th>SEX</th>
<th>AGE</th>
<th>FRESH LIVER ppm (wet weight)</th>
<th>FIXED LIVER ppm (wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ostrich (A)</td>
<td>M</td>
<td>2 yr</td>
<td>nd</td>
<td>21.7</td>
</tr>
<tr>
<td>Ostrich (B)</td>
<td>M</td>
<td>adult</td>
<td>208.8</td>
<td>137.1</td>
</tr>
<tr>
<td>Ostrich (C)</td>
<td>M</td>
<td>2 yr</td>
<td>128.7</td>
<td>nd</td>
</tr>
<tr>
<td>Ostrich (D)</td>
<td>M</td>
<td>ukn</td>
<td>146.4</td>
<td>127.8</td>
</tr>
<tr>
<td>Ostrich (E)</td>
<td>ukn</td>
<td>1 wk</td>
<td>nd</td>
<td>69.1</td>
</tr>
<tr>
<td>Emu (F)</td>
<td>F</td>
<td>ukn</td>
<td>nd</td>
<td>111.9</td>
</tr>
<tr>
<td>Emu (G)</td>
<td>ukn</td>
<td>ukn</td>
<td>nd</td>
<td>152.9</td>
</tr>
<tr>
<td>Emu (H)</td>
<td>F</td>
<td>2 yr</td>
<td>nd</td>
<td>69.7</td>
</tr>
<tr>
<td>Emu (I)</td>
<td>M</td>
<td>10 mth</td>
<td>37.1</td>
<td>13.1</td>
</tr>
<tr>
<td>Emu (J)</td>
<td>ukn</td>
<td>3 wk</td>
<td>nd</td>
<td>13.8</td>
</tr>
<tr>
<td>Rhea (K)</td>
<td>ukn</td>
<td>ukn</td>
<td>nd</td>
<td>31.7</td>
</tr>
<tr>
<td>Rhea (L)</td>
<td>ukn</td>
<td>ukn</td>
<td>nd</td>
<td>37.7</td>
</tr>
<tr>
<td>Rhea (M)</td>
<td>ukn</td>
<td>6 mth</td>
<td>89.8</td>
<td>nd</td>
</tr>
</tbody>
</table>

ukn = age or sex unknown
nd = not done
TABLE 2. SIGNIFICANT PATHOLOGICAL FINDINGS

<table>
<thead>
<tr>
<th>BREED</th>
<th>SEX</th>
<th>AGE</th>
<th>PATHOLOGICAL FINDINGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ostrich (A)</td>
<td>M</td>
<td>2 yr</td>
<td>Proventricular impaction and cloacal prolapse</td>
</tr>
<tr>
<td>Ostrich (B)</td>
<td>M</td>
<td>adult</td>
<td>Proventricular impaction, pancreatic necrosis, hemosiderosis (liver), and aspiration pneumonia</td>
</tr>
<tr>
<td>Ostrich (C)</td>
<td>M</td>
<td>2 yr</td>
<td>Hemosiderosis (liver)</td>
</tr>
<tr>
<td>Ostrich (D)</td>
<td>M</td>
<td>unknown</td>
<td>Hemosiderosis (liver, spleen)</td>
</tr>
<tr>
<td>Ostrich (E)</td>
<td>unknown</td>
<td>1 wk</td>
<td>Granulomatous hepatitis (most likely Colibacillosis)</td>
</tr>
<tr>
<td>Emu (F)</td>
<td>F</td>
<td>unknown</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>Emu (G)</td>
<td>unknown</td>
<td>unknown</td>
<td>Hemosiderosis (liver)</td>
</tr>
<tr>
<td>Emu (H)</td>
<td>F</td>
<td>2 yr</td>
<td>No pathology done</td>
</tr>
<tr>
<td>Emu (I)</td>
<td>M</td>
<td>10 mth</td>
<td>Endocarditis and septicemia</td>
</tr>
<tr>
<td>Emu (J)</td>
<td>unknown</td>
<td>3 wk</td>
<td>Undetermined cause of death</td>
</tr>
<tr>
<td>Rhea (K)</td>
<td>unknown</td>
<td>unknown</td>
<td>Mild heterophilic typhlitis, hemosiderosis (liver)</td>
</tr>
<tr>
<td>Rhea (L)</td>
<td>unknown</td>
<td>unknown</td>
<td>unkn</td>
</tr>
<tr>
<td>Rhea (M)</td>
<td>unknown</td>
<td>6 mth</td>
<td>Septicemia; hepatocellular necrosis</td>
</tr>
</tbody>
</table>

ukn = unknown
HISTOLOGIC VARIATIONS IN THE MORPHOLOGY OF PORCUPINE AND HEDGEHOG QUILLS

Michelle Winn Elliott, DVM, Robert W. Dunstan, DVM, MS, Diplomate ACVP, Wendell P. Davis, DVM
Animal Health Diagnostic Laboratory and Department of Pathology, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, 48848, USA

The North American porcupine (Erethizon dorsatum) has long been a veterinarian’s nightmare when the call comes to remove numerous porcupine quills from the mouth of a dog at the losing end of a fight with the armored creature. On the other hand, the East African pygmy hedgehog (Atelerix sp.) is the new and upcoming exotic pet. Although they are both quilled mammals, the way the quills are used for defense is quite different. The hedgehogs quills, which do not detach easily, are located along the top of the head and dorsolateral trunk. When threatened, the hedgehog rolls into a ball, and the quills bristle like a pin cushion, becoming an impenetrable barrier. Both hedgehog and porcupine quills are firm and have needle-sharp tips; however, there are conformational differences. Hedgehog quills are relatively uniform in length and diameter, while porcupine quills are quite variable. In porcupines, quills are present mainly on the dorsum and tail. When threatened, they turn their rear toward the enemy and lash out with their heavily quilled tails. The barbed quills are loosely attached, so they embed in the attacker on contact. In both species, quills are modified hairs that are thicker than normal hairs, are filled with a spongy matrix, and have a fibrous structure in the wall. The quills are more or less cylindrical, tapering at each end. Morphologic examination of skin from these two species defines their differences. At its insertion site, the hedgehog quill has the proximal taper terminating in a bulbous expansion. In hedgehogs, quills are generally in anagen, and on traction the quills break mid-shaft. The quill of the porcupine has a very sharp tip and the base, which is shaped like an arrowhead, is connected to the pelt, and epilates easily. Porcupine quills are generally in telogen. These quills are typically surrounded by abundant tricholemmal cornification (“flame follicles”). This particular pattern of cornification is characteristic of hairs in a prolonged telogen state. This difference explains why the porcupine quill is easily removed in a defensive state.
BESNOITIOSIS IN A MINIATURE DONKEY

Wendell P. Davis, DVM*, Robert W. Dunstan, DVM, MS, Diplomate ACVP, Michelle Winn Elliott, DVM
Animal Health Diagnostic Laboratory and Department of Pathology, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48848, USA

Duncan F. Peters, DVM
LaSalle Veterinary Clinic, 3620 Hwy 2E, Kalispell, MT 59901, USA

A one-year old male Miniature Donkey (Equus asinus) from a herd of eight was presented with a nine month history of pruritic dermatitis. Physical examination revealed diffuse lichenification and scales involving the skin of the face and head and dorsally from the neck to the pelvis. Multiple biopsy specimens were submitted for histologic evaluation. The main histologic alteration was the presence of multiple large, spherical, protozoal cysts, 400-500 μm in diameter, with thick (10-15 μm) cyst walls located within the superficial and deep dermis. These cysts were filled with myriads of 1 x 5 μm bradyzoites. The inflammatory response to these protozoal cysts consisted of a moderate, superficial and deep perivascular, mononuclear inflammatory cell infiltrate, with epidermal hyperplasia and compact orthokeratosis. Ultrastructurally, the bradyzoites contained apicomplexan structures (conid, polar ring, rhoptries, and microtubules) typical of those previously described for coccidia of the genus Besnoitia. This animal was treated with sulfa-drugs. Although there has been some improvement, this regimen has not resulted in clinical cure. In North America besnoitiosis is most commonly recognized in opossums; however, it has been infrequently recognized in reindeer, caribou, and Mexican burros. This appears to be the first confirmed case of besnoitiosis in a Miniature Donkey.
RADIO-TELEMETRY IN BLACK AND WHITE RHINOS IN ZIMBABWE: MANAGEMENT AND RESEARCH COMBINING TO ENHANCE LAW-ENFORCEMENT IN THE PROTECTION OF AN ENDANGERED SPECIES

Michael D. Kock* and Mark W. Atkinson
Veterinary Unit, Department of National Parks and Wildlife Management, PO BOX CY 140, Causeway, Harare, Zimbabwe

In 1993 the Department of National Parks in Zimbabwe adopted an Intensive Protection Zone (IPZ) strategy to protect the remaining black and white rhinos in the Parks and Wildlife Estate. This involved the designation of 4 areas as IPZs with all remaining rhinos either being already resident or relocated into these areas from elsewhere. IPZs have increased and better trained manpower, more vehicles and equipment with increased Governmental and NGO support. Specific management actions include ongoing dehorning and radio-collaring. As of January 1995 over 86 rhinos (black n=69; white n=17) have had radio-collars fitted in the four IPZs. The radio-collaring of rhinos has been undertaken as part of the IPZ strategy whose most important aspect is monitoring for law-enforcement, as well as behavioral monitoring and research. The former is a vital component of protection as knowledge of territories and dispersal of rhinos allows more strategic deployment of anti-poaching patrols. It also allows a rapid response if illegal activity is detected, with increased protection for rhinos in the incursion area. The addition of mortality sensors to transmitters has assisted with law-enforcement and veterinary procedures.

The attachment of telemetry devices on black and white rhinos is problematic due to the anatomy of their necks. Several methods have been devised including telemetry ear tags and horn implants. Collaring of species such as the Indian rhino ( ) has been successful but although collaring methods have been attempted on both black an white rhinos these have not been successful long term. Due to the lack of horns, due to dehorning, for implants and the need for long term monitoring to enhance law enforcement in Zimbabwe's rhino IPZs, a suitable radio-collar design was considered imperative. The initial attempts at using a stretchable elastic (heavy duty upholstery elastic) with a canvas cover were unsuccessful due to breakage and several of these rhinos being poached. A more durable nylon tube covering was designed but collars were plced on too tight. The theory behind this was that a snug collar would not rotate or have a tendency to slip off. Unfortunately initial attempts resulted in pressure necrosis and cutting of the collar insert into the dorsal neck.

Another design was tried consisting of a hose within which a steel cable was inserted attached to the transmitter. Although this design appeared to be animal friendly the majority had slipped off within 3 months. The insert collar design was retained and considerable care taken in adjusting tightness and fit. Following this the design has proven to be the best of several tried with some collars remaining on rhinos for > 16 months with no neck damage.
Tanzania devotes an unprecedented 25% of its land mass to protected wildlife areas; it also has more wildlife than almost any other country in Africa. However, the country is economically depressed and has an annual population growth rate of ~3%. This is a formula for disaster. Conflict between man and wildlife is inevitable and is already occurring in the form of habitat disturbance, transmission of disease between wildlife and domestic animals or humans, and unsustainable exploitation of wildlife for economic gain. Here we describe a program to strengthen Tanzania’s wildlife veterinary medicine program, based at Sokoine University of Agriculture (SUA), to better enable the faculty of veterinary medicine (FVM) to address problems associated with interaction between humans and wildlife. Using current physiological, pathology and surgical monitoring techniques, veterinarians can help prevent the spread of disease; identify impacts of habitat disturbance on wildlife before they become too extreme and costly to rectify; and maintain healthy, viable wildlife in nature and in game farms. Training will occur through a series of workshops, seminars and applied collaborative research programs between the FVM and specialized outside investigators. Emphasis will also be placed on scientific and grant writing. This approach should provide the skills, motivation and confidence necessary to promote FVM participation in applied wildlife veterinary medicine research that is sustainable and vital to good management. Training of undergraduate and graduate students will be improved as a result.

Human monocytic ehrlichiosis, caused by the rickettsia Ehrlichia chaffeensis, was first recognized in the United States in 1986. The reservoir and vector systems remain unknown, although recent studies have implicated white-tailed deer (Odocoileus virginianus) as possible vertebrate reservoirs and lone star ticks (Amblyomma americanum) as potential vectors. Currently, we are attempting to confirm presence of the organism at a suspected endemic site through evaluation of the mammal and tick community. A serosurvey to identify potential mammalian reservoir hosts revealed that white-tailed deer have a high prevalence of E. chaffeensis-reactive antibodies (90%+) and that raccoons and opossums are exposed at a much lower rate (17.5% and 8.3%, respectively). Seven other animal species collected; white-footed mice, cotton rats, Eastern harvest mice, golden mice, gray squirrels, chipmunks, and Eastern cottontail rabbits, were all negative for E. chaffeensis-reactive antibodies. Environmental and host sampling for ticks indicated seven species of ticks were present at the site, of which, A. americanum (nymphs and adults) was the only species found on all seropositive mammalian species. More than 99% of ticks collected by environmental sampling have been A. americanum. Findings from additional research currently underway will be presented including surveys to demonstrate natural E. chaffeensis infection in suspected mammalian reservoir hosts and tick vectors using different culture techniques and the use of PCR to determine exposure in white-tailed deer and ticks collected from the endemic site.

MERCURY CONTAMINATION IN WADING BIRDS FROM SOUTHERN FLORIDA. Maria Soledad Sepulveda, Marilyn G. Spalding, Department of Infectious Diseases, College of Veterinary Medicine; and Peter C. Frederick, Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, Florida, 32611.

With the objective of determining the variability and concentration of mercury in free-ranging nestling great egrets (Casmerodius albus), a total of 123 chicks (58 nests) belonging to eight colonies located mainly in Water Conservation Areas 3A and 3B in the Everglades, were sampled for mercury from late March to mid May, 1994. Both blood (n = 309) and growing scapular feathers (a = 69) were collected. Chicks were first bled when they were approximately 5 days old, and at various intervals until they were 25 to 30 days of age. Mercury concentration in blood and feathers averaged 1.23 mg/kg and
EVALUATION OF A POTENTIAL ENDEMIC SITE FOR THE PRESENCE OF EHRlichia Chaffeensis, the CAUSATIVE AGENT OF HUMAN MONOCYTIC EHRlichiosIs. J. Mitchell Lockhart, William R. Davidson, and David E. Stallknecht. Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602.

Human monocytic ehrlichiosis, caused by the rickettsia Ehrlichia chaffeensis, was first recognized in the United States in 1986. The reservoir and vector systems remain unknown, although recent studies have implicated white-tailed deer (Odocoileus virginianus) as possible vertebrate reservoirs and lone star ticks (Amblyomma americanum) as potential vectors. Currently, we are attempting to confirm presence of the organism at a suspected endemic site through evaluation of the mammal and tick community. A serosurvey to identify potential mammalian reservoir hosts revealed that white-tailed deer have a high prevalence of E. chaffeensis-reactive antibodies (90%+) and that raccoons and opossums are exposed at a much lower rate (17.5% and 8.3%, respectively). Seven other animal species collected; white-footed mice, cotton rats, Eastern harvest mice, golden mice, gray squirrels, chipmunks, and Eastern cottontail rabbits, were all negative for E. chaffeensis-reactive antibodies. Environmental and host sampling for ticks indicated seven species of ticks were present at the site, of which, A. americanum (nymphs and adults) was the only species found on all seropositive mammalian species. More than 99% of ticks collected by environmental sampling have been A. americanum. Findings from additional research currently underway will be presented including surveys to demonstrate natural E. chaffeensis infection in suspected mammalian reservoir hosts and tick vectors using different culture techniques and the use of PCR to determine exposure in white-tailed deer and ticks collected from the endemic site.

MERCURY CONTAMINATION IN WADING BIRDS FROM SOUTHERN FLORIDA. María Soledad Sepúlveda, Marilyn G. Spalding, Department of Infectious Diseases, College of Veterinary Medicine; and Peter C. Frederick, Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, Florida, 32611.

With the objective of determining the variability and concentration of mercury in free-ranging nestling great egrets (Casmerodius albus), a total of 123 chicks (58 nests) belonging to eight colonies located mainly in Water Conservation Areas 3A and 3B in the Everglades, were sampled for mercury from late March to mid May, 1994. Both blood (n = 309) and growing scapular feathers (a = 69) were collected. Chicks were first bled when they were approximately 5 days old, and at various intervals until they were 25 to 30 days of age. Mercury concentration in blood and feathers averaged 1.23 mg/kg and
16.08 mg/kg, respectively and were significantly correlated (n = 77, r = 0.67, P = 0.0001). Preliminary results indicate that there was no significant correlation between chick size and mercury concentration in blood. The concentrations of mercury in great egret nestlings from southern Florida are within the concentrations reported to cause adverse effects on growth and behavior in other bird species. Data on the sublethal effects of methyl mercury chloride in captive wading birds will also be presented.

EFFECT OF CANINE DISTEMPER ON AN URBAN RACCOON POPULATION: AN EXPERIMENT. Claudia A. Schubert, Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

Large-scale experiments about the effect of disease on wildlife populations are rare. We examined (1) whether the prevalence of canine distemper (CD) could be reduced in urban raccoons using field vaccination and (2) the extent to which CD might limit populations. Raccoons in a treatment area were trapped, vaccinated and released at the site of capture. Population responses were monitored in treatment and control areas using information from a municipal animal control agency. During a CD epizootic, the prevalence was significantly lower (Z = 1.280, 0.02 < P < 0.05) in the treated area (1.4%) than in the control area (8.3%). There were significantly more raccoons seropositive for CD antibody in the treatment area than in the control area during the epizootic. Canine distemper antibody prevalence tended to be higher in the treatment area than in the control area. Patterns of population change did not differ between the treatment and control areas before, during or after the epizootic (R² = 79.2, P = 0.001) indicating that CD did not limit the raccoon population. Thus, field vaccination can result in reduced disease prevalence, but will not necessarily result in increased costs for nuisance animal control.

THE ROLE OF NUTRIENT ENRICHMENT IN THE PREVALENCE OF FISH INFECTED WITH A WADING BIRD PARASITE (EUSTRONGYLIDES IGNOTUS). Donald F. Coyner, Department of Infectious Diseases, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32611.

Infection with the nematode parasite *Eustrongylides ignotus* has been shown to be a significant hazard to the reproduction of wading birds in Florida that nest near foraging sites with anthropogenic sources of nutrient input. It appears that nutrient pollution causes increases in oligochaete populations, which then transmit the parasite to fish, and birds acquire the infection from consuming infected fish. Examples of four habitat types were examined including 1) sewage plant runoff, 2) stormwater runoff, 3) dairy runoff, and 4) sugar cane runoff. Prevalences of infected fish ranged from 0 to > 30%.
16.08 mg/kg, respectively and were significantly correlated (n = 77, r = 0.67, P = 0.0001). Preliminary results indicate that there was no significant correlation between chick size and mercury concentration in blood. The concentrations of mercury in great egret nestlings from southern Florida are within the concentrations reported to cause adverse effects on growth and behavior in other bird species. Data on the sublethal effects of methyl mercury chloride in captive wading birds will also be presented.

EFFECT OF CANINE DISTEMPER ON AN URBAN RACCOON POPULATION: AN EXPERIMENT. Claudia A. Schubert, Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

Large-scale experiments about the effect of disease on wildlife populations are rare. We examined (1) whether the prevalence of canine distemper (CD) could be reduced in urban raccoons using field vaccination and (2) the extent to which CD might limit populations. Raccoons in a treatment area were trapped, vaccinated and released at the site of capture. Population responses were monitored in treatment and control areas using information from a municipal animal control agency. During a CD epizootic, the prevalence was significantly lower (Z = 1.280, 0.02 < P < 0.05) in the treated area (1.4%) than in the control area (8.3%). There were significantly more raccoons seropositive for CD antibody in the treatment area than in the control area during the epizootic. Canine distemper antibody prevalence tended to be higher in the treatment area than in the control area. Patterns of population change did not differ between the treatment and control areas before, during or after the epizootic (R² = 79.2, P = 0.001) indicating that CD did not limit the raccoon population. Thus, field vaccination can result in reduced disease prevalence, but will not necessarily result in increased costs for nuisance animal control.

THE ROLE OF NUTRIENT ENRICHMENT IN THE PREVALENCE OF FISH INFECTED WITH A WADING BIRD PARASITE (EUSTRONGYLIDES IGNOTUS). Donald F. Coyner, Department of Infectious Diseases, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32611.

Infection with the nematode parasite Eustrongylides ignotus has been shown to be a significant hazard to the reproduction of wading birds in Florida that nest near foraging sites with anthropogenic sources of nutrient input. It appears that nutrient pollution causes increases in oligochaete populations, which then transmit the parasite to fish, and birds acquire the infection from consuming infected fish. Examples of four habitat types were examined including 1) sewage plant runoff, 2) stormwater runoff, 3) dairy runoff, and 4) sugar cane runoff. Prevalences of infected fish ranged from 0 to > 30%.
16.08 mg/kg, respectively and were significantly correlated (n = 77, r = 0.67, P = 0.0001). Preliminary results indicate that there was no significant correlation between chick size and mercury concentration in blood. The concentrations of mercury in great egret nestlings from southern Florida are within the concentrations reported to cause adverse effects on growth and behavior in other bird species. Data on the sublethal effects of methyl mercury chloride in captive wading birds will also be presented.

**EFFECT OF CANINE DISTEMPER ON AN URBAN RACCOON POPULATION: AN EXPERIMENT.** Claudia A. Schubert, Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

Large-scale experiments about the effect of disease on wildlife populations are rare. We examined (1) whether the prevalence of canine distemper (CD) could be reduced in urban raccoons using field vaccination and (2) the extent to which CD might limit populations. Raccoons in a treatment area were trapped, vaccinated and released at the site of capture. Population responses were monitored in treatment and control areas using information from a municipal animal control agency. During a CD epizootic, the prevalence was significantly lower (Z = 1.280, 0.02 < P < 0.05) in the treated area (1.4%) than in the control area (8.3%). There were significantly more raccoons seropositive for CD antibody in the treatment area than in the control area during the epizootic. Canine distemper antibody prevalence tended to be higher in the treatment area than in the control area. Patterns of population change did not differ between the treatment and control areas before, during or after the epizootic (R² = 79.2, P = 0.001) indicating that CD did not limit the raccoon population. Thus, field vaccination can result in reduced disease prevalence, but will not necessarily result in increased costs for nuisance animal control.

**THE ROLE OF NUTRIENT ENRICHMENT IN THE PREVALENCE OF FISH INFECTED WITH A WADING BIRD PARASITE (EUSTRONGYLIDES IGNOTUS).** Donald F. Coyner, Department of Infectious Diseases, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32611.

Infection with the nematode parasite *Eustrongylides ignotus* has been shown to be a significant hazard to the reproduction of wading birds in Florida that nest near foraging sites with anthropogenic sources of nutrient input. It appears that nutrient pollution causes increases in oligochaete populations, which then transmit the parasite to fish, and birds acquire the infection from consuming infected fish. Examples of four habitat types were examined including 1) sewage plant runoff, 2) stormwater runoff, 3) dairy runoff, and 4) sugar cane runoff. Prevalences of infected fish ranged from 0 to > 30%.
POST-NATAL SURVIVORSHIP IN HARBOR SEALS (PHOCA VITULINA): THYROID HORMONE DYNAMICS DURING THE METABOLICALLY DEMANDING NURSING PERIOD. Martin Haulena and Pádraig J. Duignan., Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1; David J. St. Aubin, Mystic Marinelife Aquarium, 55 Coogan Blvd., Mystic, CT., 06355-1997.

The post-natal period (up to 1 year of age) is the time of greatest mortality in phocid seals. The condition of the pup at weaning is likely a significant determinant of survivorship. Thyroid hormones (TH) may be important modulators of energy mobilization, fat deposition, growth and immune development. As part of a multidisciplinary study aimed at investigating mortality factors of harbor seals (Phoca vitulina) on Sable Island, Nova Scotia, we examined thyroid function in mothers and pups to establish normal thyroid dynamics. We monitored circulating levels of free and total thyroxine (T4) and triiodothyronine (T3), and reverse-T3 in 13 free-living female harbor seals and their pups for up to 25 days after birth. Early hormone levels were significantly (p < 0.01) higher in the pups than in their mothers. Pup levels decreased progressively to maternal levels by the end of the lactation. Since high thyroid levels are associated with an increased metabolic rate allowing for mobilization of energy stores, the high pup values during a time of fat deposition suggest a different role for the hormones from that in adults. Reverse-T3 concentrations were found to be especially high in the pups immediately after birth, possibly indicating inactivation of TH. Our findings highlight the dynamic nature of thyroid hormones and their involvement in regulating metabolism through this critical period.

MORBILIVIRUS INFECTION IN CETACEANS OF THE WESTERN ATLANTIC: AN ECOSYSTEM APPROACH. Pádraig J. Duignan and Joseph R. Geraci, Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

Morbillivirus infection is now recognized as the most pathogenic viral disease of odontocete cetaceans. A longitudinal epidemiologic investigation for evidence of infection in odontocetes was conducted between 1982 and 1994 in three ecosystems of the western Atlantic. Serum samples were examined for specific antibodies by virus neutralization test and radio-immunoprecipitation, while tissues were examined for lesions by standard techniques and immuno-histochemistry. Samples were obtained from free-ranging and stranded animals and included one species from the Arctic ecosystem (Hudson Bay, Canada), three from cold temperate waters (Atlantic Canada and Gulf of Maine) and 14 from warm temperate and sub-tropical waters (Southern New England to the Gulf of Mexico). There was no evidence of infection among Hudson Bay beluga whales, Delphinapterus leucas (n = 35), sampled between 1985 and 1992. By contrast, all three species from cold temperate waters showed evidence of exposure to morbillivirus. The prevalence of infection was significantly higher (P < 0.0001) among long-finned pilot whales, Globicephala melas (92%, n = 100), than either harbor porpoises, Phocoena phocoena (19%, n = 53), or white-sided dolphins, Lagenorhynchus acutus (3%, n = 37). Clinical
POST-NATAL SURVIVORSHIP IN HARBOR SEALS (PHOCA VITULINA): THYROID HORMONE DYNAMICS DURING THE METABOLICALLY DEMANDING NURSING PERIOD. Martin Haulena and Pádraig J. Duignan., Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1; David J. St. Aubin, Mystic Marinelife Aquarium, 55 Coogan Blvd., Mystic, CT., 06355-1997.

The post-natal period (up to 1 year of age) is the time of greatest mortality in phocid seals. The condition of the pup at weaning is likely a significant determinant of survivorship. Thyroid hormones (TH) may be important modulators of energy mobilization, fat deposition, growth and immune development. As part of a multidisciplinary study aimed at investigating mortality factors of harbor seals (Phoca vitulina) on Sable Island, Nova Scotia, we examined thyroid function in mothers and pups to establish normal thyroid dynamics. We monitored circulating levels of free and total thyroxine ($T_4$) and triiodothyronine ($T_3$), and reverse-$T_3$ in 13 free-living female harbor seals and their pups for up to 25 days after birth. Early hormone levels were significantly ($p < 0.01$) higher in the pups than in their mothers. Pup levels decreased progressively to maternal levels by the end of the lactation. Since high thyroid levels are associated with an increased metabolic rate allowing for mobilization of energy stores, the high pup values during a time of fat deposition suggest a different role for the hormones from that in adults. Reverse-$T_3$ concentrations were found to be especially high in the pups immediately after birth, possibly indicating inactivation of TH. Our findings highlight the dynamic nature of thyroid hormones and their involvement in regulating metabolism through this critical period.

MORBILLIVIRUS INFECTION IN CETACEANS OF THE WESTERN ATLANTIC: AN ECOSYSTEM APPROACH. Pádraig J. Duignan and Joseph R. Geraci, Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

Morbillivirus infection is now recognized as the most pathogenic viral disease of odontocete cetaceans. A longitudinal epidemiologic investigation for evidence of infection in odontocetes was conducted between 1982 and 1994 in three ecosystems of the western Atlantic. Serum samples were examined for specific antibodies by virus neutralization test and radio-immunoprecipitation, while tissues were examined for lesions by standard techniques and immuno-histochemistry. Samples were obtained from free-ranging and stranded animals and included one species from the Arctic ecosystem (Hudson Bay, Canada), three from cold temperate waters (Atlantic Canada and Gulf of Maine) and 14 from warm temperate and sub-tropical waters (Southern New England to the Gulf of Mexico). There was no evidence of infection among Hudson Bay beluga whales, Delphinapterus leucas ($n = 35$), sampled between 1985 and 1992. By contrast, all three species from cold temperate waters showed evidence of exposure to morbillivirus. The prevalence of infection was significantly higher ($P < 0.0001$) among long-finned pilot whales, Globicephala melas ($92\%, n = 100$), than either harbor porpoises, Phocoena phocoena ($19\%, n = 53$), or white-sided dolphins, Lagenorhynchus acutus ($3\%, n = 37$). Clinical
POST-NATAL SURVIVORSHIP IN HARBOR SEALS (PHOCA VITULINA): THYROID HORMONE DYNAMICS DURING THE METABOLICALLY DEMANDING NURSING PERIOD. Martin Haulena and Pádraig J. Duignan., Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1; David J. St. Aubin, Mystic Marinelife Aquarium, 55 Coogan Blvd., Mystic, CT., 06355-1997.

The post-natal period (up to 1 year of age) is the time of greatest mortality in phocid seals. The condition of the pup at weaning is likely a significant determinant of survivorship. Thyroid hormones (TH) may be important modulators of energy mobilization, fat deposition, growth and immune development. As part of a multidisciplinary study aimed at investigating mortality factors of harbor seals (Phoca vitulina) on Sable Island, Nova Scotia, we examined thyroid function in mothers and pups to establish normal thyroid dynamics. We monitored circulating levels of free and total thyroxine (T4) and triiodothyronine (T3), and reverse-T3 in 13 free-living female harbor seals and their pups for up to 25 days after birth. Early hormone levels were significantly (p < 0.01) higher in the pups than in their mothers. Pup levels decreased progressively to maternal levels by the end of the lactation. Since high thyroid levels are associated with an increased metabolic rate allowing for mobilization of energy stores, the high pup values during a time of fat deposition suggest a different role for the hormones from that in adults. Reverse-T3 concentrations were found to be especially high in the pups immediately after birth, possibly indicating inactivation of TH. Our findings highlight the dynamic nature of thyroid hormones and their involvement in regulating metabolism through this critical period.

MORBILLIVIRUS INFECTION IN CETACEANS OF THE WESTERN ATLANTIC: AN ECOSYSTEM APPROACH. Pádraig J. Duignan and Joseph R. Geraci, Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

Morbillivirus infection is now recognized as the most pathogenic viral disease of odontocete cetaceans. A longitudinal epidemiologic investigation for evidence of infection in odontocetes was conducted between 1982 and 1994 in three ecosystems of the western Atlantic. Serum samples were examined for specific antibodies by virus neutralization test and radio-immunoprecipitation, while tissues were examined for lesions by standard techniques and immuno-histochemistry. Samples were obtained from free-ranging and stranded animals and included one species from the Arctic ecosystem (Hudson Bay, Canada), three from cold temperate waters (Atlantic Canada and Gulf of Maine) and 14 from warm temperate and sub-tropical waters (Southern New England to the Gulf of Mexico). There was no evidence of infection among Hudson Bay beluga whales, Delphinapterus leucas (n = 35), sampled between 1985 and 1992. By contrast, all three species from cold temperate waters showed evidence of exposure to morbillivirus. The prevalence of infection was significantly higher (P < 0.0001) among long-finned pilot whales, Globicephala melas (92%, n = 100), than either harbor porpoises, Phocoena phocoena (19%, n = 53), or white-sided dolphins, Lagenorhynchus acutus (3%, n = 37). Clinical
disease was also found in one pilot whale calf that stranded in 1989. Ten of 14 species from
warm temperate and sub-tropical waters showed evidence of infection including short-finned
pilot whales, *G. macrorhynchus* (64%, *n* = 25) and bottlenose dolphins, *Tursiops truncatus*
(22%, *n* = 220). We propose that differences in infection prevalence between species may
be accounted for by population size and structure, social organization and behaviour. Our
data support the hypothesis that infection may be enzootic among both pilot whale species.
We propose that pilot whales are important reservoir hosts for morbillivirus and may also
be vectors of infection between species.

**STUDIES ON THE VECTORIAL CAPACITY OF CULICOIDES SPP. (DIPTERA:
CERATOPOGONIDAE) FOR HEMORRHAGIC DISEASE VIRUSES OF WHITE-TAILED
DEER IN THE SOUTHEASTERN UNITED STATES.** Kirk E. Smith, David E. Stallknecht,
Victor F. Nettles, and William R. Davidson. Southeastern Cooperative Wildlife Disease
Study, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602,
USA.

Hemorrhagic disease (HD) is a major disease syndrome of white-tailed deer (WTD)
(*Odocoileus virginianus*) caused by either epizootic hemorrhagic disease virus (EHDV) or
bluetongue virus (BTV). These viruses are vectored by biting midges of the genus
*Culicoides*, but species acting as significant vectors for WTD have not been determined.
Through collections with light traps and from captive WTD, *Culicoides* were monitored at
one HD enzootic site over 18 months and at three sites during HD epizootics among
penned WTD. At the enzootic site, *C. lahillei* and *C. stellifer* comprised 97% of 188,486
*Culicoides* collected from WTD during summer and fall months, when acute cases of HD
in WTD occur in the southeastern United States. *Culicoides lahillei* reached very high
biting intensities during late summer/early fall; 20,840 were collected from a WTD during
a single morning. *Culicoides lahillei* also was the most abundant species collected from
WTD at HD epizootic sites. In an experimental study, 7.3% of *C. lahillei* became infected
with EHDV after feeding on viremic WTD. *Culicoides variipennis*, a confirmed vector of
BTV and EHDV, was present in low numbers at enzootic and epizootic sites. However, our
data suggests that other *Culicoides* species, particularly *C. lahillei*, also should be given
strong consideration as potential vectors of HD viruses for WTD in the southeastern United
States.

**A SEROLOGICAL SURVEY IN SMALL MAMMALS FOR VESICULAR STOMATITIS
VIRUS IN A DRY TROPICAL LOWLAND ENZOOTIC AREA IN COSTA RICA.** E. J.
Burull, School of Veterinary Medicine, University of Wisconsin-Madison, and L. Rodriguez,
Tropical Disease Research Program, School of Veterinary Medicine, National University
Heredia, Costa Rica.

Vesicular stomatitis virus, an arbovirus, appears in domestic and wild species in the Southern
US and Central and South America. Annual outbreaks of VSV occur in Costa Rican cattle,
causing economic loss due to mastitis, ill-conditioning, and similar appearance to FMD.

1995 JOINT CONFERENCE AAZV / WDA / AAWV
disease was also found in one pilot whale calf that stranded in 1989. Ten of 14 species from warm temperate and sub-tropical waters showed evidence of infection including short-finned pilot whales, *G. macrorhynchus* (64%, n = 25) and bottlenose dolphins, *Tursiops truncatus* (22%, n = 220). We propose that differences in infection prevalence between species may be accounted for by population size and structure, social organization and behaviour. Our data support the hypothesis that infection may be enzootic among both pilot whale species. We propose that pilot whales are important reservoir hosts for morbillivirus and may also be vectors of infection between species.

**STUDIES ON THE VECTORIAL CAPACITY OF CULICOIDES SPP. (DIPTERA: CERATOPOGONIDAE) FOR HEMORRHAGIC DISEASE VIRUSES OF WHITE-TAILED DEER IN THE SOUTHEASTERN UNITED STATES.** Kirk E. Smith, David E. Stallknecht, Victor F. Nettles, and William R. Davidson. Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602, USA.

Hemorrhagic disease (HD) is a major disease syndrome of white-tailed deer (WTD) (*Odocoileus virginianus*) caused by either epizootic hemorrhagic disease virus (EHDV) or bluetongue virus (BTV). These viruses are vectored by biting midges of the genus *Culicoides*, but species acting as significant vectors for WTD have not been determined. Through collections with light traps and from captive WTD, *Culicoides* were monitored at one HD enzootic site over 18 months and at three sites during HD epizootics among penned WTD. At the enzootic site, *C. lahillei* and *C. stellifer* comprised 97% of 188,486 *Culicoides* collected from WTD during summer and fall months, when acute cases of HD in WTD occur in the southeastern United States. *Culicoides lahillei* reached very high biting intensities during late summer/early fall; 20,840 were collected from a WTD during a single morning. *Culicoides lahillei* also was the most abundant species collected from WTD at HD epizootic sites. In an experimental study, 7.3% of *C. lahillei* became infected with EHDV after feeding on viremic WTD. *Culicoides variipennis*, a confirmed vector of BTV and EHDV, was present in low numbers at enzootic and epizootic sites. However, our data suggests that other *Culicoides* species, particularly *C. lahillei*, also should be given strong consideration as potential vectors of HD viruses for WTD in the southeastern United States.

**A SEROLOGICAL SURVEY IN SMALL MAMMALS FOR VESICULAR STOMATITIS VIRUS IN A DRY TROPICAL LOWLAND ENZOOTIC AREA IN COSTA RICA.** E. J. Burull, School of Veterinary Medicine, University of Wisconsin-Madison, and L. Rodriguez, Tropical Disease Research Program, School of Veterinary Medicine, National University Heredia, Costa Rica.

Vesicular stomatitis virus, an arbovirus, appears in domestic and wild species in the Southern US and Central and South America. Annual outbreaks of VSV occur in Costa Rican cattle, causing economic loss due to mastitis, ill-conditioning, and similar appearance to FMD.
disease was also found in one pilot whale calf that stranded in 1989. Ten of 14 species from warm temperate and sub-tropical waters showed evidence of infection including short-finned pilot whales, *G. macrorhynchus* (64%, n = 25) and bottlenose dolphins, *Tursiops truncatus* (22%, n = 220). We propose that differences in infection prevalence between species may be accounted for by population size and structure, social organization and behaviour. Our data support the hypothesis that infection may be enzootic among both pilot whale species. We propose that pilot whales are important reservoir hosts for morbillivirus and may also be vectors of infection between species.

STUDIES ON THE VECTORIAL CAPACITY OF *CULICOIDES* SPP. (DIPTERA: CERATOPOGONIDAE) FOR HEMORRHAGIC DISEASE VIRUSES OF WHITE-TAILED DEER IN THE SOUTHEASTERN UNITED STATES. Kirk E. Smith, David E. Stallknecht, Victor F. Nettles, and William R. Davidson. Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602, USA.

Hemorrhagic disease (HD) is a major disease syndrome of white-tailed deer (WTD) (*Odocoileus virginianus*) caused by either epizootic hemorrhagic disease virus (EHDV) or bluetongue virus (BTV). These viruses are vectored by biting midges of the genus *Culicoides*, but species acting as significant vectors for WTD have not been determined. Through collections with light traps and from captive WTD, *Culicoides* were monitored at one HD enzootic site over 18 months and at three sites during HD epizootics among penned WTD. At the enzootic site, *C. lahillei* and *C. stellifer* comprised 97% of 188,486 *Culicoides* collected from WTD during summer and fall months, when acute cases of HD in WTD occur in the southeastern United States. *Culicoides lahillei* reached very high biting intensities during late summer/early fall; 20,840 were collected from a WTD during a single morning. *Culicoides lahillei* also was the most abundant species collected from WTD at HD epizootic sites. In an experimental study, 7.3% of *C. lahillei* became infected with EHDV after feeding on viremic WTD. *Culicoides variipennis*, a confirmed vector of BTV and EHDV, was present in low numbers at enzootic and epizootic sites. However, our data suggests that other *Culicoides* species, particularly *C. lahillei*, also should be given strong consideration as potential vectors of HD viruses for WTD in the southeastern United States.

A SEROLOGICAL SURVEY IN SMALL MAMMALS FOR VESICULAR STOMATITIS VIRUS IN A DRY TROPICAL LOWLAND ENZOOTIC AREA IN COSTA RICA. E. J. Burull, School of Veterinary Medicine, University of Wisconsin-Madison, and L. Rodriguez, Tropical Disease Research Program, School of Veterinary Medicine, National University Heredia, Costa Rica.

Vesicular stomatitis virus, an arbovirus, appears in domestic and wild species in the Southern US and Central and South America. Annual outbreaks of VSV occur in Costa Rican cattle, causing economic loss due to mastitis, ill-conditioning, and similar appearance to FMD.
VSV-NJ infection has been topographically associated, appearing predominantly at the premontane wet and tropical dryland forest elevations. VSV-IN seroprevalence has not varied with environment. Cattle, insect, and small mammal surveys were conducted in VSV-NJ enzootic areas in 1988 and in 1993-1994. The premontane small mammal survey found seroprevalence to VSV in one species, *Sigmodon hispidus*. Tropical dry forest insect surveys found 13 sandfly types at the study site. Only *Lutzomyia shannoni*, a phlebotomine sandfly, contained cow blood. Cattle here originated from other areas and had equal seroprevalence to VSV-NJ and VSV-IN in 1993. In the current study, a small mammal survey was conducted in June and July of 1994. Three one-week captures were performed. A total of 66 individuals belonging to the orders Rodentia, Marsupialia, and Reptilia were caught. Rodentia species present in the area included *Sigmodon hispidus*, *Liomys salvini*, *Ototylomys phyllotis*, and *Oryzomys*. Blood was collected for VSV neutralizing antibody analysis, PCR, and blood parasite analysis. All field rodent and reptilian species were predominantly positive to VSV-IN, with some seroprevalence to VSV-NJ. *Sigmodon hispidus* had the highest seroprevalence to VSV-IN. Blood parasites were noted in several species. These results indicate the possibility of two different transmission cycles for VSV-IN and VSV-NJ.

**PATHOGEN SHARING AMONG SYMPATRIC POPULATIONS OF BIGHORN SHEEP (OVIS CANADENSIIS), MULE DEER (ODOCOILEUS HEMIONUS), AND CATTLE.**

Randall S. Singer\(^1\), Walter M. Boyce\(^1\), David A. Jessup\(^2\), and Ian Gardner\(^1\). 1School of Veterinary Medicine, University of California, Davis, California 95616; 2California Department of Fish and Game, 1701 Nimbus Rd., Suite D, Rancho Cordova, California, 95670.

From 1991 to 1994, sympatric populations of free-ranging bighorn sheep (*Ovis canadensis*, n = 28), free-ranging mule deer (*Odocoileus hemionus*, n = 38), and domestic cattle (n = 26) in the San Bernadino mountains of southern California were sampled for exposure to selected pathogens, including bluetongue virus (BTV), bovine herpes virus 1, bovine respiratory syncytial virus, bovine virus diarrhea-mucosal disease virus, parainfluenza-3 virus, *Leptospira interrogans* serovars and *Babesia* spp. In addition, all individuals were examined for the presence of *Psoroptes* spp. mites. The most notable findings included: 1) cattle titers to *Leptospira interrogans* serovars suggesting active infection with *L. pomona* and *L. hardjo*, with no seropositive bighorn sheep or mule deer; 2) a high antibody prevalence to BTV in cattle (75%) with no reactors among bighorn sheep and mule deer; 3) *Psoroptes* spp. isolation from 50% of the bighorn sheep, but complete absence of the mite in mule deer and cattle; and 4) seropositive antibody responses in bighorn sheep and mule deer to recently discovered *Babesia* spp. with no evidence of antibody responses in cattle. These results suggest that the pathogens studied are not being shared between these sympatric domestic and wildlife populations.
VSV-NJ infection has been topographically associated, appearing predominantly at the premontane wet and tropical dryland forest elevations. VSV-IN seroprevalence has not varied with environment. Cattle, insect, and small mammal surveys were conducted in VSV-NJ enzootic areas in 1988 and in 1993-1994. The premontane small mammal survey found seroprevalence to VSV in one species, Sigmodon hispidus. Tropical dry forest insect surveys found 13 sandfly types at the study site. Only Lutzomyia shannoni, a phlebotomine sandfly, contained cow blood. Cattle here originated from other areas and had equal seroprevalence to VSV-NJ and VSV-IN in 1993. In the current study, a small mammal survey was conducted in June and July of 1994. Three one-week captures were performed. A total of 66 individuals belonging to the orders Rodentia, Marsupialia, and Reptilia were caught. Rodentia species present in the area included Sigmodon hispidus, Liomys salvini, Ototylomys phyllotis, and Oryzomys. Blood was collected for VSV neutralizing antibody analysis, PCR, and blood parasite analysis. All field rodent and reptilian species were predominantly positive to VSV-IN, with some seroprevalence to VSV-NJ. Sigmodon hispidus had the highest seroprevalence to VSV-IN. Blood parasites were noted in several species. These results indicate the possibility of two different transmission cycles for VSV-IN and VSV-NJ.

PATHOGEN SHARING AMONG SYMPATRIC POPULATIONS OF BIGHORN SHEEP (OVIS CANADENSIS), MULE DEER (ODOCOILEUS HEMIONUS), AND CATTLE. Randall S. Singer, Walter M. Boyce, David A. Jessup, and Ian Gardner. 1School of Veterinary Medicine, University of California, Davis, California 95616; 2 California Department of Fish and Game, 1701 Nimbus Rd., Suite D, Rancho Cordova, California, 95670.

From 1991 to 1994, sympatric populations of free-ranging bighorn sheep (Ovis canadensis, n = 28), free-ranging mule deer (Odocoileus hemionus, n = 38), and domestic cattle (n = 26) in the San Bernardino mountains of southern California were sampled for exposure to selected pathogens, including bluetongue virus (BTV), bovine herpes virus 1, bovine respiratory syncytial virus, bovine virus diarrhea-mucosal disease virus, parainfluenza-3 virus, Leptospira interrogans serovars and Babesia spp. In addition, all individuals were examined for the presence of Psoroptes spp. mites. The most notable findings included: 1) cattle titers to Leptospira interrogans serovars suggesting active infection with L. pomona and L. hardjo, with no seropositive bighorn sheep or mule deer; 2) a high antibody prevalence to BTV in cattle (75%) with no reactors among bighorn sheep and mule deer; 3) Psoroptes spp. isolation from 50% of the bighorn sheep, but complete absence of the mite in mule deer and cattle; and 4) seropositive antibody responses in bighorn sheep and mule deer to recently discovered Babesia spp. with no evidence of antibody responses in cattle. These results suggest that the pathogens studied are not being shared between these sympatric domestic and wildlife populations.
THE PREVALENCE OF ENTEROBACTERIA AND FUNGI ISOLATED FROM THE CHOANA AND CLOACA OF WILD YELLOW-NAPE AMAZON CHICKS (AMAZONA AUROPALLIATA). Christina Sigurdson-Scott, Avian Medical Center of Sacramento, 6114 Greenback Lane, Citrus Heights, CA 95621 and Kim L. Joyner, Mariana Aviaries/FUNDAVES, 10a. Avenue, 2-32, Zone 14, Guatemala City, Guatemala.

The incidence of enterobacteria and fungi was determined from choanal and cloacal swabs of twenty-five wild yellow-naped amazon chicks (Amazona auropalliata) at ages 0-2 weeks, 2-4 weeks, and 6-8 weeks of age. The chicks were located within a 33 hectare region in the Pacific lowlands of Guatemala. Gram-negative enteric bacteria were isolated from 20% (12/59) of choanal samples and 47% (28/59) of cloacal samples. Citrobacter spp., Enterobacter spp., and Klebsiella spp. were the most frequently encountered isolates. Fungi were isolated from 6.8% (4/59) of the choanal samples and 27% (16/59) of the cloacal samples. The nest litter was also sampled and determined to have gram-negative enterics in 79% (26/33) of the samples and fungi in 73% (24/33) of the samples.

ASPECTS OF BOVINE TUBERCULOSIS (MYCOBACTERIUM BOVIS) INFECTIONS IN FERAL POPULATIONS OF FERRETS (MUSTELA FURO), STOATS (M. ERMINEA) AND CATS (FELIS CATUS) IN OTAGO AND SOUTHLAND, NEW ZEALAND. Justine Ragg, Henrik Moller and Ken Waldrup. AgResearch, Invermay Agricultural Centre, Puddle Alley, Private Bag 50034, Mosgiel, New Zealand.

Twenty-one properties around the Otago region of the South Island of New Zealand were surveyed for the presence of lesions due to Mycobacterium bovis infection (Tb) in feral cats, ferrets and stoats during 1993 and 1994. In total, 1286 predators were necropsied for tuberculous lesions and general ecological information. The properties surveyed were chosen based on their status as Tb-endemic or Tb-free. No Tb infected cats, ferrets or stoats were found in Tb-free areas, but prevalence rates in Tb-endemic areas were 0.5% (n=214) for cats, 17.6% (n=544) for ferrets and 4.55%, (n=66) for stoats. More adult (22.4%) ferrets were infected with Tb compared to juvenile (2.5%) ferrets (Chi-square, p < 0.001), and a higher proportion of male ferrets (14.8%) were infected with Tb than females (9.5%) (Chi-square, p=0.029). Tb prevalences in adult ferrets increased 32.3% from winter to summer (Multiple regression; p=0.0142). Lymph nodes associated with an oral route of inoculation (submandibular, retropharyngeal, and mesenteric, 64%) contributed to 71.2% of all single site infections. DNA restriction endonuclease analysis of culture isolates showed that 7 of 8 ferrets sampled showed a different biotype of M. bovis than other wildlife (brush-tailed possum and feral pig) or domestic stock (cattle and deer) at the Table Hill study site.
THE PREVALENCE OF ENTEROBACTERIA AND FUNGI ISOLATED FROM THE
CHOANA AND CLOACA OF WILD YELLOW-NAPE AMazon CHICKS (AMAZONA
AUROPALLIATA). Christina Sigurdson-Scott, Avian Medical Center of Sacramento, 6114
Greenback Lane, Citrus Heights, CA 95621 and Kim L. Joyner, Mariana
Aviaries/FUNDAVES, 10a. Avenue, 2-32, Zone 14, Guatemala City, Guatemala.

The incidence of enterobacteria and fungi was determined from choanal and cloacal swabs
of twenty-five wild yellow-naped amazon chicks (Amazona auropalliata) at ages 0-2 weeks,
2-4 weeks, and 6-8 weeks of age. The chicks were located within a 33 hectare region in the
Pacific lowlands of Guatemala. Gram-negative enteric bacteria were isolated from
20%(12/59) of choanal samples and 47%(28/59) of cloacal samples. Citrobacter spp.,
Enterobacter spp., and Klebsiella spp. were the most frequently encountered isolates. Fungi
were isolated from 6.8%(4/59) of the choanal samples and 27%(16/59) of the cloacal
samples. The nest litter was also sampled and determined to have gram-negative enterics
in 79%(26/33) of the samples and fungi in 73%(24/33) of the samples.

ASPECTS OF BOVINE TUBERCULOSIS (MYCOBACTERIUM BOVIS) INFECTIONS IN
FERAL POPULATIONS OF FERRETS (MUSTELA FURO), STOATS (M. ERMINEA)
AND CATS (FELIS CATUS) IN OTAGO AND SOUTHLAND, NEW ZEALAND. Justine
Ragg, Henrik Moller and Ken Waldrup. AgResearch, Invermay Agricultural Centre, Puddle
Alley, Private Bag 50034, Mosgiel, New Zealand.

Twenty-one properties around the Otago region of the South Island of New Zealand were
surveyed for the presence of lesions due to Mycobacterium bovis infection (Tb) in feral cats,
ferrets and stoats during 1993 and 1994. In total, 1286 predators were necropsied for
tuberculous lesions and general ecological information. The properties surveyed were
chosen based on their status as Tb-endemic or Tb-free. No Tb infected cats, ferrets or
stoats were found in Tb-free areas, but prevalence rates in Tb-endemic areas were 0.5%
(n=214) for cats, 17.6% (n=544) for ferrets and 4.55%, (n=66) for stoats. More adult
(22.4%) ferrets were infected with Tb compared to juvenile (2.5%) ferrets (Chi-square, p
< 0.001), and a higher proportion of male ferrets (14.8%) were infected with Tb than
females (9.5%) (Chi-square, p=0.029). Tb prevalences in adult ferrets increased 32.3%
from winter to summer (Multiple regression; p=0.0142). Lymph nodes associated with an
oral route of inoculation (submandibular, retropharyngeal, and mesenteric, 64%) contributed
to 71.2% of all single site infections. DNA restriction endonuclease analysis of culture
isolates showed that 7 of 8 ferrets sampled showed a different biotype of M. bovis than
other wildlife (brush-tailed possum and feral pig) or domestic stock (cattle and deer) at the
Table Hill study site.
DETECTION AND QUANTIFICATION OF CLOSTRIDIUM BOTULINUM TYPE C TOXIN GENE IN WETLAND SEDIMENTS USING MOLECULAR TECHNIQUES. Judy L. Williamson1, Tonie E. Rocke1, and Judd M. Aiken2. 1National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711; 2Department of Animal Health and Biomedical Sciences, University of Wisconsin-Madison, Madison, WI 53706.

Avian botulism, a paralytic disease resulting from the ingestion of a neurotoxin (C1 toxin) produced by Clostridium botulinum type C, kills thousands of wild waterfowl each year in the United States. Clostridium botulinum is a ubiquitous, spore-forming anaerobe found in the soil and bottom sediments of rivers, lakes, and estuaries. The structural gene for the type C neurotoxin is carried by specific bacteriophages (Tox+) which must infect C. botulinum for toxin gene expression and protein production. Traditional methods for the isolation and quantification of bacteria in environmental samples have proven unsatisfactory for C. botulinum. In this study, we have modified standard molecular techniques for detection of the C1 toxin gene in wetland sediments. Polymerase chain reaction (PCR) procedures were developed for detection of the C1 toxin gene in extracted DNA and toxin gene expression in extracted RNA. The amplification products from these PCR procedures provide information on the levels of C1 toxin gene and gene expression in wetland sediments collected from various avian botulism outbreak and non-outbreak sites. This information, when correlated with known environmental conditions, may provide insight into the requirements for type C neurotoxin production and ultimately, a means for evaluating the risk of avian botulism in wetlands.

INVESTIGATIONS OF KERATOCONJUNCTIVITIS IN MULE DEER (ODOCOILEUS HEMIONUS) FROM ZION NATIONAL PARK, UTAH. Shelli A. Dubay1, E.S. Williams1, W.W. Mills1, H. Van Campen1, S. Fedorchak2, A. Boerger-Fields1, and J.L.Cavender1. 1Department of Veterinary Sciences, University of Wyoming. 1174 Snowy Range Road. Laramie, Wyoming, 89070, USA. and 2Resource Management Division. Zion National Park, Springdale, Utah, 84767, USA.

During January, 1995, 20 mule deer were captured in Zion National Park, Utah where an outbreak of infectious keratoconjunctivitis was observed during the winters of 1992/93 and 1993/94. Twelve males and eight females were captured. Blood, eye swabs, and eye parasites (Thelazia californiensis) were collected. T. californiensis was recovered from eight of 90 (40%) deer captured. Culturette swabs of conjunctiva were used for bacterial isolation. They were streaked onto both Columbia blood and MacConkey agar plates, and bacterial species were identified. Enterobacter agglomerans, Micrococcus spp., Bacillus spp., and Streptomyces spp. were commonly isolated. Swabs used for Chlamydia spp. isolations were placed in Bovarnick's media containing gentamycin which was then inoculated onto McCoy cells and signs of cytopathic effect were noted. Chlamydia spp. were not detected in the samples. Sera were tested for antibodies to Chlamydia spp., bluetongue virus, bovine respiratory syncytial virus, bovine viral diarrhea virus, epizootic hemorrhagic disease virus, infectious bovine rhinotracheitis virus, parainfluenza 3 virus, Brucella spp., and five serovars of Leptospira interrogans. Antibodies were detected against epizootic hemorrhagic disease...
DETECTION AND QUANTIFICATION OF CLOSTRIDIUM BOTULINUM TYPE C TOXIN GENE IN WETLAND SEDIMENTS USING MOLECULAR TECHNIQUES. Judy L. Williamson, Tonie E. Rocke, and Judd M. Aiken. 1National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711; 2Department of Animal Health and Biomedical Sciences, University of Wisconsin-Madison, Madison, WI 53706.

Avian botulism, a paralytic disease resulting from the ingestion of a neurotoxin (C1 toxin) produced by Clostridium botulinum type C, kills thousands of wild waterfowl each year in the United States. Clostridium botulinum is a ubiquitous, spore-forming anaerobe found in the soil and bottom sediments of rivers, lakes, and estuaries. The structural gene for the type C neurotoxin is carried by specific bacteriophages (Tox+) which must infect C. botulinum for toxin gene expression and protein production. Traditional methods for the isolation and quantification of bacteria in environmental samples have proven unsatisfactory for C. botulinum. In this study, we have modified standard molecular techniques for detection of the C1 toxin gene in wetland sediments. Polymerase chain reaction (PCR) procedures were developed for detection of the C1 toxin gene in extracted DNA and toxin gene expression in extracted RNA. The amplification products from these PCR procedures provide information on the levels of C1 toxin gene and gene expression in wetland sediments collected from various avian botulism outbreak and non-outbreak sites. This information, when correlated with known environmental conditions, may provide insight into the requirements for type C neurotoxin production and ultimately, a means for evaluating the risk of avian botulism in wetlands.

INVESTIGATIONS OF KERATOCONJUNCTIVITIS IN MULE DEER (ODOCOILEUS HEMIONUS) FROM ZION NATIONAL PARK, UTAH. Shelli A. Dubay, E.S. Williams, W.W. Mills, H. Van Campen, S. Fedorchak, A. Boerger-Fields, and J.L. Cavender. 1Department of Veterinary Sciences, University of Wyoming. 1174 Snowy Range Road, Laramie, Wyoming, 89070, USA. and 2Resource Management Division. Zion National Park, Springdale, Utah, 84767, USA.

During January, 1995, 20 mule deer were captured in Zion National Park, Utah where an outbreak of infectious keratoconjunctivitis was observed during the winters of 1992/93 and 1993/94. Twelve males and eight females were captured. Blood, eye swabs, and eye parasites (Thelazia californiensis) were collected. T. californiensis was recovered from eight of 90 (40%) deer captured. Culturette swabs of conjunctiva were used for bacterial isolation. They were streaked onto both Columbia blood and MacConkey agar plates, and bacterial species were identified. Enterobacter agglomerans, Micrococcus spp., Bacillus spp., and Streptomyces spp. were commonly isolated. Swabs used for Chlamydia spp. isolations were placed in Bovarnick’s media containing gentamycin which was then inoculated onto McCoy cells and signs of cytopathic effect were noted. Chlamydia spp. were not detected in the samples. Sera were tested for antibodies to Chlamydia spp., bluetongue virus, bovine respiratory syncytial virus, bovine viral diarrhea virus, epizootic hemorrhagic disease virus, infectious bovine rhinotracheitis virus, parainfluenza 3 virus, Brucella spp., and five serovars of Leptospira interrogans. Antibodies were detected against epizootic hemorrhagic disease
DETECTION AND QUANTIFICATION OF CLOSTRIDIUM BOTULINUM TYPE C TOXIN GENE IN WETLAND SEDIMENTS USING MOLECULAR TECHNIQUES. Judy L. Williamson¹, Tonie E. Rocke¹, and Judd M. Aiken². ¹National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711; ²Department of Animal Health and Biomedical Sciences, University of Wisconsin-Madison, Madison, WI 53706.

Avian botulism, a paralytic disease resulting from the ingestion of a neurotoxin (C₁ toxin) produced by Clostridium botulinum type C, kills thousands of wild waterfowl each year in the United States. Clostridium botulinum is a ubiquitous, spore-forming anaerobe found in the soil and bottom sediments of rivers, lakes, and estuaries. The structural gene for the type C neurotoxin is carried by specific bacteriophages (Tox⁺) which must infect C. botulinum for toxin gene expression and protein production. Traditional methods for the isolation and quantification of bacteria in environmental samples have proven unsatisfactory for C. botulinum. In this study, we have modified standard molecular techniques for detection of the C₁ toxin gene in wetland sediments. Polymerase chain reaction (PCR) procedures were developed for detection of the C₁ toxin gene in extracted DNA and toxin gene expression in extracted RNA. The amplification products from these PCR procedures provide information on the levels of C₁ toxin gene and gene expression in wetland sediments collected from various avian botulism outbreak and non-outbreak sites. This information, when correlated with known environmental conditions, may provide insight into the requirements for type C neurotoxin production and ultimately, a means for evaluating the risk of avian botulism in wetlands.

INVESTIGATIONS OF KERATOCONJUNCTIVITIS IN MULE DEER (ODOCOILEUS HEMIONUS) FROM ZION NATIONAL PARK, UTAH. Shelli A. Dubay¹, E.S. Williams¹, W.W. Mills¹, H. Van Campen¹, S. Fedorchak², A. Boerger-Fields¹, and J.L. Cavender¹. ¹Department of Veterinary Sciences, University of Wyoming. 1174 Snowy Range Road. Laramie, Wyoming, 89070, USA. and ²Resource Management Division. Zion National Park, Springdale, Utah, 84767, USA.

During January, 1995, 20 mule deer were captured in Zion National Park, Utah where an outbreak of infectious keratoconjunctivitis was observed during the winters of 1992/93 and 1993/94. Twelve males and eight females were captured. Blood, eye swabs, and eye parasites (Thelazia californiensis) were collected. T. californiensis was recovered from eight of 90 (40%) deer captured. Culturette swabs of conjunctiva were used for bacterial isolation. They were streaked onto both Columbia blood and MacConkey agar plates, and bacterial species were identified. Enterobacter agglomerans, Micrococcus spp., Bacillus spp., and Streptomyces spp. were commonly isolated. Swabs used for Chlamydia spp. isolations were placed in Bovarnick's media containing gentamycin which was then inoculated onto McCoy cells and signs of cytopathic effect were noted. Chlamydia spp. were not detected in the samples. Sera were tested for antibodies to Chlamydia spp., bluetongue virus, bovine respiratory syncytial virus, bovine viral diarrhea virus, epizootic hemorrhagic disease virus, infectious bovine rhinotracheitis virus, parainfluenza 3 virus, Brucella spp., and five serovars of Leptospira interrogans. Antibodies were detected against epizootic hemorrhagic disease
virus and parainfluenza 3 virus. Clinical signs of keratoconjunctivitis were not detected in captured deer, nor were bacterial species which have been implicated in keratoconjunctivitis outbreaks in free-ranging wildlife.

INDICES OF CONDITION IN A WILD POPULATION OF THE MOUNTAIN BUSHTAIL POSSUM (TRICHOSURUS CANINUS) IN AUSTRALIA. Karen L. Viggers, Division of Biochemistry and Molecular Biology, The Australian National University, Canberra, ACT, 0200, Australia; David B. Lindenmayer, Centre for Resource and Environmental studies, The Australian National University, Canberra, ACT 9299, Australia; and Ross Cunningham, Statistical Consulting Unit, The Graduate School, The Australian National University, Canberra, ACT, 0200, Australia.

Estimates of body condition and physiological indices may be useful to assess or monitor the health status of individual animals as well as populations of animals. An index of condition may be derived for any species by examining the relationship between body weight and a measure of skeletal size. In this paper we derive and compare condition indices for the mountain brushtail possum, Trichosurus caninus, from several populations throughout the geographic range of the species in eastern Australia. To validate condition indices derived in this way, an independent measure of condition should be used. Future studies are planned to measure tritiated water space in a population of T. caninus as an indirect measure of percentage body fat. These results will be examined for correlation with the condition indices derived for the same animals. We then intend to assess the usefulness of condition indices for: 1) estimating body condition in individual animals, and 2) monitoring the condition of a population over time. This approach may have application to the conservation of endangered species or populations that are in decline.
virus and parainfluenza 3 virus. Clinical signs of keratoconjunctivitis were not detected in captured deer, nor were bacterial species which have been implicated in keratoconjunctivitis outbreaks in free-ranging wildlife.

INDICES OF CONDITION IN A WILD POPULATION OF THE MOUNTAIN BUSHTAIL POSSUM (TRICHOSURUS CANINUS) IN AUSTRALIA. Karen L. Viggers, Division of Biochemistry and Molecular Biology, The Australian National University, Canberra, ACT, 0200, Australia; David B. Lindenmayer, Centre for Resource and Environmental studies, The Australian National University, Canberra, ACT 9299, Australia; and Ross Cunningham, Statistical Consulting Unit, The Graduate School, The Australian National University, Canberra, ACT, 0200, Australia.

Estimates of body condition and physiological indices may be useful to assess or monitor the health status of individual animals as well as populations of animals. An index of condition may be derived for any species by examining the relationship between body weight and a measure of skeletal size. In this paper we derive and compare condition indices for the mountain brushtail possum, Trichosurus caninus, from several populations throughout the geographic range of the species in eastern Australia. To validate condition indices derived in this way, an independent measure of condition should be used. Future studies are planned to measure tritiated water space in a population of T. caninus as an indirect measure of percentage body fat. These results will be examined for correlation with the condition indices derived for the same animals. We then intend to assess the usefulness of condition indices for: 1) estimating body condition in individual animals, and 2) monitoring the condition of a population over time. This approach may have application to the conservation of endangered species or populations that are in decline.
Terrestrial Mammals


More than 10,000 dead hares were collected by the SAGIR network (national sanitary surveillance system for wildlife) between 1986 and 1994. In spite of unavoidable bias, the results of laboratory tests performed on this sample show that six main causes were responsible for 70% of the mortality in the free ranging hare populations during this period: pseudotuberculosis, E.B.H.S., traumas, coccidiosis, pasteurellosis and tularemia. The geographical repartition and importance, the variation of the annual prevalence during the study period and the evolution of cases number during the year are described for each one of these causes of mortality.


A study of European brown hare syndrome (EHBS) was conducted in Poland (Czempin). From 1993 until 1994, 100 blood and 78 spleen samples of European brown hares (Lepus europaeus) were tested for prevalence of EBHS and rabbit haemorrhagic disease (RHDV) antibodies and EBHS virus antigen with two ELISA testkits (Svin, Denmark). Thirty-eight percent of the serum samples were seropositive for EBHS, and 7.6% of the organ materials were antigen positive for EBHS virus. Three of the sera were seropositive against RHDV, whereas 2 of them were also seropositive for EBHSV. EBHSV seropositive hares were most frequently found during summer period (April-September). Negative staining electron microscopy of liver and spleen homogenates revealed the presence of calicivirus. Histopathological findings corresponded with the clinical picture of chronic EBHS. A pathohistological picture related to EBHS was found in 22 out of 98 investigated hares and corresponded in 50% of the animals which reacted positively in the EBHSV Ag ELISA and in 29% of the animals which reacted positively in the EBHSV Ab ELISA. These investigations done in western Poland are the first showing that caliciviruses are present in European brown hare populations in East Europe and may be one of the causes for increased mortality in the Poland hare population over the past 10 years.
RELATIONSHIPS AMONG FREE-RANGING CARNIVORES, HUMANS AND HEALTH IN WESTERN EUROPE: A REVIEW. Marc Artois, CNEVA-Nancy, BP 9, 54220 Malzéville, France.

Primitive natural habitats no longer exist in western Europe. Only medium sized top predators such as the red fox, European badger and wild cat have survived unaided. Now, some nearly-extirpated species such as the boreal lynx, the river otter and grey wolf are making a comeback, either through re-introduction or natural re-colonization after recent efficient protection. Others species such as the brown bear of the Pyrenees and the Apennines and the European mink in both the eastern and western part of its range are still threatened.

Contagious diseases circulate in these populations. Some are without apparent regulatory effects, such as pox and herpes virus infections, while others such as fox rabies and feline leukemia virus are potential threats to populations. The influence of these diseases on population dynamics is the topic of current research, both in the field and through modelling.

The close co-habitation of humans and wild carnivores has made disease in these animals an increasing concern with respect to wildlife management, human health and health of domestic animals. Eradication of rabies in foxes has evolved from massive killing of foxes to vaccination of foxes. New approaches such as immunocontraception are on the horizon. Special protection from disease may be required for some endangered species together with programs to limit competition and preserve gene pools. Veterinarians charged with these responsibilities require relevant information and training.

PRIVATE-SECTOR INTERSTATE TRANSLOCATION OF WILD CANIDS: A DOCUMENTED DISEASE THREAT. William R. Davidson, Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine and D.B. Warnell School of Forest Resources, The University of Georgia, Athens, Georgia 30602; Lisa Conti, Florida Department of Health and Rehabilitative Services, 1317 Winewood Boulevard, Tallahassee, Florida 32399; William B. Johnston, Division of Epidemiology, Alabama Department of Public Health, 434 Monroe Street, Montgomery, Alabama 36130; and Jean S. Smith, Viral and Rickettsial Zoonoses Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333.

Since about 1980, a large number of private fox-chasing enclosures have been built in the southeastern United States. Red foxes (Vulpes vulpes), gray foxes (Urocyon cinereogargenteus), and coyotes (Canis latrans) are commonly translocated and stocked in these enclosures; species and sources of animals differ among states and enclosures, but both legal and illegal interstate translocations are known to occur. Although data on pathogens in translocated wild canids are limited, there is mounting evidence that this practice poses
RELATIONSHIPS AMONG FREE-RANGING CARNIVORES, HUMANS AND HEALTH IN WESTERN EUROPE: A REVIEW. Marc Artois, CNEVA-Nancy, BP 9, 54220 Malzéville, France.

Primitive natural habitats no longer exist in western Europe. Only medium sized top predators such as the red fox, European badger and wild cat have survived unaided. Now, some nearly-extirpated species such as the boreal lynx, the river otter and grey wolf are making a comeback, either through re-introduction or natural re-colonization after recent efficient protection. Others species such as the brown bear of the Pyrenees and the Apennines and the European mink in both the eastern and western part of its range are still threatened.

Contagious diseases circulate in these populations. Some are without apparent regulatory effects, such as pox and herpes virus infections, while others such as fox rabies and feline leukemia virus are potential threats to populations. The influence of these diseases on population dynamics is the topic of current research, both in the field and through modelling.

The close co-habitation of humans and wild carnivores has made disease in these animals an increasing concern with respect to wildlife management, human health and health of domestic animals. Eradication of rabies in foxes has evolved from massive killing of foxes to vaccination of foxes. New approaches such as immunocontraception are on the horizon. Special protection from disease may be required for some endangered species together with programs to limit competition and preserve gene pools. Veterinarians charged with these responsibilities require relevant information and training.

PRIVATE-SECTOR INTERSTATE TRANSLOCATION OF WILD CANIDS: A DOCUMENTED DISEASE THREAT. William R. Davidson, Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine and D.B. Warnell School of Forest Resources, The University of Georgia, Athens, Georgia 30602; Lisa Conti, Florida Department of Health and Rehabilitative Services, 1317 Winewood Boulevard, Tallahassee, Florida 32399; William B. Johnston, Division of Epidemiology, Alabama Department of Public Health, 434 Monroe Street, Montgomery, Alabama 36130; and Jean S. Smith, Viral and Rickettsial Zoonoses Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333.

Since about 1980, a large number of private fox-chasing enclosures have been built in the southeastern United States. Red foxes (Vulpes vulpes), gray foxes (Urocyon cinereoargenteus), and coyotes (Canis latrans) are commonly translocated and stocked in these enclosures; species and sources of animals differ among states and enclosures, but both legal and illegal interstate translocations are known to occur. Although data on pathogens in translocated wild canids are limited, there is mounting evidence that this practice poses...
significant disease risks. Shipments of red foxes illegally imported from midwestern states have been infected with Echinococcus multilocularis, a human pathogen not endemic in the Southeast. In Alabama and Florida, rabies has been diagnosed in unvaccinated foxhounds directly linked to enclosures containing coyotes; the viruses were identified as the urban dog/coyote ecotype which currently is epizootic in Texas. In an event unrelated to fox-chasing enclosures, gray foxes infected with a rabies virus ecotype presently restricted to western Texas were shipped to a private animal collection in Montana. These findings reaffirm a previous conclusion that private-sector translocation of wild canids poses potential health risks to indigenous wildlife, domestic animals, and humans. We recommend that appropriate state agencies move in unison to strictly regulate or prohibit both exportation and importation of live wild canids by the private-sector.

FECAL AND SEROLOGIC SURVEY OF SELECTED CANINE PATHOGENS IN GREAT LAKES TIMBER WOLVES (CANIS LUPUS LYCAON). Kerry Beheler-Amass, Wildlife Health Program, Bureau of Wildlife Management, Wisconsin Dept of Natural Resources (WDNR), Madison WI 53707; Richard Thiel, WDNR, Sandhill Outdoor Skills Center, Babcock WI 54413; Ronald Schultz, WDNR Endangered Resources, Woodruff WI 54568; Adrian Wydeven, WDNR Endangered Resources, Park Falls WI 54552; Bruce Kohn, WDNR Research, Rhinelander WI 54501; Stephen Schmitt, Michigan Dept of Natural Resources (MDNR) Rose Lake Wildlife Disease Laboratory, East Lansing MI 48823; James Hamill, MDNR, Crystal Falls MI 49920; and Sanjay Kapil, Dept of Veterinary Diagnostic Investigations, College of Veterinary Medicine, Kansas State University, Manhattan KS 66506-5601.

Sera from 43 timber wolves (Canis lupus lycaon) captured in leg-hold traps and fitted with radio collars in northern Wisconsin, northeastern Minnesota, and northern Michigan from 1985 to 1989, and from 1991 to 1994 were assayed for titers to the following canine pathogens: canine parvovirus, canine distemper virus, infectious canine hepatitis, Lyme disease, and dirofilarisis or heartworm disease. Forty-six wolf fecal samples field collected from 1991 to 1994 were assayed for fecal shed canine parvovirus and examined for endoparasites. Canine parvovirus positive antibody titers, measured as >160 by hemagglutination inhibition, were detected in 23 (53%) samples. Canine distemper virus positive titers, measured as >1:10 by indirect immunofluorescence assay, were detected in 14 (33%) samples. Infectious canine hepatitis virus positive titers, measured as >1:10 by serum neutralization assay, were detected in 13 (30%) samples. Lyme disease exposure was detected in 9 of 19 (47%) samples using a qualitative ALICE assay. All wolf sera were negative for canine heartworm antigen and circulating microfilaria. Canine parvovirus fecal shed antigen, measured positive from 1:32 to >1:4096 by hemagglutination assay, was detected in 13 (28%) field collected fecal samples. The most common endoparasite eggs identified by fecal flotation (# of samples with parasite) were Isospora species (coccidia) (19), Uncinaria stenocephalia (northern hookworm) (12), Echinococcus granuloses (rabbit tapeworm) (10), and Alaria canis (intestinal trematode) (9). Fecal samples also yielded ectoparasites including Sarcoptes scabiei var. canis and Trichodectes canis. This is the first comprehensive report of canine pathogens present in Wisconsin, Michigan, and northeastern Minnesota timber wolves.
significant disease risks. Shipments of red foxes illegally imported from midwestern states have been infected with *Echinococcus multilocularis*, a human pathogen not endemic in the Southeast. In Alabama and Florida, rabies has been diagnosed in unvaccinated foxhounds directly linked to enclosures containing coyotes; the viruses were identified as the urban dog/coyote ecotype which currently is epizootic in Texas. In an event unrelated to fox-chasing enclosures, gray foxes infected with a rabies virus ecotype presently restricted to western Texas were shipped to a private animal collection in Montana. These findings reaffirm a previous conclusion that private-sector translocation of wild canids poses potential health risks to indigenous wildlife, domestic animals, and humans. We recommend that appropriate state agencies move in unison to strictly regulate or prohibit both exportation and importation of live wild canids by the private-sector.

**Fecal and Serologic Survey of Selected Canine Pathogens in Great Lakes Timber Wolves (Canis lupus lycaon).** Kerry Beheler-Amass, Wildlife Health Program, Bureau of Wildlife Management, Wisconsin Dept of Natural Resources (WDNR), Madison WI 53707; Richard Thiel, WDNR, Sandhill Outdoor Skills Center, Babcock WI 54413; Ronald Schultz, WDNR Endangered Resources, Woodruff WI 54568; Adrian Wydeven, WDNR Endangered Resources, Park Falls WI 54552; Bruce Kohn, WDNR Research, Rhinelander WI 54501; Stephen Schmitt, Michigan Dept of Natural Resources (MDNR) Rose Lake Wildlife Disease Laboratory, East Lansing MI 48823; James Hamill, MDNR, Crystal Falls MI 49920; and Sanjay Kapil, Dept of Veterinary Diagnostic Investigations, College of Veterinary Medicine, Kansas State University, Manhattan KS 66506-5601.

Sera from 43 timber wolves (*Canis lupus lycaon*) captured in leg-hold traps and fitted with radio collars in northern Wisconsin, northeastern Minnesota, and northern Michigan from 1985 to 1989, and from 1991 to 1994 were assayed for titers to the following canine pathogens: canine parvovirus, canine distemper virus, infectious canine hepatitis, Lyme disease, and dirofilarisis or heartworm disease. Forty-six wolf fecal samples field collected from 1991 to 1994 were assayed for fecal shed canine parvovirus and examined for endoparasites. Canine parvovirus positive antibody titers, measured as >1:160 by hemagglutination inhibition, were detected in 23 (53%) samples. Canine distemper virus positive titers, measured as >1:10 by indirect immunofluorescence assay, were detected in 14 (33%) samples. Infectious canine hepatitis virus positive titers, measured as >1:10 by serum neutralization assay, were detected in 13 (30%) samples. Lyme disease exposure was detected in 9 of 19 (47%) samples using a qualitative ALICE assay. All wolf sera were negative for canine heartworm antigen and circulating microfilaria. Canine parvovirus fecal shed antigen, measured positive from 1:32 to >1:4096 by hemagglutination assay, was detected in 13 (28%) field collected fecal samples. The most common endoparasite eggs identified by fecal flotation (# of samples with parasite) were *Isospora* species (coccidia) (19), *Uncinaria stenocephalia* (northern hookworm) (12), *Echinococcus granuloses* (rabbit tapeworm) (10), and *Alaria canis* (intestinal trematode) (9). Fecal samples also yielded ectoparasites including *Sarcoptes scabiei* var. canis and *Trichodectes canis*. This is the first comprehensive report of canine pathogens present in Wisconsin, Michigan, and northeastern Minnesota timber wolves.
CONTINUATION OF AN ORAL RABIES VACCINATION TRIAL OF RACCOONS (PROCYON LOTOR) ON CAPE COD, MASSACHUSETTS. Alison Robbins, Steven Rowell, Sara Levine, Tufts University School of Veterinary Medicine, 200 Westboro Road, North Grafton, MA 01536; Bryan Windmiller, Tufts University, Department of Biology, Medford, MA 02155; Michael Niezgoda, Charles Rupprecht, Centers for Disease Control, 1600 Clifton Road, Mailstop G-33, Atlanta, GA 30333; Michael McGuill, Massachusetts Department of Public Health, 305 South Street, Boston, MA 02130.

Tufts University School of Veterinary Medicine and the Massachusetts Department of Public Health, in collaboration with the Centers for Disease Control, are conducting a field trial of the vaccinia-rabies glycoprotein recombinant virus (V-RG) as a means of preventing the spread of raccoon rabies onto the Cape Cod peninsula of Massachusetts. As the project moves into its second year, research objectives include: 1) estimate the proportion of raccoons and other potential vector species that consumed vaccine-laden baits and the variation in seroprevalence among species, sex/age class, and habitat type; 2) evaluate bait distribution techniques and strategies in varied habitat; 3) evaluate effectiveness of the vaccination barrier as it becomes challenged by the approaching rabies epizootic. Thirty thousand (30,000) vaccine laden baits were distributed throughout a 16,000 hectare study site located astride the Cape Cod Canal by hand, from cars, and helicopters during two zone wide distribution campaigns in 1994. Post baiting serum samples were collected from live-trapped, sedated mammals within the study site and analyzed for presence of rabies virus neutralizing antibodies. The overall percentage of antibody positive raccoons was 36% and 52% after the first and second baiting respectively. Uptake of bait was significantly slower in the fall than the spring. Non-target species taking laden baits placed on tracking plates differed significantly between spring and fall distributions.

NEW YORK STATE ORAL RABIES VACCINATION: FIRST EVALUATION IN AN ENZOOTIC AREA. Cathleen Hanlon, Brian Laniewicz, Amy Willsey, Charles Trimarchi, Charles Rupprecht¹, Guthrie Birkhead, John Debbie. New York State Dept. of Health, Albany, NY. ¹Centers for Disease Control, Atlanta, GA.

The first North American evaluation of oral rabies vaccination (ORV) in an enzootic raccoon rabies area was initiated in October 1994 in southern Albany and Renssealer Counties. The overall objective is to assess the effect of ORV upon enzootic raccoons. The 600 sq km vaccination area is divided by the Hudson River, with the Albany County site receiving a fishmeal polymer (FMP) bait with a paraffin ampule vaccine chamber and the Rensselaer County site receiving a FMP bait with a sachet vaccine chamber. As a result of vaccine packaging delays, distribution of 31,200 baits, rather than the planned 60,000 occurred in October 1994, with a second baiting throughout the entire area in April. Preliminary results indicate an overall rabies virus neutralizing antibody rate of 48% (13/27) in the vaccination area versus 0% (0/7) in the surveillance areas among raccoons. Following Autumn 1994 baiting, 30 persons reported incidences in which 24 handled bait or bait remnants. Thirteen of 24 persons handled a bait following primary contact by a dog or cat.
CONTINUATION OF AN ORAL RABIES VACCINATION TRIAL OF RACCOONS (PROCYON LOTOR) ON CAPE COD, MASSACHUSETTS. Alison Robbins, Steven Rowell, Sara Levine, Tufts University School of Veterinary Medicine, 200 Westboro Road, North Grafton, MA 01536; Bryan Windmiller, Tufts University, Department of Biology, Medford, MA 02155; Michael Niezgoda, Charles Rupprecht, Centers for Disease Control, 1600 Clifton Road, Mailstop G-33, Atlanta, GA 30333; Michael McGuill, Massachusetts Department of Public Health, 305 South Street, Boston, MA 02130.

Tufts University School of Veterinary Medicine and the Massachusetts Department of Public Health, in collaboration with the Centers for Disease Control, are conducting a field trial of the vaccinia-rabies glycoprotein recombinant virus (V-RG) as a means of preventing the spread of raccoon rabies onto the Cape Cod peninsula of Massachusetts. As the project moves into its second year, research objectives include: 1) estimate the proportion of raccoons and other potential vector species that consumed vaccine-laden baits and the variation in seroprevalence among species, sex/age class, and habitat type; 2) evaluate bait distribution techniques and strategies in varied habitat; 3) evaluate effectiveness of the vaccination barrier as it becomes challenged by the approaching rabies epizootic. Thirty thousand (30,000) vaccine laden baits were distributed throughout a 16,000 hectare study site located astride the Cape Cod Canal by hand, from cars, and helicopters during two zone wide distribution campaigns in 1994. Post baiting serum samples were collected from live-trapped, sedated mammals within the study site and analyzed for presence of rabies virus neutralizing antibodies. The overall percentage of antibody positive raccoons was 36% and 52% after the first and second baiting respectively. Uptake of bait was significantly slower in the fall than the spring. Non-target species taking laden baits placed on tracking plates differed significantly between spring and fall distributions.

NEW YORK STATE ORAL RABIES VACCINATION: FIRST EVALUATION IN AN ENZOOTIC AREA. Cathleen Hanlon, Brian Laniewicz, Amy Willsey, Charles Trimarchi, Charles Rupprecht, Guthrie Birkhead, John Debbie. New York State Dept. of Health, Albany, NY. ¹Centers for Disease Control, Atlanta, GA.

The first North American evaluation of oral rabies vaccination (ORV) in an enzootic raccoon rabies area was initiated in October 1994 in southern Albany and Renssealer Counties. The overall objective is to assess the effect of ORV upon enzootic raccoons. The 600 sq km vaccination area is divided by the Hudson River, with the Albany County site receiving a fishmeal polymer (FMP) bait with a paraffin ampule vaccine chamber and the Rensselaer County site receiving a FMP bait with a sachet vaccine chamber. As a result of vaccine packaging delays, distribution of 31,200 baits, rather than the planned 60,000 occurred in October 1994, with a second baiting throughout the entire area in April. Preliminary results indicate an overall rabies virus neutralizing antibody rate of 48% (13/27) in the vaccination area versus 0% (0/7) in the surveillance areas among raccoons. Following Autumn 1994 baiting, 30 persons reported incidences in which 24 handled bait or bait remnants. Thirteen of 24 persons handled a bait following primary contact by a dog or cat.
Eleven persons, including one five year old male, found and handled bait or remnants without assistance from an animal. An adult, male hunter reported possible contact with vaccine upon accidentally placing his hand directly upon a partially consumed bait, with no untoward effect. These preliminary data will be instrumental in further developing mechanisms to minimize human and domestic animal contact. Current trends in rabies cases are promising, although not yet statistically significant. Research to date has demonstrated the potential of ORV and defined some basic variables, which must be judiciously and responsibly refined during the continued development of this adjunct wildlife rabies control method.

Marine Animals

DISSEMINATED CARCINOMATOSIS IN STRANDED CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS), SOUTHERN CALIFORNIA, 1994. H.J. Holshuh, Comparative Medical and Veterinary Services, Department of Health Services, County of Los Angeles, 7323 Descanso Ave., Downey, CA 90242; Donald D. Zumwalt and Jackie M. Ott, Marine Mammal Care Center at Fort MacArthur, 3601 S. Gaffey St., San Pedro, CA 90731.

Marine mammal mortality surveillance was re-initiated in 1993 in the Los Angeles basin after nearly a decade of lost data when the Marine Mammal Care Center was opened at Fort MacArthur. Among those stranded pinnipeds that were received dead or died during attempted rehabilitation, 82 California sea lions (Zalophus californianus), 19 harbor seals (Phoca vitulina) and 23 northern elephant seals (Mirounga angustirostris) have been necropsied. In 1994, 7 female and one male adult sea lions died at the Center. Six of these parous females were discovered to suffer disseminated neoplasia. Gross and histologic pathology of these cases will be shown as well as available toxicologic results. While this entity is not new nor unique to our local ecosystem, clusters of neoplasia could have important implications relating to the possible effects of carcinogenic pollutants in the marine environment.

METASTATIC CARCINOMA OF PROBABLE TRANSITIONAL CELL ORIGIN IN FREE-LIVING CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS). Frances Gulland, The Marine Mammal Center, Marin Headlands, Sausalito, CA 94965, Linda Lowenstine and John Trupkiewicz, Department of Veterinary Pathology, University of California, Davis, CA 95616; and Terry Spraker, Wildlife Pathology International, 2905 Stanford Road, Fort Collins, CO 80521.

Sixty-four cases of widely metastatic carcinoma of probable transitional cell origin were identified in 370 California sea lions (Zalophus californianus) that stranded alive along the California coast. This is the highest prevalence (17.3%) of neoplasia in a pinniped population reported to date. Live animals were usually emaciated, with perineal edema and
Eleven persons, including one five year old male, found and handled bait or remnants without assistance from an animal. An adult, male hunter reported possible contact with vaccine upon accidently placing his hand directly upon a partially consumed bait, with no untoward effect. These preliminary data will be instrumental in further developing mechanisms to minimize human and domestic animal contact. Current trends in rabies cases are promising, although not yet statistically significant. Research to date has demonstrated the potential of ORV and defined some basic variables, which must be judiciously and responsibly refined during the continued development of this adjunct wildlife rabies control method.

Marine Animals

DISSEMINATED CARCINOMATOSIS IN STRANDED CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS), SOUTHERN CALIFORNIA, 1994. H.J. Holshuh, Comparative Medical and Veterinary Services, Department of Health Services, County of Los Angeles, 7323 Descanso Ave., Downey, CA 90242; Donald D. Zumwalt and Jackie M. Ott, Marine Mammal Care Center at Fort MacArthur, 3601 S. Gaffey St., San Pedro, CA 90731.

Marine mammal mortality surveillance was re-initiated in 1993 in the Los Angeles basin after nearly a decade of lost data when the Marine Mammal Care Center was opened at Fort MacArthur. Among those stranded pinnipeds that were received dead or died during attempted rehabilitation, 82 California sea lions (Zalophus californianus), 19 harbor seals (Phoca vitulina) and 23 northern elephant seals (Mirounga angustirostris) have been necropsied. In 1994, 7 female and one male adult sea lions died at the Center. Six of these parous females were discovered to suffer disseminated neoplasia. Gross and histologic pathology of these cases will be shown as well as available toxicologic results. While this entity is not new nor unique to our local ecosystem, clusters of neoplasia could have important implications relating to the possible effects of carcinogenic pollutants in the marine environment.

METASTATIC CARCINOMA OF PROBABLE TRANSITIONAL CELL ORIGIN IN FREE-LIVING CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS). Frances Gulland, The Marine Mammal Center, Marin Headlands, Sausalito, CA 94965, Linda Lowenstine and John Trupkiewicz, Department of Veterinary Pathology, University of California, Davis, CA 95616; and Terry Spraker, Wildlife Pathology International, 2905 Stanford Road, Fort Collins, CO 80521.

Sixty-four cases of widely metastatic carcinoma of probable transitional cell origin were identified in 370 California sea lions (Zalophus californianus) that stranded alive along the California coast. This is the highest prevalence (17.3%) of neoplasia in a pinniped population reported to date. Live animals were usually emaciated, with perineal edema and
Eleven persons, including one five year old male, found and handled bait or remnants without assistance from an animal. An adult, male hunter reported possible contact with vaccine upon accidently placing his hand directly upon a partially consumed bait, with no untoward effect. These preliminary data will be instrumental in further developing mechanisms to minimize human and domestic animal contact. Current trends in rabies cases are promising, although not yet statistically significant. Research to date has demonstrated the potential of ORV and defined some basic variables, which must be judiciously and responsibly refined during the continued development of this adjunct wildlife rabies control method.

Marine Animals

DISSEMINATED CARCINOMATOSIS IN STRANDED CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS), SOUTHERN CALIFORNIA, 1994. H.J. Holshuh, Comparative Medical and Veterinary Services, Department of Health Services, County of Los Angeles, 7323 Descanso Ave., Downey, CA 90242; Donald D. Zumwalt and Jackie M. Ott, Marine Mammal Care Center at Fort MacArthur, 3601 S. Gaffey St., San Pedro, CA 90731.

Marine mammal mortality surveillance was re-initiated in 1993 in the Los Angeles basin after nearly a decade of lost data when the Marine Mammal Care Center was opened at Fort MacArthur. Among those stranded pinnipeds that were received dead or died during attempted rehabilitation, 82 California sea lions (Zalophus californianus), 19 harbor seals (Phoca vitulina) and 23 northern elephant seals (Mirounga angustirostris) have been necropsied. In 1994, 7 female and one male adult sea lions died at the Center. Six of these parous females were discovered to suffer disseminated neoplasia. Gross and histologic pathology of these cases will be shown as well as available toxicologic results. While this entity is not new nor unique to our local ecosystem, clusters of neoplasia could have important implications relating to the possible effects of carcinogenic pollutants in the marine environment.

METASTATIC CARCINOMA OF PROBABLE TRANSITIONAL CELL ORIGIN IN FREE-LIVING CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS). Frances Gulland, The Marine Mammal Center, Marin Headlands, Sausalito, CA 94965, Linda Lowenstine and John Trupkiewicz, Department of Veterinary Pathology, University of California, Davis, CA 95616; and Terry Spraker, Wildlife Pathology International, 2905 Stanford Road, Fort Collins, CO 80521.

Sixty-four cases of widely metastatic carcinoma of probable transitional cell origin were identified in 370 California sea lions (Zalophus californianus) that stranded alive along the California coast. This is the highest prevalence (17.3%) of neoplasia in a pinniped population reported to date. Live animals were usually emaciated, with perineal edema and
hind-flipper paralysis or paresis. On post mortem examination, large caseous masses were observed in the sub-lumbar lymph nodes, often extending around the ureters resulting in hydrourete. Metastases were wide-spread, yet a single primary neoplastic focus was never identified. The carcinoma was considered to be of transitional cell origin due to the typical morphology of invasive nests and cords of pleiomorphic epithelial cells. Possible etiology of this neoplasm is discussed.


An acute fibrinopurulent pneumonia is found in Stellar sea lion (\textit{Eumetopias juatus}), California sea lion (\textit{Zalophus californianus}) and northern fur seal (\textit{Callorhinus ursinus}) neonatal pups. These pups are usually in good body condition, but occasionally pups in poor condition also are found with pneumonia. The lungs are either unilaterally or bilaterally affected. Pneumonic lung is dark red, firm and edematous and occasionally covered with a thin layer of fibrin. On cut surface a red mucoid exudate can be expressed from cut bronchioli. Histologically alveolar spaces are dilated and filled with neutrophils, edema and fibrin. Alveolar capillaries are often congested and occasionally hemorrhage is found within pulmonary parenchyma. In 1,683 fur seal pups necropsied from 1986-1994 on the Pribilof Islands, 43 pups were found with this condition (0.03%). The animals with pneumonia were cultured and a \( \beta \) hemolytic \textit{E. coli} was isolated. During the summer of 1994, 37 Stellar sea lion pups were necropsied from southeast Alaska and one had pneumonia in which \( \beta \) hemolytic \textit{E. coli} was isolated (0.03%). In 1992, 123 California sea lion pups were necropsied from San Miguel Island and pneumonia with associated \( \beta \) hemolytic \textit{E. coli} was found in two (0.02%). Pathogenic strains of \( \beta \) hemolytic \textit{E. coli} are associated with pneumonia and fulminating septicemia of newborn and young mammals. Most strains are host specific. A limited number of well-defined serotypes are closely associated with specific disease entities in an animal host. A variety of epizootiological factors and etiological agents other than \textit{E. coli} can be present in the animal at the same time.

MULTIFOCAL NECROTIZING MYOPATHY OF NORTHERN FUR SEALS (\textit{CALLORHINUS URSINUS}) FROM ST. PAUL ISLAND, ALASKA. T. R. Spraker, Department of Pathology, Colorado State University, Fort Collins, Colorado 80523; D. L. DeGhetto and G. A Antonelis; National Marine Mammal Laboratory, 7600 Sand Point Way NE, Seattle, Washington 98115

During July-October 1986-1994, 1,917 fur seal pups (\textit{Callorhinus ursinus}) were necropsied from rookeries on the Pribilof Islands of Alaska. A condition characterized by multifocal
hind-flipper paralysis or paresis. On post mortem examination, large caseous masses were observed in the sub-lumbar lymph nodes, often extending around the ureters resulting in hydroureter. Metastases were wide-spread, yet a single primary neoplastic focus was never identified. The carcinoma was considered to be of transitional cell origin due to the typical morphology of invasive nests and cords of pleiomorphic epithelial cells. Possible etiology of this neoplasm is discussed.


An acute fibrinopurulent pneumonia is found in Stellar sea lion (Eumetopias jubatus), California sea lion (Zalophus californianus) and northern fur seal (Callorhinus ursinus) neonatal pups. These pups are usually in good body condition, but occasionally pups in poor condition also are found with pneumonia. The lungs are either unilaterally or bilaterally affected. Pneumonic lung is dark red, firm and edematous and occasionally covered with a thin layer of fibrin. On cut surface a red mucoid exudate can be expressed from cut bronchioli. Histologically alveolar spaces are dilated and filled with neutrophils, edema and fibrin. Alveolar capillaries are often congested and occasionally hemorrhage is found within pulmonary parenchyma. In 1,683 fur seal pups necropsied from 1986-1994 on the Pribilof Islands, 43 pups were found with this condition (0.03%). The animals with pneumonia were cultured and a β hemolytic E. coli was isolated. During the summer of 1994, 37 Stellar sea lion pups were necropsied from southeast Alaska and one had pneumonia in which β hemolytic E. coli was isolated (0.03%). In 1992, 123 California sea lion pups were necropsied from San Miguel Island and pneumonia with associated β hemolytic E. coli was found in two (0.02%). Pathogenic strains of β hemolytic E. coli are associated with pneumonia and fulminating septicemia of newborn and young mammals. Most strains are host specific. A limited number of well-defined serotypes are closely associated with specific disease entities in an animal host. A variety of epizootiological factors and etiological agents other than E. coli can be present in the animal at the same time.

**MULTIFOCAL NECROTIZING MYOPATHY OF NORTHERN FUR SEALS (CALLORHINUS URSINUS) FROM ST. PAUL ISLAND, ALASKA.** T. R. Spraker, Department of Pathology, Colorado State University, Fort Collins, Colorado 80523; D. L. DeGhetto and G. A Antonelis; National Marine Mammal Laboratory, 7600 Sand Point Way NE, Seattle, Washington 98115

During July-October 1986-1994, 1,917 fur seal pups (Callorhinus ursinus) were necropsied from rookeries on the Pribilof Islands of Alaska. A condition characterized by multifocal
hind-flipper paralysis or paresis. On post mortem examination, large caseous masses were observed in the sub-lumbar lymph nodes, often extending around the ureters resulting in hydrourereter. Metastases were wide-spread, yet a single primary neoplastic focus was never identified. The carcinoma was considered to be of transitional cell origin due to the typical morphology of invasive nests and cords of pleiomorphic epithelial cells. Possible etiology of this neoplasm is discussed.


An acute fibrinopurulent pneumonia is found in Stellar sea lion (Eumetopias jubatus), California sea lion (Zalophus californianus) and northern fur seal (Callorhinus ursinus) neonatal pups. These pups are usually in good body condition, but occasionally pups in poor condition also are found with pneumonia. The lungs are either unilaterally or bilaterally affected. Pneumonic lung is dark red, firm and edematous and occasionally covered with a thin layer of fibrin. On cut surface a red mucoid exudate can be expressed from cut bronchioli. Histologically alveolar spaces are dilated and filled with neutrophils, edema and fibrin. Alveolar capillaries are often congested and occasionally hemorrhage is found within pulmonary parenchyma. In 1,683 fur seal pups necropsied from 1986-1994 on the Pribilof Islands, 43 pups were found with this condition (0.03%). The animals with pneumonia were cultured and a $\beta$ hemolytic E. coli was isolated. During the summer of 1994, 37 Stellar sea lion pups were necropsied from southeast Alaska and one had pneumonia in which $\beta$ hemolytic E. coli was isolated (0.03%). In 1992, 123 California sea lion pups were necropsied from San Miguel Island and pneumonia with associated $\beta$ hemolytic E. coli was found in two (0.02%). Pathogenic strains of $\beta$ hemolytic E. coli are associated with pneumonia and fulminating septicemia of newborn and young mammals. Most strains are host specific. A limited number of well-defined serotypes are closely associated with specific disease entities in an animal host. A variety of epizootiological factors and etiological agents other than E. coli can be present in the animal at the same time.

MULTIFOCAL NECROTIZING MYOPATHY OF NORTHERN FUR SEALS (CALLORHINUS URSINUS) FROM ST. PAUL ISLAND, ALASKA. T. R. Spraker, Department of Pathology, Colorado State University, Fort Collins, Colorado 80523; D. L. DeGhetto and G. A Antonelis; National Marine Mammal Laboratory, 7600 Sand Point Way NE, Seattle, Washington 98115

During July-October 1986-1994, 1,917 fur seal pups (Callorhinus ursinus) were necropsied from rookeries on the Pribilof Islands of Alaska. A condition characterized by multifocal
necrosis of skeletal muscle and myocardium was found in 1990 and 1991. This condition was called white muscle syndrome (WMS). Gross lesions were in skeletal muscle of the pectoral girdle, cervical region, intercostal and abdominal wall and characterized by 2 to 3 mm, multiple, linear, white foci randomly scattered throughout. Of the 98 animals with WMS, 23 animals also had 1 to 2 mm, multiple, round to elongated, white foci within the myocardium. The histological lesions of WMS were characterized by multifocal necrosis of myocytes and myocardial cells with calcification. The cause of WMS was not determined. The epidemiology, gross and histologic lesions and laboratory work suggest that the etiology was not an infectious agent, vitamin A or E deficiency, trace mineral deficiency or toxicity, heavy metal toxicity or aromatic hydrocarbon toxicity. The cause of WMS may be an unidentified (inorganic) toxin.

**ALBATROSS AS SENTINELS OF ORGANOCHLORINE POLLUTION IN THE NORTH PACIFIC OCEAN.** Rosalind M. Rolland and Theo Colborn, World Wildlife Fund, 1250 Twenty-Fourth St. NW, Washington, DC 20037; John P. Giesy, Heidi Auman, Dave Verbrugge, Dept. of Fish and Wildlife, Pesticide Research Center, Institute of Environmental Toxicology, Michigan State University, E. Lansing, MI 48824; Paul D. Jones, ESR:Environmental, PO Box 30-547, Lower Hutt, New Zealand; Cheryl L. Summer and Jim P. Ludwig, The SERE Group LTD, Box 556, Eureka, MI 58833

This paper examines the biological effects and concentrations of organochlorine contaminants in Laysan and black-footed albatrosses nesting on Midway Atoll in the North Pacific Ocean. From 1992-1995, albatross tissues and eggs were analyzed for total and coplanar PCBs, dioxins, furans, dioxin equivalents (TCDD-EQ), and DDT and metabolites. Organochlorine contaminants have been found in all samples examined to date. The black-footed albatross samples were 2-4 times more contaminated with PCBs and DDT group compounds compared to the Laysan samples. The TCDD-EQ for the black-foot samples indicates that they are at or above the threshold where reproductive effects are seen in sensitive fish-eating avian species. Although fledgling rates are meeting replacement levels in both species, the black-foots show a higher rate of eggshell cracking, decreased egg viability and embryonic defects when compared to the Laysans. The use of albatrosses to monitor trends of organochlorine contamination in the marine environment will be discussed.

**DIAGNOSTIC FINDINGS ON LAYSAN ALBATROSS ON MIDWAY WITH SPECIFIC REFERENCE TO LEAD.** Thierry M. Work, National Wildlife Health Center -Hawaii Field Station, PO Box 50167, Honolulu, HI 96850 and Milt Smith, National Wildlife Health Center, 6006 Schroeder Rd., Madison, WI 53711

Clinical, pathologic, toxicologic, and epizootiologic aspects of mortality causes in Laysan albatross chicks on Midway atoll, Hawaii, were investigated in 1993 and 1994. Referent values for hematology including hematocrit, total white cell count and differential were formulated for healthy Laysan albatross adults and chicks. Control populations came from
necrosis of skeletal muscle and myocardium was found in 1990 and 1991. This condition was called white muscle syndrome (WMS). Gross lesions were in skeletal muscle of the pectoral girdle, cervical region, intercostal and abdominal wall and characterized by 2 to 3 mm, multiple, linear, white foci randomly scattered throughout. Of the 98 animals with WMS, 23 animals also had 1 to 2 mm, multiple, round to elongated, white foci within the myocardium. The histological lesions of WMS were characterized by multifocal necrosis of myocytes and myocardial cells with calcification. The cause of WMS was not determined. The epidemiology, gross and histologic lesions and laboratory work suggest that the etiology was not an infectious agent, vitamin A or E deficiency, trace mineral deficiency or toxicity, heavy metal toxicity or aromatic hydrocarbon toxicity. The cause of WMS may be an unidentified (inorganic) toxin.

ALBATROSS AS SENTINELS OF ORGANOCHLORINE POLLUTION IN THE NORTH PACIFIC OCEAN. Rosalind M. Rolland and Theo Colborn, World Wildlife Fund, 1250 Twenty-Fourth St. NW, Washington, DC 20037; John P. Giesy, Heidi Auman, Dave Verbrugge, Dept. of Fish and Wildlife, Pesticide Research Center, Institute of Environmental Toxicology, Michigan State University, E. Lansing, MI 48824; Paul D. Jones, ESR:Environmental, PO Box 30-547, Lower Hutt, New Zealand; Cheryl L. Summer and Jim P. Ludwig, The SERE Group LTD, Box 556, Eureka, MI 58833

This paper examines the biological effects and concentrations of organochlorine contaminants in Laysan and black-footed albatrosses nesting on Midway Atoll in the North Pacific Ocean. From 1992-1995, albatross tissues and eggs were analyzed for total and coplanar PCBs, dioxins, furans, dioxin equivalents (TCDD-EQ), and DDT and metabolites. Organochlorine contaminants have been found in all samples examined to date. The black-footed albatross samples were 2-4 times more contaminated with PCBs and DDT group compounds compared to the Laysan samples. The TCDD-EQ for the black-foot samples indicates that they are at or above the threshold where reproductive effects are seen in sensitive fish-eating avian species. Although fledgling rates are meeting replacement levels in both species, the black-feet show a higher rate of eggshell cracking, decreased egg viability and embryonic defects when compared to the Laysans. The use of albatrosses to monitor trends of organochlorine contamination in the marine environment will be discussed.

DIAGNOSTIC FINDINGS ON LAYSAN ALBATROSS ON MIDWAY WITH SPECIFIC REFERENCE TO LEAD. Thierry M. Work, National Wildlife Health Center -Hawaii Field Station, PO Box 50167, Honolulu, HI 96850 and Milt Smith, National Wildlife Health Center, 6006 Schroeder Rd., Madison, WI 53711

Clinical, pathologic, toxicologic, and epizootiologic aspects of mortality causes in Laysan albatross chicks on Midway atoll, Hawaii, were investigated in 1993 and 1994. Referent values for hematology including hematocrit, total white cell count and differential were formulated for healthy Laysan albatross adults and chicks. Control populations came from
necrosis of skeletal muscle and myocardium was found in 1990 and 1991. This condition was called white muscle syndrome (WMS). Gross lesions were in skeletal muscle of the pectoral girdle, cervical region, intercostal and abdominal wall and characterized by 2 to 3 mm, multiple, linear, white foci randomly scattered throughout. Of the 98 animals with WMS, 23 animals also had 1 to 2 mm, multiple, round to elongated, white foci within the myocardium. The histological lesions of WMS were characterized by multifocal necrosis of myocytes and myocardial cells with calcification. The cause of WMS was not determined. The epidemiology, gross and histologic lesions and laboratory work suggest that the etiology was not an infectious agent, vitamin A or E deficiency, trace mineral deficiency or toxicity, heavy metal toxicity or aromatic hydrocarbon toxicity. The cause of WMS may be an unidentified (inorganic) toxin.

ALBATROSS AS SENTINELS OF ORGANOCHLORINE POLLUTION IN THE NORTH PACIFIC OCEAN. Rosalind M. Rolland and Theo Colborn, World Wildlife Fund, 1250 Twenty-Fourth St. NW, Washington, DC 20037; John P. Giesy, Heidi Auman, Dave Verbrugge, Dept. of Fish and Wildlife, Pesticide Research Center, Institute of Environmental Toxicology, Michigan State University, E. Lansing, MI 48824; Paul D. Jones, ESR: Environmental, PO Box 30-547, Lower Hutt, New Zealand; Cheryl L. Summer and Jim P. Ludwig, The SERE Group LTD, Box 556, Eureka, MI 58833

This paper examines the biological effects and concentrations of organochlorine contaminants in Laysan and black-footed albatrosses nesting on Midway Atoll in the North Pacific Ocean. From 1992-1995, albatross tissues and eggs were analyzed for total and coplanar PCBs, dioxins, furans, dioxin equivalents (TCDD-EQ), and DDT and metabolites. Organochlorine contaminants have been found in all samples examined to date. The black-footed albatross samples were 2-4 times more contaminated with PCBs and DDT group compounds compared to the Laysan samples. The TCDD-EQ for the black-foot samples indicates that they are at or above the threshold where reproductive effects are seen in sensitive fish-eating avian species. Although fledgling rates are meeting replacement levels in both species, the black-footos show a higher rate of eggshell cracking, decreased egg viability and embryonic defects when compared to the Laysans. The use of albatrosses to monitor trends of organochlorine contamination in the marine environment will be discussed.

DIAGNOSTIC FINDINGS ON LAYSAN ALBATROSS ON MIDWAY WITH SPECIFIC REFERENCE TO LEAD. Thierry M. Work, National Wildlife Health Center - Hawaii Field Station, PO Box 50167, Honolulu, HI 96850 and Milt Smith, National Wildlife Health Center, 6006 Schroeder Rd., Madison, WI 53711

Clinical, pathologic, toxicologic, and epizootiologic aspects of mortality causes in Laysan albatross chicks on Midway atoll, Hawaii, were investigated in 1993 and 1994. Referent values for hematology including hematocrit, total white cell count and differential were formulated for healthy Laysan albatross adults and chicks. Control populations came from
Laysan and Tern Islands as well as Kauai. We found that blood parameters for adults and chicks varied with location (island) of collection and season. When compared to healthy adults, healthy albatross chicks had lower PCVs, absolute and relative heterophil, eosinophil and basophil counts, glucose and AST and relatively higher absolute and relative lymphocyte counts, WBC counts, and globulin. When compared to "healthy" chicks, sick chicks on Midway had higher absolute and relative heterophil counts, glucose, AST and albumin/globulin ratios and lower total white and absolute and relative lymphocyte counts. Chicks with elevated blood lead had depressed ALAD activity and, using this enzyme as an endpoint, we determined 0.06 μg/ml to be the no-effect blood lead level in albatross chicks. Blood lead levels in adults were within background limits or undetectable. Necropsy findings in 1993 revealed suspect lead poisoning as the most common cause of death followed by necrotizing enteritis, trauma and miscellaneous. In 1993 blood lead was partially responsible for clinical signs in ill chicks. In 1994, the most common diagnosis was necrotizing enteritis followed by trauma, suspect lead poisoning and miscellaneous. Exposure to peeling paint from abandoned buildings were risk factors in both years determining whether albatross chicks had elevated tissue lead levels. Similarly, prevalence of birds with elevated tissue lead levels was highest in areas of Midway atoll with the highest densities of buildings. The cause of the necrotizing enteritis remains undetermined.

Wild Birds

ISOLATION OF MYCOPLASMA GALLISEPTICUM FROM HOUSE FINCHES WITH CONJUNCTIVITIS. David H. Ley, J. Edward Berkhoff, and Judith M. McLaren, North Carolina State University College of Veterinary Medicine, 4700 Hillsborough St., Raleigh, NC 27606.

An unprecedented outbreak of conjunctivitis in house finches (Carpodacus mexicanus) has been observed in several Eastern states (CT, DE, MA, MD, NC, NJ, PA, VA, and WV) beginning with reports from Maryland and Virginia in February 1994. Predominant gross and microscopic lesions consisted of conjunctival swelling with serous to mucopurulent exudate and chronic lymphoplasmacytic response. For mycoplasma culture, conjunctival swabs were inoculated to Frey's broth with 15% swine serum. From June to Sept. 1994, Mycoplasma gallisepticum was isolated from specimens of seven accessions originating in VA, DE and NC. Specimens from 10 of 22 house finches and one of two bluejays (Cyanocitta cristata) were positive for Mycoplasma gallisepticum by direct immunofluorescence of colonies on agar medium. Incubation times required for isolation were notably slow, ranging from 13 to 40 days, and generally required 2 or more broth passages. A commercial polymerase chain reaction test kit was used to confirm that isolates were Mycoplasma gallisepticum. The occurrence of Mycoplasma gallisepticum associated with conjunctivitis in house finches may not only have substantial impact on the management and health maintenance of this population, but also other wild birds and poultry.
Laysan and Tern Islands as well as Kauai. We found that blood parameters for adults and chicks varied with location (island) of collection and season. When compared to healthy adults, healthy albatross chicks had lower PCVs, absolute and relative heterophil, eosinophil and basophil counts, glucose and AST and relatively higher absolute and relative lymphocyte counts, WBC counts, and globulin. When compared to "healthy" chicks, sick chicks on Midway had higher absolute and relative heterophil counts, glucose, AST and albumin/globulin ratios and lower total white and absolute and relative lymphocyte counts. Chicks with elevated blood lead had depressed ALAD activity and, using this enzyme as an endpoint, we determined 0.06 μg/ml to be the no-effect blood lead level in albatross chicks. Blood lead levels in adults were within background limits or undetectable. Necropsy findings in 1993 revealed suspect lead poisoning as the most common cause of death followed by necrotizing enteritis, trauma and miscellaneous. In 1993 blood lead was partially responsible for clinical signs in ill chicks. In 1994, the most common diagnosis was necrotizing enteritis followed by trauma, suspect lead poisoning and miscellaneous. Exposure to peeling paint from abandoned buildings were risk factors in both years determining whether albatross chicks had elevated tissue lead levels. Similarly, prevalence of birds with elevated tissue lead levels was highest in areas of Midway atoll with the highest densities of buildings. The cause of the necrotizing enteritis remains undetermined.

Wild Birds

**ISOLATION OF MYCOPLASMA GALLISEPTICUM FROM HOUSE FINCHES WITH CONJUNCTIVITIS.** David H. Ley, J. Edward Berkhoff, and Judith M. McLaren, North Carolina State University College of Veterinary Medicine, 4700 Hillsborough St., Raleigh, NC 27606.

An unprecedented outbreak of conjunctivitis in house finches (Carpodacus mexicanus) has been observed in several Eastern states (CT, DE, MA, MD, NC, NJ, PA, VA, and WV) beginning with reports from Maryland and Virginia in February 1994. Predominant gross and microscopic lesions consisted of conjunctival swelling with serous to mucopurulent exudate and chronic lymphoplasmacytic response. For mycoplasma culture, conjunctival swabs were inoculated to Frey's broth with 15% swine serum. From June to Sept. 1994, Mycoplasma gallisepticum was isolated from specimens of seven accessions originating in VA, DE and NC. Specimens from 10 of 22 house finches and one of two bluejays (Cyanocitta cristata) were positive for Mycoplasma gallisepticum by direct immunofluorescence of colonies on agar medium. Incubation times required for isolation were notably slow, ranging from 13 to 40 days, and generally required 2 or more broth passages. A commercial polymerase chain reaction test kit was used to confirm that isolates were Mycoplasma gallisepticum. The occurrence of Mycoplasma gallisepticum associated with conjunctivitis in house finches may not only have substantial impact on the management and health maintenance of this population, but also other wild birds and poultry.
FIELD INVESTIGATION OF MYCOPLASMA GALLISEPTICUM IN HOUSE FINCHES (CARPODACUS MEXICANUS) FROM MARYLAND AND GEORGIA. M. Page Luttrell1, David E. Stallknecht1, John R. Fischer1, Stanley H. Kleven2, and Victor F. Nettles1 1Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602; 2Poultry Disease Research Center, Department of Avian Medicine, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30605.

A field study investigating the occurrence of Mycoplasma gallisepticum (MG) in house finches (Carpodacus mexicanus) was conducted in Maryland and Georgia. Eighty-eight finches were captured and examined grossly and microscopically for MG-related conjunctivitis. Serum samples were obtained, and swabs from conjunctiva, sinus were inoculated into two mycoplasma broth media for culture and polymerase chain reaction (PCR). From Maryland, 12 of 57 birds had gross conjunctival lesions. MG was isolated from 9 of the 12 affected and from 3 birds without gross lesions. Fourteen of 22 finches tested by PCR were positive for MG. Sixteen of 38 birds tested by the serum plate agglutination test (SPA) were positive for MG, and nine of these had hemagglutination inhibition (HI) titers of 1:40 or 1:80. From Georgia, 3 of 31 finches examined had gross lesions; two of these were both culture and PCR positive for MG. Twelve birds were positive by SPA, and two of these had HI titers of 1:80. Histologic findings in birds with gross conjunctivitis from both locations were characterized by extensive epithelial and lymphoid hyperplasia as well as lymphoplasmacytic inflammation in conjunctival tissues; keratitis was rarely present. The source of MG infection in house finches is unknown, and further research is warranted to determine the prevalence and impact of this newly-described disease.

OVERVIEW OF CONJUNCTIVITIS IN HOUSE FINCHES IN THE EASTERN UNITED STATES, 1994-1995. John R. Fischer, Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, GA 30602; Kathryn A. Converse, National Wildlife Health Center, National Biological Service, 6006 Schroeder Road, Madison, WI 53711.

In February 1994, observations of house finches (Carpodacus mexicanus) with conjunctivitis were first reported in suburban Washington, D.C.. Since that time, hundreds of sick finches have been reported from the states of New Hampshire, Vermont, New York, Connecticut, Massachusetts, Rhode Island, New Jersey, Delaware, Pennsylvania, Maryland, Virginia, West Virginia, North Carolina, and South Carolina. Reports typically are being received from suburban areas of large cities where bird feeding is common and press releases concerning the problem have appeared in newspapers. Many affected birds have been submitted to wildlife rehabilitators and diagnostic laboratories. Typical gross lesions include unilateral or bilateral conjunctival swelling with serous to mucoid drainage, and occasional unilateral mucoid nasal exudate. Microscopically, lymphoplasmacytic inflammation, epithelial hyperplasia, and lymphoid hyperplasia of conjunctiva are common, whereas keratitis and
FIELD INVESTIGATION OF MYCOPLASMA GALLISEPTICUM IN HOUSE FINCHES (CARPODACUS MEXICANUS) FROM MARYLAND AND GEORGIA. M. Page Luttrell1, David E. Stallknecht1, John R. Fischer1, Stanley H. Kleven2, and Victor F. Nettles1 1Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602; 2Poultry Disease Research Center, Department of Avian Medicine, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30605.

A field study investigating the occurrence of Mycoplasma gallisepticum (MG) in house finches (Carpodacus mexicanus) was conducted in Maryland and Georgia. Eighty-eight finches were captured and examined grossly and microscopically for MG-related conjunctivitis. Serum samples were obtained, and swabs from conjunctiva, sinus were inoculated into two mycoplasma broth media for culture and polymerase chain reaction (PCR). From Maryland, 12 of 57 birds had gross conjunctival lesions. MG was isolated from 9 of the 12 affected and from 3 birds without gross lesions. Fourteen of 22 finches tested by PCR were positive for MG. Sixteen of 38 birds tested by the serum plate agglutination test (SPA) were positive for MG, and nine of these had hemagglutination inhibition (HI) titers of 1:40 or 1:80. From Georgia, 3 of 31 finches examined had gross lesions; two of these were both culture and PCR positive for MG. Twelve birds were positive by SPA, and two of these had HI titers of 1:80. Histologic findings in birds with gross conjunctivitis from both locations were characterized by extensive epithelial and lymphoid hyperplasia as well as lymphoplasmacytic inflammation in conjunctival tissues; keratitis was rarely present. The source of MG infection in house finches is unknown, and further research is warranted to determine the prevalence and impact of this newly-described disease.

OVERVIEW OF CONJUNCTIVITIS IN HOUSE FINCHES IN THE EASTERN UNITED STATES, 1994-1995. John R. Fischer, Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, GA 30602; Kathryn A. Converse, National Wildlife Health Center, National Biological Service, 6006 Schroeder Road, Madison, WI 53711.

In February 1994, observations of house finches (Carpodacus mexicanus) with conjunctivitis were first reported in suburban Washington, D.C. Since that time, hundreds of sick finches have been reported from the states of New Hampshire, Vermont, New York, Connecticut, Massachusetts, Rhode Island, New Jersey, Delaware, Pennsylvania, Maryland, Virginia, West Virginia, North Carolina, and South Carolina. Reports typically are being received from suburban areas of large cities where bird feeding is common and press releases concerning the problem have appeared in newspapers. Many affected birds have been submitted to wildlife rehabilitators and diagnostic laboratories. Typical gross lesions include unilateral or bilateral conjunctival swelling with serous to mucoid drainage, and occasional unilateral mucoid nasal exudate. Microscopically, lymphoplasmacytic inflammation, epithelial hyperplasia, and lymphoid hyperplasia of conjunctiva are common, whereas keratitis and
rhinitis are rare. *Mycoplasma gallisepticum* (MG) is the only pathogen that has been isolated from affected birds. Experiments to confirm MG as the causative agent of house finch conjunctivitis are underway.

**SEX RATIOS AND BILL DEFORMITIES OF DOUBLE-CRESTED CORMORANT CHICKS.** L. Sileo, E. J. Burull, and T. E. Creekmore, National Wildlife Health Center, National Biological Service, 6006 Schroeder Rd., Madison, WI 53711; K. L. Stromborg, P. D. Allen, and C.J. Dykstra, U. S. Fish and Wildlife Service, 1015 Challenger Ct., Green Bay, WI 54311; P. van Tuinen, Department of Pathology, Medical College of Wisconsin, Milwaukee, WI 53226; R. K. Upper Mississippi Science Center, National Biological Service, P.O. Box 818, LaCrosse, WI 54602.

About 85% of double-crested cormorant chicks (*Phalacrocorax auritus*) with crossed bills examined from a contaminated colony in Wisconsin from 1988-1992 were females. Approximately 50% of the normal nestlings were female. During 1994, we collected tissue samples from 70 nestling cormorants at five colonies for cytogenetic and histological determination of sex. Necropsies confirmed previous results; the sex ratio of normal nestlings was even, whereas most cross-billed nestlings had gonads that morphologically resembled ovaries. Cytogenetic techniques indicated that the genotypic sex ratio of normal nestlings was even, but the sample was too small for complete reliability. Histological examinations revealed two explanations for the skewed macroscopic sex ratio of cross-billed chicks. First, nestlings with undeterminable macroscopic sex were more likely to have testes. Second, several gonads that grossly resembled ovaries were in fact testes. Although these results are preliminary, it appears that the skewed macroscopic sex ratio is real and it is not genetic in origin.


An epizootic of neurotropic-velogenic Newcastle disease (NVND) occurred in double-crested cormorants (*Phalacrocorax auritus*) on lakes Michigan, Huron, Superior and Ontario and lakes in Minnesota, North Dakota, South Dakota, and Nebraska during summer 1992. We examined 85 cormorants at the National Wildlife Health Center. Gross lesions were not observed. Virus isolation was used to confirm presumptive histopathologic diagnoses of NVND. A high correlation was found between characteristic microscopic lesions in the brain and spinal cord of moribund cormorants and isolation of NVND virus. In the face of
rhinitis are rare. **Mycoplasma gallisepticum** (MG) is the only pathogen that has been isolated from affected birds. Experiments to confirm MG as the causative agent of house finch conjunctivitis are underway.

**SEX RATIOS AND BILL DEFORMITIES OF DOUBLE-CRESTED CORMORANT CHICKS.** L. Sileo, E. J. Burull, and T. E. Creekmore, National Wildlife Health Center, National Biological Service, 6006 Schroeder Rd., Madison, WI 53711; K. L. Stromborg, P. D. Allen, and C.J. Dykstra, U. S. Fish and Wildlife Service, 1015 Challenger Ct., Green Bay, WI 54311; P. van Tuinen, Department of Pathology, Medical College of Wisconsin, Milwaukee, WI 53226; R. K. Upper Mississippi Science Center, National Biological Service, P.O. Box 818, LaCrosse, WI 54602.

About 85% of double-crested cormorant chicks (**Phalacrocorax auritus**) with crossed bills examined from a contaminated colony in Wisconsin from 1988-1992 were females. Approximately 50% of the normal nestlings were female. During 1994, we collected tissue samples from 70 nesting cormorants at five colonies for cytogenetic and histological determination of sex. Necropsies confirmed previous results; the sex ratio of normal nestlings was even, whereas most cross-billed nestlings had gonads that morphologically resembled ovaries. Cytogenetic techniques indicated that the genotypic sex ratio of normal nestlings was even, but the sample was too small for complete reliability. Histological examinations revealed two explanations for the skewed macroscopic sex ratio of cross-billed chicks. First, nestlings with undeterminable macroscopic sex were more likely to have testes. Second, several gonads that grossly resembled ovaries were in fact testes. Although these results are preliminary, it appears that the skewed macroscopic sex ratio is real and it is not genetic in origin.

**SUMMARY OF MICROSCOPIC CHANGES AND VIRUS ISOLATION FROM CORMORANTS SUBMITTED TO THE NWHC DURING THE 1992 OUTBREAK OF NEUROTROPIC-VELOGENIC NEWCASTLE DISEASE IN CORMORANTS IN THE GREAT LAKES AND UPPER MID-WEST STATES.** Carol U. Meteyer¹, Doug E. Docherty¹, J. Christian Franson¹, Dennis A. Senne², Linda C. Glaser¹. ¹National Wildlife Health Center, 6006 Schroeder Rd., Madison, WI 53711; ²Diagnostic Virology Laboratory, National Veterinary Services Laboratories, Animal and Plant Health Inspection Service, USDA, Ames, Iowa 50010.

An epizootic of neurotropic-velogenic Newcastle disease (NVND) occurred in double-crested cormorants (**Phalacrocorax auritus**) on lakes Michigan, Huron, Superior and Ontario and lakes in Minnesota, North Dakota, South Dakota, and Nebraska during summer 1992. We examined 85 cormorants at the National Wildlife Health Center. Gross lesions were not observed. Virus isolation was used to confirm presumptive histopathologic diagnoses of NVND. A high correlation was found between characteristic microscopic lesions in the brain and spinal cord of moribund cormorants and isolation of NVND virus. In the face of
rhinitis are rare. *Mycoplasma gallisepticum* (MG) is the only pathogen that has been isolated from affected birds. Experiments to confirm MG as the causative agent of house finch conjunctivitis are underway.

**SEX RATIOS AND BILL DEFORMITIES OF DOUBLE-CRESTED CORMORANT CHICKS.** L. Sileo, E. J. Burull, and T. E. Creekmore, National Wildlife Health Center, National Biological Service, 6006 Schroeder Rd., Madison, WI 53711; K. L. Stromborg, P. D. Allen, and C.J. Dykstra, U. S. Fish and Wildlife Service, 1015 Challenger Ct., Green Bay, WI 54311; P. van Tuinen, Department of Pathology, Medical College of Wisconsin, Milwaukee, WI 53226; R. K. Upper Mississippi Science Center, National Biological Service, P.O. Box 818, LaCrosse, WI 54602.

About 85% of double-crested cormorant chicks (*Phalacrocorax auritus*) with crossed bills examined from a contaminated colony in Wisconsin from 1988-1992 were females. Approximately 50% of the normal nestlings were female. During 1994, we collected tissue samples from 70 nestling cormorants at five colonies for cytogenetic and histological determination of sex. Necropsies confirmed previous results; the sex ratio of normal nestlings was even, whereas most cross-billed nestlings had gonads that morphologically resembled ovaries. Cytogenetic techniques indicated that the genotypic sex ratio of normal nestlings was even, but the sample was too small for complete reliability. Histological examinations revealed two explanations for the skewed macroscopic sex ratio of cross-billed chicks. First, nestlings with undeterminable macroscopic sex were more likely to have testes. Second, several gonads that grossly resembled ovaries were in fact testes. Although these results are preliminary, it appears that the skewed macroscopic sex ratio is real and it is not genetic in origin.

**SUMMARY OF MICROSCOPIC CHANGES AND VIRUS ISOLATION FROM CORMORANTS SUBMITTED TO THE NWHC DURING THE 1992 OUTBREAK OF NEUROTROPIC-VELOGENIC NEWCASTLE DISEASE IN CORMORANTS IN THE GREAT LAKES AND UPPER MID-WEST STATES.** Carol U. Meteyer¹, Doug E. Docherty¹, J. Christian Franson¹, Dennis A. Senne², Linda C. Glaser¹. ¹National Wildlife Health Center, 6006 Schroeder Rd., Madison, WI 53711; ²Diagnostic Virology Laboratory, National Veterinary Services Laboratories, Animal and Plant Health Inspection Service, USDA, Ames, Iowa 50010.

An epizootic of neurotropic-velogenic Newcastle disease (NVND) occurred in double-crested cormorants (*Phalacrocorax auritus*) on lakes Michigan, Huron, Superior and Ontario and lakes in Minnesota, North Dakota, South Dakota, and Nebraska during summer 1992. We examined 85 cormorants at the National Wildlife Health Center. Gross lesions were not observed. Virus isolation was used to confirm presumptive histopathologic diagnoses of NVND. A high correlation was found between characteristic microscopic lesions in the brain and spinal cord of moribund cormorants and isolation of NVND virus. In the face of
epizootic NVND histopathology provided preliminary evidence of the spread of NVND to new geographic areas - important information for governmental agencies and the public.

NEUROTROPIC VELOGENIC NEWCASTLE DISEASE IN CORMORANTS: PATHOLOGY AND VIRUS CHARACTERIZATION. Scott D. Fitzgerald¹, Willie M. Reed¹, Monty Banerjee¹ and Brundaban Panigrahf.¹ Animal Health Diagnostic Laboratory and Dept. of Pathology, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824 USA; ²National Veterinary Services Laboratories, Veterinary Services, A.P.H.I.S., U.S. Dept. of Agriculture, Ames, Iowa 50010 USA.

An epizootic of mortality occurred in a nesting colony of double-crested cormorants located on Snake Island, Michigan. Affected juvenile birds were dehydrated and emaciated. Most birds had subcutaneous edema and hemorrhage, petechial hemorrhages in the skeletal muscles and brain, mottled enlarged and congested spleen and liver, and atrophied thymus and bursa. Histologically, principal alterations were severe lymphocytic meningoencephalitis and myelitis, lymphoid tissue depletion, and multi-systemic perivascular hemorrhage. The paramyxovirus isolated was inoculated into chicken embryos, and inoculated by 3 different routes into 6-week-old chickens to calculate pathogenicity indices. This highly pathogenic Newcastle disease virus has appeared in numerous Great Lakes localities and poses a threat to the health of free-ranging waterfowl throughout the Mississippi Flyway.


Field studies have shown that avian malaria (Plasmodium relictum) is significantly limiting native forest bird populations in Hawaii, but little is known about the direct effects of malaria infections on morbidity and mortality. We conducted challenge experiments with juvenile Iiwi (Vestiaria coccinea, N = 33), juvenile Apapane (Himitone sanguinea, N = 16) and adult Common Amakihi (Hemignathus virens, N = 30) to measure the impact of this disease on morbidity and mortality. Birds were divided randomly into treatment and control groups and exposed to multiple infective mosquito bites (high dose), single infective bites (low dose) and uninfected bites (control). Mortality ranged between 50% and 100%, depending on species and dose, and was caused by intense malarial anemia and secondary shock. Both sex and initial body weight had significant effects on survivorship of Iiwi and Amakihi. Behavioral observations of infected Apapane identified changes in activity budgets of infected birds, with significant increases in sedentary behaviors that paralleled increased parasitemias and decreased body weight and fat levels. These observations confirm the lethality of malarial infections in Hawaiian birds and explain some of the current elevational distributions of these species.
epizootic NVND histopathology provided preliminary evidence of the spread of NVND to new geographic areas - important information for governmental agencies and the public.

NEUROTROPIC VELOGENIC NEWCASTLE DISEASE IN CORMORANTS: PATHOLOGY AND VIRUS CHARACTERIZATION. Scott D. Fitzgerald¹, Willie M. Reed¹, Monty Banerjee¹ and Brundaban Panigrahy². ¹Animal Health Diagnostic Laboratory and Dept. of Pathology, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824 USA; ²National Veterinary Services Laboratories, Veterinary Services, A.P.H.I.S., U.S. Dept. of Agriculture, Ames, Iowa 50010 USA.

An epizootic of mortality occurred in a nesting colony of double-crested cormorants located on Snake Island, Michigan. Affected juvenile birds were dehydrated and emaciated. Most birds had subcutaneous edema and hemorrhage, petechial hemorrhages in the skeletal muscles and brain, mottled enlarged and congested spleen and liver, and atrophied thymus and bursa. Histologically, principal alterations were severe lymphocytic meningoencephalitis and myelitis, lymphoid tissue depletion, and multi-systemic perivascular hemorrhage. The paramyxovirus isolated was inoculated into chicken embryos, and inoculated by 3 different routes into 6-week-old chickens to calculate pathogenicity indices. This highly pathogenic Newcastle disease virus has appeared in numerous Great Lakes localities and poses a threat to the health of free-ranging waterfowl throughout the Mississippi Flyway.


Field studies have shown that avian malaria (Plasmodium relictum) is significantly limiting native forest bird populations in Hawaii, but little is known about the direct effects of malaria infections on morbidity and mortality. We conducted challenge experiments with juvenile Iiwi (Vestiaria coccinea, N = 33), juvenile Apapane (Himitone sanguinea, N = 16) and adult Common Amakihi (Hemignathus virens, N = 30) to measure the impact of this disease on morbidity and mortality. Birds were divided randomly into treatment and control groups and exposed to multiple infective mosquito bites (high dose), single infective bites (low dose) and uninfective bites (control). Mortality ranged between 50% and 100%, depending on species and dose, and was caused by intense malarial anemia and secondary shock. Both sex and initial body weight had significant effects on survivorship of Iiwi and Amakihi. Behavioral observations of infected Apapane identified changes in activity budgets of infected birds, with significant increases in sedentary behaviors that paralleled increased parasitemias and decreased body weight and fat levels. These observations confirm the lethality of malarial infections in Hawaiian birds and explain some of the current elevational distributions of these species.
epizootic NVND histopathology provided preliminary evidence of the spread of NVND to new geographic areas - important information for governmental agencies and the public.

NEUROTROPIC VELOGENIC NEWCASTLE DISEASE IN CORMORANTS: PATHOLOGY AND VIRUS CHARACTERIZATION. Scott D. Fitzgerald¹, Willie M. Reed¹, Monty Banerjee¹ and Brundaban Panigrahy². ¹Animal Health Diagnostic Laboratory and Dept. of Pathology, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824 USA; ²National Veterinary Services Laboratories, Veterinary Services, A.P.H.I.S., U.S. Dept. of Agriculture, Ames, Iowa 50010 USA.

An epizootic of mortality occurred in a nesting colony of double-crested cormorants located on Snake Island, Michigan. Affected juvenile birds were dehydrated and emaciated. Most birds had subcutaneous edema and hemorrhage, petechial hemorrhages in the skeletal muscles and brain, mottled enlarged and congested spleen and liver, and atrophied thymus and bursa. Histologically, principal alterations were severe lymphocytic meningoencephalitis and myelitis, lymphoid tissue depletion, and multi-systemic perivascular hemorrhage. The paramyxovirus isolated was inoculated into chicken embryos, and inoculated by 3 different routes into 6-week-old chickens to calculate pathogenicity indices. This highly pathogenic Newcastle disease virus has appeared in numerous Great Lakes localities and poses a threat to the health of free-ranging waterfowl throughout the Mississippi Flyway.


Field studies have shown that avian malaria (Plasmodium relictum) is significantly limiting native forest bird populations in Hawaii, but little is known about the direct effects of malaria infections on morbidity and mortality. We conducted challenge experiments with juvenile Iiwi (Vestiaria coccinea, N = 33), juvenile Apapane (Himitone sanguinea, N = 16) and adult Common Amakihi (Hemignathus virens, N = 30) to measure the impact of this disease on morbidity and mortality. Birds were divided randomly into treatment and control groups and exposed to multiple infective mosquito bites (high dose), single infective bites (low dose) and uninfective bites (control). Mortality ranged between 50% and 100%, depending on species and dose, and was caused by intense malarial anemia and secondary shock. Both sex and initial body weight had significant effects on survivorship of Iiwi and Amakihi. Behavioral observations of infected Apapane identified changes in activity budgets of infected birds, with significant increases in sedentary behaviors that paralleled increased parasitemias and decreased body weight and fat levels. These observations confirm the lethality of malarial infections in Hawaiian birds and explain some of the current elevational distributions of these species.
During the winter of 1994-95, two bald eagle mortality events attracted public attention. In each event, deaths occurred in a restricted geographic site over a several week period. In each event, the deaths of multiple birds were linked by similar clinical signs, and gross and microscopic lesions. Both events have defied definitive diagnosis to date. In Arkansas, twenty-eight bald eagles were found sick or dead in two adjacent coves of DeGray Lake from November 24, 1994, to January 15, 1995. Sick birds were described as uncoordinated, weak, or unable to fly. The Arkansas bald eagles were in good body condition and had green stained fluid in the upper gastrointestinal tract. The consistent microscopic lesion in these birds was a spongiform change in the white matter of the brain and spinal cord, compatible with myelinic edema. In Wisconsin, 11 bald eagles were found sick or dead in Sauk and Columbia counties from January 1 to February 19, 1995. Sick birds displayed tremors and twitching. The Wisconsin eagles were in good body condition and consistently had a pale, soft liver that corresponded with microscopic evidence of pronounced hepatic fatty change in a periportal to diffuse distribution. Each Wisconsin eagle also had minimal to mild cerebral vasculitis and perivasculitis. No biologic or chemical agent has been confirmed as the cause of either mortality event.

Diagnostic findings for 133 great horned owls (Bubo virginianus) examined from 1975 to 1994 at the National Wildlife Health Center were reviewed. The carcasses were collected from 24 states, but most (58%) came from Colorado (n=21), Missouri (n=12), Oregon (n=12), Wyoming (n=11), Illinois (n=10), and Wisconsin (n=10). The most common cause of death was trauma, diagnosed in 47 (35%) cases. Twelve of these were shot. Forty-two (32%) of the owls were emaciated, but a presumptive cause for emaciation was found in only 16 (38%) of these. Other diagnoses included hydrogen sulfide and agricultural pesticide poisonings (n=12), electrocution (n=9), and infectious diseases (n=8). Liver lead concentrations were determined for 60 (45%) carcasses; all were indicative of normal background exposure (<2 ppm, wet weight). These findings indicate that although disease and exposure to environmental contaminants account for some of the deaths in great horned owls in the United States, trauma and starvation may be more important mortality factors in this species.
During the winter of 1994-95, two bald eagle mortality events attracted public attention. In each event, deaths occurred in a restricted geographic site over a several week period. In each event, the deaths of multiple birds were linked by similar clinical signs, and gross and microscopic lesions. Both events have defied definitive diagnosis to date. In Arkansas, twenty-eight bald eagles were found sick or dead in two adjacent coves of DeGray Lake from November 24, 1994, to January 15, 1995. Sick birds were described as uncoordinated, weak, or unable to fly. The Arkansas bald eagles were in good body condition and had green stained fluid in the upper gastrointestinal tract. The consistent microscopic lesion in these birds was a spongiform change in the white matter of the brain and spinal cord, compatible with myelinic edema. In Wisconsin, 11 bald eagles were found sick or dead in Sauk and Columbia counties from January 1 to February 19, 1995. Sick birds displayed tremors and twitching. The Wisconsin eagles were in good body condition and consistently had a pale, soft liver that corresponded with microscopic evidence of pronounced hepatic fatty change in a periportal to diffuse distribution. Each Wisconsin eagle also had minimal to mild cerebral vasculitis and perivasculitis. No biologic or chemical agent has been confirmed as the cause of either mortality event.

MORTALITY FACTORS IN GREAT HORNED OWLS (BUBO VIRGINIANUS) SUBMITTED TO THE NATIONAL WILDLIFE HEALTH CENTER FROM 1975 TO 1994. S. E. Little and J. C. Franson, National Biological Service, National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711, USA. 1Present address: Southeastern Cooperative Wildlife Disease Study, The University of Georgia, Athens, GA 30602, USA.

Diagnostic findings for 133 great horned owls (Bubo virginianus) examined from 1975 to 1994 at the National Wildlife Health Center were reviewed. The carcasses were collected from 24 states, but most (58%) came from Colorado (n=21), Missouri (n=12), Oregon (n=12), Wyoming (n=11), Illinois (n=10), and Wisconsin (n=10). The most common cause of death was trauma, diagnosed in 47 (35%) cases. Twelve of these were shot. Forty-two (32%) of the owls were emaciated, but a presumptive cause for emaciation was found in only 16 (38%) of these. Other diagnoses included hydrogen sulfide and agricultural pesticide poisonings (n=12), electrocution (n=9), and infectious diseases (n=8). Liver lead concentrations were determined for 60 (45%) carcasses; all were indicative of normal background exposure (<2 ppm, wet weight). These findings indicate that although disease and exposure to environmental contaminants account for some of the deaths in great horned owls in the United States, trauma and starvation may be more important mortality factors in this species.
CAUSES OF MORTALITY IN OWLS IN HAWAII, 1992-1994. Thierry M. Work, National Biological Survey, National Wildlife Health Center, Honolulu Field Station, PO Box 50167, Honolulu, Hawaii 96850, USA. and Jon Hale United States Fish and Wildlife Service, Ecological Services, Environmental Contaminants Branch, PO Box 50167, Honolulu, Hawaii 96850, USA.

Eighty one barn owls and 5 Hawaiian owls (pueo) were submitted to the National Wildlife Health Center Honolulu Field Station (HFS) from November, 1992 through August 1994. We used gross, clinical and microscopic pathology, microbiology and toxicology to determine probable cause of death in 74 barn owls and 5 pueos from Kauai, Oahu, Lanai, Molokai, Maui and Hawaii. In barn owls, the most common cause of death was trauma (50%) followed by infectious disease (28%) and emaciation (22%). Cause of death was not determined in 7 barn owls. Most traumas apparently resulted from vehicular collisions. Trichomoniasis was the predominant infectious disease and appears to be a significant cause of death in barn owls in Hawaii. Pasteurellosis and aspergillosis were less commonly encountered. No predisposing cause of emaciation was detected.

Of 28 identified barn owl stomach contents, 64% contained mainly insects of the family Tetigoniidae and Gryllidae, 18% contained mainly rodents, and the remainder had mixtures of rodents and insects or grass. Three Pueos died of trauma and one each died of emaciation and pasteurellosis. Analysis of 22 livers and kidneys for organochlorine contaminants and 20 brains for cholinesterase activity failed to implicate organochlorine, organophosphorus or carbamate pesticides as causes of death in pueos or barn owls.

RETICULOENDOTHELIOSIS IN CAPTIVE GREATER (TYMPANUCHUS CUPIDO PINNATUS) AND ATTWATER'S (T. CUPIDO ATTWATERI) PRAIRIE CHICKENS. Mark L. Drew, Department of Large Animal Medicine and Surgery, College of Veterinary Medicine, Texas A&M University, College Station, Texas; David L. Graham, Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, Texas; Clifton P. Griffin and Nova J. Silvy, Department of Wildlife and Fisheries Science, College of Agriculture and Life Sciences, Texas A&M University, College Station, Texas; Adly Fadly and Richard Witter, Avian Disease and Oncology Laboratory, United States Department of Agriculture, 3606 E. Mount Hope Road, East Lansing, Michigan.

In 1991, a captive propagation program was started for prairie chickens using wild caught Greater Prairie chickens (Tymanuchus cupido pinnatus). Eggs from nests of wild Attwater's Prairie chickens (T. cupido attwateri) were collected in 1993 to create a breeding flock of this subspecies. From 1993 to 1994, 3 greater prairie chickens have been euthanized or found dead with multiple subcutaneous nodules on the face, legs and feet. Neoplastic masses were found in multiple organs of all birds at necropsy. Histologic sections of these masses revealed pleomorphic lymphoreticular cells suggestive of reticuloendotheliosis. Reticuloendotheliosis virus was demonstrated in one tumor by PCR and viremia was evident in 53% of greater and Attwater's prairie chickens in captivity. Management and genetic concerns arising from the presence of this disease that affect the captive breeding of these birds will be discussed.
CAUSES OF MORTALITY IN OWLS IN HAWAII, 1992-1994. Thierry M. Work, National Biological Survey, National Wildlife Health Center, Honolulu Field Station, PO Box 50167, Honolulu, Hawaii 96850, USA. and Jon Hale United States Fish and Wildlife Service, Ecological Services, Environmental Contaminants Branch, PO Box 50167, Honolulu, Hawaii 96850, USA.

Eighty one barn owls and 5 Hawaiian owls (pueo) were submitted to the National Wildlife Health Center Honolulu Field Station (HFS) from November, 1992 through August 1994. We used gross, clinical and microscopic pathology, microbiology and toxicology to determine probable cause of death in 74 barn owls and 5 pueos from Kauai, Oahu, Lanai, Molokai, Maui and Hawaii. In barn owls, the most common cause of death was trauma (50%) followed by infectious disease (28%) and emaciation (22%). Cause of death was not determined in 7 barn owls. Most traumas apparently resulted from vehicular collisions. Trichomoniasis was the predominant infectious disease and appears to be a significant cause of death in barn owls in Hawaii. Pasteurellosis and aspergillosis were less commonly encountered. No predisposing cause of emaciation was detected.

Of 28 identified barn owl stomach contents, 64% contained mainly insects of the family Tetigoniidae and Gryllidae, 18% contained mainly rodents, and the remainder had mixtures of rodents and insects or grass. Three Pueos died of trauma and one each died of emaciation and pasteurellosis. Analysis of 22 livers and kidneys for organochlorine contaminants and 20 brains for cholinesterase activity failed to implicate organochlorine, organophosphorus or carbamate pesticides as causes of death in pueos or barn owls.

RETICULOENDOTHELIOSIS IN CAPTIVE GREATER (TYMPANUCHUS CUPIDO PINNATUS) AND ATTWATER’S (T. CUPIDO ATTWATERI) PRAIRIE CHICKENS. Mark L. Drew, Department of Large Animal Medicine and Surgery, College of Veterinary Medicine, Texas A&M University, College Station, Texas; David L. Graham, Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, Texas; Clifton P. Griffin and Nova J. Silvy, Department of Wildlife and Fisheries Science, College of Agriculture and Life Sciences, Texas A&M University, College Station, Texas; Adly Fadly and Richard Witter, Avian Disease and Oncology Laboratory, United States Department of Agriculture, 3606 E. Mount Hope Road, East Lansing, Michigan.

In 1991, a captive propagation program was started for prairie chickens using wild caught Greater Prairie chickens (Tympanuchus cupido pinnatus). Eggs from nests of wild Attwater’s Prairie chickens (T. cupido attwateri) were collected in 1993 to create a breeding flock of this subspecies. From 1993 to 1994, 3 greater prairie chickens have been euthanized or found dead with multiple subcutaneous nodules on the face, legs and feet. Neoplastic masses were found in multiple organs of all birds at necropsy. Histologic sections of these masses revealed pleomorphic lymphoreticular cells suggestive of reticuloendotheliosis. Reticuloendotheliosis virus was demonstrated in one tumor by PCR and viremia was evident in 53% of greater and Attwater’s prairie chickens in captivity. Management and genetic concerns arising from the presence of this disease that affect the captive breeding of these birds will be discussed.
Wild Ungulates


Moxidectin administered as a pour-on application at the rate of 500 ug/kg was highly efficacious against all mature and immature lungworm (Dictyocaulus) and abomasal nematodes in young red deer and wapiti hybrid (elk X red) deer. Efficacies of 100% were found for mature and immature lungworm, L5 Ostertagia-type and adult Ostertagia-type nematodes in the abomasum. Efficacies observed against Early and Late L4 Ostertagia-type parasites in the abomasum were 99.5% and 99% in red deer and 99.3% and 98% in wapiti hybrid deer respectively. There were no adverse side-effects observed in any treated animals.

EFFECTS OF TYPE AND PARASITIC INFECTION ON THE ORAL ABSORPTION AND EFFICACY OF ALBENDAZOLE IN DEER (CERVUS ELAPHUS). Ken Waldrup, Colin Mackintosh, Mike Duffy, Rob Labes and Peter Johnstone. AgResearch, Invermay Agricultural Centre, Puddle Alley, Private Bag 50034, Mosgiel, New Zealand.

Albendazole was administered as an oral drench at the rate of 10 mg/kg to parasitized and non-parasitized red deer, wapiti hybrid (F1, elk X red) deer and "pure" elk. Efficacy of this treatment against lungworm (Dictyocaulus) was shown to be approximately 90% in red deer, 60% in wapiti hybrid deer and negligible in elk. Pharmacokinetic analysis of plasma samples taken during this experiment showed a difference in the uptake of albendazole relative to type.

EXPERIMENTAL INFECTION OF WHITE-TAILED DEER WITH LEPTOSPIRA GRIPPOTYPHOSA. Charlotte F. Quist, Wayne A. Roberts, Cathy A. Brown, Kirk E. Smith, and Victor F. Nettles. Southeastern Cooperative Wildlife Disease Study and Athens Diagnostic Laboratory, College of Veterinary Medicine, The University of Georgia, Athens, GA 30602, USA.

The Southeastern Cooperative Wildlife Disease Study (SCWDS) has received numerous inquiries regarding the potential of wild mammals, particularly white-tailed deer (Odocoileus virginianus), to serve as reservoirs of leptospirosis. Although antibody prevalence to all serovars is low, serologic data from white-tailed deer in the southeastern United States indicate that Leptospira interrogaans serovar grippotyphosa is the second most common serovar after L. pomona. However, there are no clinical reports of L. grippotyphosa in white-tailed deer, nor have any experimental studies been done. The objective of this study was to evaluate the clinical and serologic response of white-tailed deer to L. grippotyphosa.
Wild Ungulates


Moxidectin administered as a pour-on application at the rate of 500 ug/kg was highly efficacious against all mature and immature lungworm (Dictyocaulus) and abomasal nematodes in young red deer and wapiti hybrid (elk X red) deer. Efficacies of 100% were found for mature and immature lungworm, L5 Ostertagia-type and adult Ostertagia-type nematodes in the abomasum. Efficacies observed against Early and Late L4 Ostertagia-type parasites in the abomasum were 99.5% and 99% in red deer and 99.3% and 98% in wapiti hybrid deer respectively. There were no adverse side-effects observed in any treated animals.

EFFECTS OF TYPE AND PARASITIC INFECTION ON THE ORAL ABSORPTION AND EFFICACY OF ALBENDAZOLE IN DEER (Cervus elaphus). Ken Waldrup, Colin Mackintosh, Mike Duffy, Rob Labes and Peter Johnstone. AgResearch, Invermay Agricultural Centre, Puddle Alley, Private Bag 50034, Mosgiel, New Zealand.

Albendazole was administered as an oral drench at the rate of 10 mg/kg to parasitized and non-parasitized red deer, wapiti hybrid (F1, elk X red) deer and "pure" elk. Efficacy of this treatment against lungworm (Dictyocaulus) was shown to be approximately 90% in red deer, 60% in wapiti hybrid deer and negligible in elk. Pharmacokinetic analysis of plasma samples taken during this experiment showed a difference in the uptake of albendazole relative to type.

EXPERIMENTAL INFECTION OF WHITE-TAILED DEER WITH LePTOSPIRA GRIppOTYPHOSa. Charlotte F. Quist,1 Wayne A. Roberts,2 Cathy A. Brown,2 Kirk E. Smith,1 and Victor F. Nettles.1 1Southeastern Cooperative Wildlife Disease Study and 2Athens Diagnostic Laboratory, College of Veterinary Medicine, The University of Georgia, Athens, GA 30602, USA.

The Southeastern Cooperative Wildlife Disease Study (SCWDS) has received numerous inquiries regarding the potential of wild mammals, particularly white-tailed deer (Odocoileus virginianus), to serve as reservoirs of leptospirosis. Although antibody prevalence to all serovars is low, serologic data from white-tailed deer in the southeastern United States indicate that Leptospira interrogans serovar grippotyphosa is the second most common serovar after L. pomona. However, there are no clinical reports of L. grippotyphosa in white-tailed deer, nor have any experimental studies been done. The objective of this study was to evaluate the clinical and serologic response of white-tailed deer to L. grippotyphosa.
Wild Ungulates


Moxidectin administered as a pour-on application at the rate of 500 μg/kg was highly efficacious against all mature and immature lungworm (Dictyocaulus) and abomasal nematodes in young red deer and wapiti hybrid (elk X red) deer. Efficacies of 100% were found for mature and immature lungworm, L5 Ostertagia-type and adult Ostertagia-type nematodes in the abomasum. Efficacies observed against Early and Late L4 Ostertagia-type parasites in the abomasum were 99.5% and 99% in red deer and 99.3% and 98% in wapiti hybrid deer respectively. There were no adverse side-effects observed in any treated animals.

Effects of Type and Parasitic Infection on the Oral Absorption and Efficacy of Albendazole in Deer (Cervus elaphus). Ken Waldrup, Colin Mackintosh, Mike Duffy, Rob Labes and Peter Johnstone. AgResearch, Invermay Agricultural Centre, Puddle Alley, Private Bag 50034, Mosgiel, New Zealand.

Albendazole was administered as an oral drench at the rate of 10 mg/kg to parasitized and non-parasitized red deer, wapiti hybrid (F1, elk X red) deer and "pure" elk. Efficacy of this treatment against lungworm (Dictyocaulus) was shown to be approximately 90% in red deer, 60% in wapiti hybrid deer and negligible in elk. Pharmacokinetic analysis of plasma samples taken during this experiment showed a difference in the uptake of albendazole relative to type.

Experimental Infection of White-Tailed Deer with Leptospira Grippotyphosa. Charlotte F. Quist,1 Wayne A. Roberts,2 Cathy A. Brown,2 Kirk E. Smith,1 and Victor F. Nettles.1 1Southeastern Cooperative Wildlife Disease Study and 2Athens Diagnostic Laboratory, College of Veterinary Medicine, The University of Georgia, Athens, GA 30602, USA.

The Southeastern Cooperative Wildlife Disease Study (SCWDS) has received numerous inquiries regarding the potential of wild mammals, particularly white-tailed deer (Odocoileus virginianus), to serve as reservoirs of leptospirosis. Although antibody prevalence to all serovars is low, serologic data from white-tailed deer in the southeastern United States indicate that Leptospira interrogans serovar grippotyphosa is the second most common serovar after L. pomona. However, there are no clinical reports of L. grippotyphosa in white-tailed deer, nor have any experimental studies been done. The objective of this study was to evaluate the clinical and serologic response of white-tailed deer to L. grippotyphosa.
Five 5-to-6-month-old fawns were inoculated subcutaneously with $4 \times 10^8$ viable *L. grippotyphosa* organisms; three additional fawns were housed with them as contact controls. After $3 \ 1/2$ weeks, three inoculated deer and one control deer were euthanized and necropsied. The remaining two inoculated animals were euthanized after 6 weeks. Clinical illness was not observed, nor were there any significant differences in body temperatures or leukocyte parameters between inoculated and control deer. All inoculated fawns developed antibodies by 10 days post-infection, with titers ranging from 1:100 to 1:800. Titers began receding at 20 days post-infection, but were still detectable at 6 weeks. Contact control animals remained serologically negative. Cross reactions to other *Leptospira* serovars, viz., *pomona, hardjo, icterohemorrhagicae, and canicola*, were not seen. This study indicates that antibody titers to *L. grippotyphosa* found in wild deer may be the result of actual exposure to this organism; however, lack of seroconversion in contact control fawns suggests that shedding of the organism may not occur.

**A MODEL FOR HEMORRHAGIC DISEASE IN WHITE-TAILED DEER BASED ON HERD IMMUNITY TO EPIZOOTIC HEMORRHAGIC DISEASE AND BLUETONGUE VIRUSES.** David E. Stallknecht, Victor F. Nettles, and William R. Davidson, Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, the University of Georgia, Athens, Georgia 30602.

Hemorrhagic disease (HD), which is caused by viruses in the epizootic hemorrhagic disease (EHD) and bluetongue (BT) serogroups, is the most important viral disease affecting white-tailed deer (*Odocoileus virginianus*) in the United States. Although these viruses are widely distributed over much of this species range, much regional variation exists in the extent of exposure and in the severity of the disease. A simple 3-step model was developed to represent the relationship between herd immunity and severity of disease. In the southeastern United States, reports of HD-related mortality occur most frequently from areas of limited exposure to few EHD and BT virus serotypes. In areas of increased exposure to multiple serotypes, reported disease occurs less frequently with reports of HD-related morbidity exceeding those relating to mortality. In areas of extremely high exposure to multiple serotypes, little or no disease is reported. Results indicate that exposure does not equate with disease and that herd immunity patterns can be used as a predictor of disease risk.

**EXPERIMENTAL INFECTION OF DEER WITH BOVINE VIRAL DIARRHEA VIRUS.** Elizabeth Williams¹, Hana Van Campen¹, Tom Thorne², Walt Cook¹, and Glenn Stout³, ¹Department of Veterinary Sciences, University of Wyoming, Laramie, Wyoming 82070; ²Wyoming Game and Fish Department, Research Laboratory, Box 3312, University Station, Laramie, Wyoming 82071; ³Wyoming Game and Fish Department, Sybille Wildlife Research and Conservation Education Unit, Wheatland, Wyoming 82201.

Bovine viral diarrhea (BVD) virus infection is an economically significant disease of cattle. Deer have been implicated as reservoirs of this virus for cattle with little scientific evidence.
Five 5-to-6-month-old fawns were inoculated subcutaneously with $4 \times 10^8$ viable *L. grippotyphosa* organisms; three additional fawns were housed with them as contact controls. After 3 1/2 weeks, three inoculated deer and one control deer were euthanized and necropsied. The remaining two inoculated animals were euthanized after 6 weeks. Clinical illness was not observed, nor were there any significant differences in body temperatures or leukocyte parameters between inoculated and control deer. All inoculated fawns developed antibodies by 10 days post-infection, with titers ranging from 1:100 to 1:800. Titers began receding at 20 days post-infection, but were still detectable at 6 weeks. Contact control animals remained serologically negative. Cross reactions to other *Leptospira* serovars, viz., pomona, hardjo, icterohemorrhagicae, and canicola, were not seen. This study indicates that antibody titers to *L. grippotyphosa* found in wild deer may be the result of actual exposure to this organism; however, lack of seroconversion in contact control fawns suggests that shedding of the organism may not occur.

A MODEL FOR HEMORRHAGIC DISEASE IN WHITE-TAILED DEER BASED ON HERD IMMUNITY TO EPIZOOTIC HEMORRHAGIC DISEASE AND BLUETONGUE VIRUSES. David E. Stallknecht, Victor F. Nettles, and William R. Davidson, Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, the University of Georgia, Athens, Georgia 30602.

Hemorrhagic disease (HD), which is caused by viruses in the epizootic hemorrhagic disease (EHD) and bluetongue (BT) serogroups, is the most important viral disease affecting white-tailed deer (*Odocoileus virginianus*) in the United States. Although these viruses are widely distributed over much of this species range, much regional variation exists in the extent of exposure and in the severity of the disease. A simple 3-step model was developed to represent the relationship between herd immunity and severity of disease. In the southeastern United States, reports of HD-related mortality occur most frequently from areas of limited exposure to few EHD and BT virus serotypes. In areas of increased exposure to multiple serotypes, reported disease occurs less frequently with reports of HD-related morbidity exceeding those relating to mortality. In areas of extremely high exposure to multiple serotypes, little or no disease is reported. Results indicate that exposure does not equate with disease and that herd immunity patterns can be used as a predictor of disease risk.

EXPERIMENTAL INFECTION OF DEER WITH BOVINE VIRAL DIARRHEA VIRUS. Elizabeth Williams, Hana Van Campen, Tom Thorne, Walt Cook, and Glenn Stout, 1Department of Veterinary Sciences, University of Wyoming, Laramie, Wyoming 82070; 2Wyoming Game and Fish Department, Research Laboratory, Box 3312, University Station, Laramie, Wyoming 82071; 3Wyoming Game and Fish Department, Sybille Wildlife Research and Conservation Education Unit, Wheatland, Wyoming 82201.

Bovine viral diarrhea (BVD) virus infection is an economically significant disease of cattle. Deer have been implicated as reservoirs of this virus for cattle with little scientific evidence.
Five 5-to-6-month-old fawns were inoculated subcutaneously with $4 \times 10^8$ viable L. 
grippotyphosa organisms; three additional fawns were housed with them as contact controls.
After 3 1/2 weeks, three inoculated deer and one control deer were euthanized and 
necropsied. The remaining two inoculated animals were euthanized after 6 weeks. Clinical 
ilness was not observed, nor were there any significant differences in body temperatures or 
leukocyte parameters between inoculated and control deer. All inoculated fawns developed 
antibodies by 10 days post-infection, with titers ranging from 1:100 to 1:800. Titers began 
receding at 20 days post-infection, but were still detectable at 6 weeks. Contact control 
animals remained serologically negative. Cross reactions to other Leptospira serovars, viz., 
pomona, hardjo, icterohemorrhagiae, and canicola, were not seen. This study indicates that 
antibody titers to L. grippotyphosa found in wild deer may be the result of actual exposure 
to this organism; however, lack of seroconversion in contact control fawns suggests that 
shedding of the organism may not occur.

A MODEL FOR HEMORRHAGIC DISEASE IN WHITE-TAILED DEER BASED ON 
HERD IMMUNITY TO EPIZOOTIC HEMORRHAGIC DISEASE AND BLUETONGUE 
VIRUSES. David E. Stallknecht, Victor F. Nettles, and William R. Davidson, Southeastern 
Cooperative Wildlife Disease Study, College of Veterinary Medicine, the University of 
Georgia, Athens, Georgia 30602.

Hemorrhagic disease (HD), which is caused by viruses in the epizootic hemorrhagic disease 
(EHD) and bluetongue (BT) serogroups, is the most important viral disease affecting 
white-tailed deer (Odocoileus virginianus) in the United States. Although these viruses are 
widely distributed over much of this species range, much regional variation exists in the 
extent of exposure and in the severity of the disease. A simple 3-step model was developed 
to represent the relationship between herd immunity and severity of disease. In the 
southeastern United States, reports of HD-related mortality occur most frequently from 
areas of limited exposure to few EHD and BT virus serotypes. In areas of increased 
exposure to multiple serotypes, reported disease occurs less frequently with reports of HD-
related morbidity exceeding those relating to mortality. In areas of extremely high exposure 
to multiple serotypes, little or no disease is reported. Results indicate that exposure does 
not equate with disease and that herd immunity patterns can be used as a predictor of 
disease risk.

EXPERIMENTAL INFECTION OF DEER WITH BOVINE VIRAL DIARRHEA VIRUS. 
Elizabeth Williams¹, Hana Van Campen¹, Tom Thorne², Walt Cook¹, and Glenn Stout³, 
¹Department of Veterinary Sciences, University of Wyoming, Laramie, Wyoming 82070; 
²Wyoming Game and Fish Department, Research Laboratory, Box 3312, University Station, 
Laramie, Wyoming 82071; ³Wyoming Game and Fish Department, Sybille Wildlife Research 
and Conservation Education Unit, Wheatland, Wyoming 82201.

Bovine viral diarrhea (BVD) virus infection is an economically significant disease of cattle. 
Deer have been implicated as reservoirs of this virus for cattle with little scientific evidence.
To determine the susceptibility of deer to infection with BVD virus, four mule deer (*Odocoileus hemionus*) and one white-tailed deer (*O. virginianus*) fawns were inoculated intranasally with NY-1 strain of BVD virus. All deer were febrile at 2 days postinoculation, but none of the animals developed significant clinical illness. Virus was isolated from the white blood cells of four of five fawns from day 2-15 postinoculation indicating systemic infection. In addition, virus was isolated from nasal swabs suggesting BVD virus could be transmitted by this route. Four of five fawns had serum neutralization titers to NADL-BVD virus prior to inoculation and all developed >4-fold serum neutralization titers to BVD virus by 3 weeks postinoculation. These preliminary findings indicate that mule and white-tailed deer are susceptible to infection with BVD virus and that the pathogenesis of the acute infection is similar to that in calves. Additional studies of the pathogenesis and epizootiology of pestivirus infections in deer are underway.

**SPONGIFORM ENCEPHALOPATHY IN FREE-RANGING CERVIDS IN COLORADO.**

T. R. Spraker, Department of Pathology, Colorado State University, Fort Collins, Colorado 80523; M. W. Miller, Colorado Division of Wildlife, Fort Collins, Colorado 80523; E. S. Williams, Wyoming State Diagnostic Laboratory, University of Wyoming, Laramie, Wyoming 82070; D. M. Getzy, Department of Pathology, Colorado State University, Fort Collins, Colorado 80523; W. J. Adrian, G. G. Schoonveld and R. A. Spowart, Colorado Division of Wildlife, Fort Collins, Colorado 80523.

Between March 1981 and March 1995, 44 cases of a spongiform encephalopathy was diagnosed in 37 mule deer (*Odocoileus hemionus*), 6 Rocky Mountain elk (*Cervus elaphus nelsoni*) and a white-tailed deer (*O. virginianus*) from northcentral Colorado. This is the first known outbreaks of a spongiform encephalopathy in free-ranging mammals. Clinical signs included emaciation, excessive salivation, behavioral changes, ataxia and weakness. Severe emaciation with total loss and/or serious atrophy of adipose tissue were the only consistent gross finding. Spongiform encephalopathy characterized by microcavitation of grey and white matter, intraneuronal vacuolation and neuronal degeneration was found microscopically in all cases. Scrapie associated prion protein was found in brains of 12 animals, 6 using an immunohistochemical staining procedure and in another 6 by Western blot. Ages ranged from 2.5 to 7.5 yrs for mule deer and from 1.5 to 10.5 yrs for elk. Mule deer appeared to be the primary species affected and accounted for 84% of all cases. Males were disproportionately over represented among mule deer cases. Seasonality of cases were apparent: 37 of the 44 cases were submitted during October-April. Mule deer submissions were clustered near two population centers (Fort Collins and Estes Park). Forty-one of these 44 cases were submitted since 1990. Prevalence estimates, host range (including domestic animals), distribution, origins and management implications of spongiform encephalopathy in free-ranging wild deer and elk remain unknown.
To determine the susceptibility of deer to infection with BVD virus, four mule deer (Odocoileus hemionus) and one white-tailed deer (O. virginianus) fawns were inoculated intranasally with NY-1 strain of BVD virus. All deer were febrile at 2 days postinoculation, but none of the animals developed significant clinical illness. Virus was isolated from the white blood cells of four of five fawns from day 2-15 postinoculation indicating systemic infection. In addition, virus was isolated from nasal swabs suggesting BVD virus could be transmitted by this route. Four of five fawns had serum neutralization titers to NADL-BVD virus prior to inoculation and all developed >4-fold serum neutralization titers to BVD virus by 3 weeks postinoculation. These preliminary findings indicate that mule and white-tailed deer are susceptible to infection with BVD virus and that the pathogenesis of the acute infection is similar to that in calves. Additional studies of the pathogenesis and epizootiology of pestivirus infections in deer are underway.

SPONGIFORM ENCEPHALOPATHY IN FREE-RANGING CERVIDS IN COLORADO.
T. R. Spraker, Department of Pathology, Colorado State University, Fort Collins, Colorado 80523; M. W. Miller, Colorado Division of Wildlife, Fort Collins, Colorado 80523; E. S. Williams, Wyoming State Diagnostic Laboratory, University of Wyoming, Laramie, Wyoming 82070; D. M. Getzy, Department of Pathology, Colorado State University, Fort Collins, Colorado 80523; W. J. Adrian, G. G. Schoonveld and R. A. Spowart, Colorado Division of Wildlife, Fort Collins, Colorado 80523.

Between March 1981 and March 1995, 44 cases of a spongiform encephalopathy was diagnosed in 37 mule deer (Odocoileus hemionus), 6 Rocky Mountain elk (Cervus elaphus nelsoni) and a white-tailed deer (O. virginianus) from northcentral Colorado. This is the first known outbreaks of a spongiform encephalopathy in free-ranging mammals. Clinical signs included emaciation, excessive salivation, behavioral changes, ataxia and weakness. Severe emaciation with total loss and/or serious atrophy of adipose tissue were the only consistent gross finding. Spongiform encephalopathy characterized by microcavitation of grey and white matter, intraneuronal vacuolation and neuronal degeneration was found microscopically in all cases. Scrapie associated prion protein was found in brains of 12 animals, 6 using an immunohistochemical staining procedure and in another 6 by Western blot. Ages ranged from 2.5 to 7.5 yrs for mule deer and from 1.5 to 10.5 yrs for elk. Mule deer appeared to be the primary species affected and accounted for 84% of all cases. Males were disproportionately over represented among mule deer cases. Seasonality of cases were apparent: 37 of the 44 cases were submitted during October-April. Mule deer submissions were clustered near two population centers (Fort Collins and Estes Park). Forty-one of these 44 cases were submitted since 1990. Prevalence estimates, host range (including domestic animals), distribution, origins and management implications of spongiform encephalopathy in free-ranging wild deer and elk remain unknown.
EVALUATION OF WILDLIFE SPECIES FOR THE SURVEILLANCE OF LYME DISEASE. Robert G. McLean, Division Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, P.O. Box 2087, Fort Collins, Colorado 80522; James S. Gill, University of Osteopathic Medicine and Health, 3200 Grand Ave., Des Moines, Iowa 50312; Rexford D. Lord and Jan G. Humphreys, Biology Department, Indiana University of Pennsylvania, Indiana, Pennsylvania 15705.

Lyme disease, caused by Borrelia burgdorferi, has emerged as the leading vector-borne disease in the United States and its distribution is expanding. Because this continuing expansion will greatly increase the public health risk in newly emerging states, reliable surveillance to accurately determine the geographic distribution and to monitor spatial and temporal changes are needed. Through cooperative programs, we are determining the best wildlife species to use as sentinels for state-wide surveillance of B. burgdorferi. Three wildlife species were sampled in Pennsylvania and the statewide distribution of B. burgdorferi and specific habitats that support transmission were determined. White-tailed deer were found to be an effective sentinel for determining the specific geographic distribution of B. burgdorferi in Minnesota and for state-wide surveillance in other states. The best method was the serologic testing of hunter-killed deer by ELISA with western immunoblotting for confirmation.

INFECTIOUS, PARASITIC AND OTHER DISEASES OF FREE LIVING MOOSE (ALCES ALCES) IN SWEDEN. Dolores Gavier-Widen1, Torsten Mörner1, and Malik Merza2, 1Division of Wildlife, 2Department of Virology, National Veterinary Institute, P.O. Box 7073, S-75007 Uppsala, Sweden.

A number of diseases were identified by post mortem examination and laboratory investigations on approximately 900 moose (Alces alces) necropsied at the National Veterinary Institute between 1986 and 1995. Only a few diseases are presently known to be caused by viral infection. Malignant catarrhal fever is seen occasionally. Cutaneous fibropapillomas are found in about 0.5% of the moose population; they are caused by a papillomavirus specific for moose and different from all bovine papillomaviruses. Endemic ethmoturbinate tumor, most of them carcinomas, have a similar presentation to that of cattle in Sweden and are probably caused by a retroviral infection. Tick born encephalitis seldom causes disease in the free ranging moose, even though the proportion of seropositive individuals is high within endemic areas. Recently, a previously unknown retrovirus, the Alces leucotropic oncovirus (ALOV) has been repeatedly isolated from animals with a chronic wasting syndrome. The syndrome involves ulceration of the oral mucosa, enteritis and cell depletion of lymphoid organs. It occurs mostly in the South of Sweden. Much research and resources have been dedicated to finding the cause of the disease and a number of experimental infections aimed at elucidating the role of the recently isolated ALOV virus in the development of the disease are currently going on. Bacterial infections secondary to traumatic lesions are occasionally seen. Pneumonia caused by Klebsiella pneumonia or Pasteurella multocida occurs only sporadically. Fusobacterium necrophorum is involved in deep necrosis of gingival mucosa in association with food impaction. Infection
EVALUATION OF WILDLIFE SPECIES FOR THE SURVEILLANCE OF LYME DISEASE. Robert G. McLean, Division Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, P.O. Box 2087, Fort Collins, Colorado 80522; James S. Gill, University of Osteopathic Medicine and Health, 3200 Grand Ave., Des Moines, Iowa 50312; Rexford D. Lord and Jan G. Humphreys, Biology Department, Indiana University of Pennsylvania, Indiana, Pennsylvania 15705.

Lyme disease, caused by Borrelia burgdorferi, has emerged as the leading vector-borne disease in the United States and its distribution is expanding. Because this continuing expansion will greatly increase the public health risk in newly emerging states, reliable surveillance to accurately determine the geographic distribution and to monitor spatial and temporal changes are needed. Through cooperative programs, we are determining the best wildlife species to use as sentinels for state-wide surveillance of B. burgdorferi. Three wildlife species were sampled in Pennsylvania and the statewide distribution of B. burgdorferi and specific habitats that support transmission were determined. White-tailed deer were found to be an effective sentinel for determining the specific geographic distribution of B. burgdorferi in Minnesota and for state-wide surveillance in other states. The best method was the serologic testing of hunter-killed deer by ELISA with western immunoblotting for confirmation.

INFECTIOUS, PARASITIC AND OTHER DISEASES OF FREE LIVING MOOSE (Alces Alces) IN SWEDEN. Dolores Gavier-Widén¹, Torsten Mörner¹, and Malik Merza², ¹Division of Wildlife, ²Department of Virology, National Veterinary Institute, P.O. Box 7073, S-75007 Uppsala, Sweden.

A number of diseases were identified by post mortem examination and laboratory investigations on approximately 900 moose (Alces alces) necropsied at the National Veterinary Institute between 1986 and 1995. Only a few diseases are presently known to be caused by viral infection. Malignant catarrhal fever is seen occasionally. Cutaneous fibropapillomas are found in about 0.5% of the moose population; they are caused by a papillomavirus specific for moose and different from all bovine papillomaviruses. Endemic ethmoturbinate tumor, most of them carcinomas, have a similar presentation to that of cattle in Sweden and are probably caused by a retroviral infection. Tick born encephalitis seldom causes disease in the free ranging moose, even though the proportion of seropositive individuals is high within endemic areas. Recently, a previously unknown retrovirus, the Alces leucotropic oncovirus (ALOV) has been repeatedly isolated from animals with a chronic wasting syndrome. The syndrome involves ulceration of the oral mucosa, enteritis and cell depletion of lymphoid organs. It occurs mostly in the South of Sweden. Much research and resources have been dedicated to finding the cause of the disease and a number of experimental infections aimed at elucidating the role of the recently isolated ALOV virus in the development of the disease are currently going on. Bacterial infections secondary to traumatic lesions are occasionally seen. Pneumonia caused by Klebsiella pneumonia or Pasteurella multocida occurs only sporadically. Fusobacterium necrophorus is involved in deep necrosis of gingival mucosa in association with food impaction. Infection
with *Mycobacterium avium* is found sporadically. Approximately 30% of the moose population is infected with the nematode *Elaphostrongylus alces*, sometimes causing granulomatous inflammation in the meninges and spinal nerves, and high mortality with nervous signs and emaciation among calves. Other common parasitic diseases are chorioptic mange, subcutaneous nodules of *Onchocerca* spp. and parasitic pneumonia caused by *Dictyocaulus viviparus*. Intestinal parasites rarely cause disease in free living moose. Calves can suffer from enteritis by severe infestation with coccidia. Repeatedly observed postmortem findings with unknown etiology include interstitial nephritis, keratitis, cataract, meningitis and infertility. Diagnostic methods for specific viral diseases of moose are currently under development at the National Veterinary Institute of Sweden; moose cells for viral isolation, monoclonal antibodies, anti-moose IgG and a serum bank are currently available for routine diagnostic purposes.

**EFFECT OF STRESS ON PASTEURELLA HAEMOLYTICA CYTOTOXIN DEPENDENT KILLING OF NEUTROPHILS FROM BIGHORN SHEEP.** Brita J. Kraabel and Michael W. Miller, Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, Colorado 80526

Peripheral blood neutrophils from ten Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) were exposed to culture supernatants from one domestic and three bighorn sheep *Pasteurella haemolytica* isolates. Cytotoxicity of the isolates was determined prior to the administration of adrenocorticotrophic hormone (ACTH) to the bighorn sheep and compared to the cytotoxicity after administration of ACTH. Neutrophils from ACTH treated bighorn sheep were more susceptible to the cytotoxic effects of three of the four isolates. White blood cell counts from treated bighorn sheep demonstrated increased number of neutrophils and decreased number of lymphocytes and eosinophils when compared to white cell counts prior to ACTH administration (*p* = 0.02). Cytotoxin dependent killing of bighorn sheep neutrophils was enhanced by prior administration of ACTH.

**BRUCELLOSIS IN YELLOWSTONE NATIONAL PARK BISON: 1995 SAMPLING OF OUT-MIGRATING BISON (BISON BISON).** Thomas J. Roffe, National Wildlife Health Center, 6006 Schroeder Rd, Madison, WI 53711; Jack C. Rhyan, National Veterinary Services Laboratory, PO Box 844, Ames, IA 50010; Keith Aune, Montana Fish Wildlife and Parks, Montana State University, Bozeman, MT 59717; Michael Philo, Animal and Plant Health Inspection Service, 9439 Owl Way, Bozeman, MT 59715; Darla R. Ewalt, National Veterinary Services Laboratory, PO Box 844, Ames, IA 50010.

The issue of brucellosis in ungulates of the Yellowstone Ecosystem is controversial because of the varying perceptions of risk of transmission from infected wildlife to cattle. While bison and elk of the ecosystem are known to be infected, infection prevalence, relationship to serology, and mode of transmission for Yellowstone National Park (YNP) bison are debated. Bison migrating out of YNP into Montana are destroyed because they are viewed as a threat to Montana’s brucellosis-free status for cattle, and over 400 have been killed this
with *Mycobacterium avium* is found sporadically. Approximately 30% of the moose population is infected with the nematode *Elaphostrongylus alces*, sometimes causing granulomatous inflammation in the meninges and spinal nerves, and high mortality with nervous signs and emaciation among calves. Other common parasitic diseases are chorioptic mange, subcutaneous nodules of *Onchocerca* spp. and parasitic pneumonia caused by *Dictyocaulus viviparus*. Intestinal parasites rarely cause disease in free living moose. Calves can suffer from enteritis by severe infestation with coccidia. Repeatedly observed post mortem findings with unknown etiology include interstitial nephritis, keratitis, cataract, meningitis and infertility. Diagnostic methods for specific viral diseases of moose are currently under development at the National Veterinary Institute of Sweden; moose cells for viral isolation, monoclonal antibodies, anti-moose IgG and a serum bank are currently available for routine diagnostic purposes.

**EFFECT OF STRESS ON PASTEURLELLA HAEMOLYTICA CYTOTOXIN DEPENDENT KILLING OF NEUTROPHILS FROM BIGHORN SHEEP.** Brita J. Kraabel and Michael W. Miller, Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, Colorado 80526

Peripheral blood neutrophils from ten Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) were exposed to culture supernatants from one domestic and three bighorn sheep *Pasteurella haemolytica* isolates. Cytotoxicity of the isolates was determined prior to the administration of adrenocorticotrophic hormone (ACTH) to the bighorn sheep and compared to the cytotoxicity after administration of ACTH. Neutrophils from ACTH treated bighorn sheep were more susceptible to the cytotoxic effects of three of the four isolates. White blood cell counts from treated bighorn sheep demonstrated increased number of neutrophils and decreased number of lymphocytes and eosinophils when compared to white cell counts prior to ACTH administration (p = 0.02). Cytotoxin dependent killing of bighorn sheep neutrophils was enhanced by prior administration of ACTH.

**BRUCELLOSIS IN YELLOWSTONE NATIONAL PARK BISON: 1995 SAMPLING OF OUT-MIGRATING BISON (BISON BISON).** Thomas J. Roffe, National Wildlife Health Center, 6006 Schroeder Rd, Madison, WI 53711; Jack C. Rhyan, National Veterinary Services Laboratory, PO Box 844, Ames, IA 50010; Keith Aune, Montana Fish Wildlife and Parks, Montana State University, Bozeman, MT 59717; Michael Philo, Animal and Plant Health Inspection Service, 9439 Owl Way, Bozeman, MT 59715; Darla R. Ewalt, National Veterinary Services Laboratory, PO Box 844, Ames, IA 50010.

The issue of brucellosis in ungulates of the Yellowstone Ecosystem is controversial because of the varying perceptions of risk of transmission from infected wildlife to cattle. While bison and elk of the ecosystem are known to be infected, infection prevalence, relationship to serology, and mode of transmission for Yellowstone National Park (YNP) bison are debated. Bison migrating out of YNP into Montana are destroyed because they are viewed as a threat to Montana's brucellosis-free status for cattle, and over 400 have been killed this
with *Mycobacterium avium* is found sporadically. Approximately 30% of the moose population is infected with the nematode *Elaphostrongylus alces*, sometimes causing granulomatous inflammation in the meninges and spinal nerves, and high mortality with nervous signs and emaciation among calves. Other common parasitic diseases are choriomycotic mange, subcutaneous nodules of *Onchocerca* spp. and parasitic pneumonia caused by *Dictyocaulus viviparus*. Intestinal parasites rarely cause disease in free living moose. Calves can suffer from enteritis by severe infection with coccidia. Repeatedly observed post mortem findings with unknown etiology include interstitial nephritis, keratitis, cataract, meningitis and infertility. Diagnostic methods for specific viral diseases of moose are currently under development at the National Veterinary Institute of Sweden; moose cells for viral isolation, monoclonal antibodies, anti-moose IgG and a serum bank are currently available for routine diagnostic purposes.

**EFFECT OF STRESS ON PASTEURELLA HAEMOLYTICA CYTOTOXIN DEPENDENT KILLING OF NEUTROPHILS FROM BIGHORN SHEEP.** Brita J. Kraabel and Michael W. Miller, Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, Colorado 80526

Peripheral blood neutrophils from ten Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) were exposed to culture supernatants from one domestic and three bighorn sheep *Pasteurella haemolytica* isolates. Cytotoxicity of the isolates was determined prior to the administration of adrenocorticotrophic hormone (ACTH) to the bighorn sheep and compared to the cytotoxicity after administration of ACTH. Neutrophils from ACTH treated bighorn sheep were more susceptible to the cytotoxic effects of three of the four isolates. White blood cell counts from treated bighorn sheep demonstrated increased number of neutrophils and decreased number of lymphocytes and eosinophils when compared to white cell counts prior to ACTH administration ($p = 0.02$). Cytokine dependent killing of bighorn sheep neutrophils was enhanced by prior administration of ACTH.

**BRUCELLOSIS IN YELLOWSTONE NATIONAL PARK BISON: 1995 SAMPLING OF OUT-MIGRATING BISON (BISON BISON).** Thomas J. Roffe, National Wildlife Health Center, 6006 Schroeder Rd, Madison, WI 53711; Jack C. Ryhan, National Veterinary Services Laboratory, PO Box 844, Ames, IA 50010; Keith Aune, Montana Fish Wildlife and Parks, Montana State University, Bozeman, MT 59717; Michael Philo, Animal and Plant Health Inspection Service, 9439 Owl Way, Bozeman, MT 59715; Darla R. Ewalt, National Veterinary Services Laboratory, PO Box 844, Ames, IA 50010.

The issue of brucellosis in ungulates of the Yellowstone Ecosystem is controversial because of the varying perceptions of risk of transmission from infected wildlife to cattle. While bison and elk of the ecosystem are known to be infected, infection prevalence, relationship to serology, and mode of transmission for Yellowstone National Park (YNP) bison are debated. Bison migrating out of YNP into Montana are destroyed because they are viewed as a threat to Montana's brucellosis-free status for cattle, and over 400 have been killed this
year. We collected samples from 10 of these bison: 5 females (4 card test positive, 1 card test negative) and 5 males (3 card test positive, 2 card test negative). Critical in our assessment of infection was a field sampling protocol that included collection of 18-20 lymph nodes and 12-15 other tissues, swabs and fluids from each carcass. This protocol was completed in 8 of the 10 bison collected thus far. The remaining 2 (card test negative males) had only blood samples taken. Multiple tissues from fetuses were also collected. One card-positive female had a retained placenta, necro-suppurative placentitis/metritis, and immunohistochemical evidence of Brucella bacteria in the uterus, placenta and exudate. Bacteriology and confirmatory serology are currently pending on all animals. Final results, including those from additional animals collected this year, will be reported.

ENZOOTIC BRUCELLOSIS (BRUCELLA SUIS BIOTYPE 2) IN WILD BOARS (SUS SCROFA) IN BELGIUM. J. Godfroid and F. Boelaert, National Veterinary Research Institute, Groeselenberg, 99, B-1180 Brussels, Belgium; C. Saegerman and X. Patigny, Veterinary Services, rue des Champs Elysées, 4, B-5590 Ciney, Belgium.

We describe the first case of enzootic Brucella suis biotype 2 brucellosis in wild boars (Sus scrofa) in Western Europe, where a potential Brucella suis biotype 2 reservoir in hares (Lepus capensis) exists. In 1994, 13 B. suis biotype 2 strains were isolated from 141 wild boars (9.2%) in the absence of visible pathological changes. The serological diagnosis of brucellosis is still problematic in suidae. In our study the Slow Agglutination Test (SAT) was found to be unsatisfactory. The Rose Bengal Test (RBT) and the Complement Fixation Test (CFT) were found to be satisfactory at the population level, if used in parallel and if the sample size is sufficient. We have developed an indirect ELISA for the detection of serum antibodies directed against the "smooth" Brucella lipopolysaccharide (S-LPS). In our study, the ELISA detected antibodies in all bacteriological positive wild boars and was used on poor quality sera that could not be analyzed by classical tests. Fifty-six sera (39%) were classified positive by the ELISA. According to the biology of the B. suis biotype 2 infection in suidae and to the excellent specificity of the ELISA in domestic pigs, our results suggest that the prevalence of the B. suis biotype 2 infection in wild boars is very high. The proposed ELISA should be evaluated in the context of B. suis infections in both domestic (or feral) pigs and wild boars. The contamination of cattle and domestic pigs by direct or indirect contact with infected wild boars or hares might be possible. Therefore, the implication of B. suis biotype 2 besides Yersinia enterocolitica 0:9 in the occurrence of (false?) positive serological reactions in brucellosis screening tests or eradication programs in cattle and in domestic pigs should be considered.


Rinderpest virus was first introduced into sub-Saharan Africa at the end of the 19th Century. Periodic outbreaks of the virus up until the 1950's had profound effects on ecosystem function; in particular, the virus kept ungulate numbers at low densities and
We collected samples from 10 of these bison: 5 females (4 card test positive, 1 card test negative) and 5 males (3 card test positive, 2 card test negative). Critical in our assessment of infection was a field sampling protocol that included collection of 18-20 lymph nodes and 12-15 other tissues, swabs and fluids from each carcass. This protocol was completed in 8 of the 10 bison collected thus far. The remaining 2 (card test negative males) had only blood samples taken. Multiple tissues from fetuses were also collected. One card-positive female had a retained placenta, necro-suppurative placentitis/metritis, and immunohistochemical evidence of Brucella bacteria in the uterus, placenta and exudate. Bacteriology and confirmatory serology are currently pending on all animals. Final results, including those from additional animals collected this year, will be reported.

ENZOOTIC BRUCELLOSIS (BRUCELLA SUIS BIOTYPE 2) IN WILD BOARS (SUS SCROFA) IN BELGIUM. J. Godfroid and F. Boelaert, National Veterinary Research Institute, Groeselenberg, 99, B-1180 Brussels, Belgium; C. Saegerman and X. Patigny, Veterinary Services, rue des Champs Elysées, 4, B-5590 Ciney, Belgium.

We describe the first case of enzootic Brucella suis biotype 2 brucellosis in wild boars (Sus scrofa) in Western Europe, where a potential Brucella suis biotype 2 reservoir in hares (Lepus capensis) exists. In 1994, 13 B. suis biotype 2 strains were isolated from 141 wild boars (9.2%) in the absence of visible pathological changes. The serological diagnosis of brucellosis is still problematic in suidae. In our study the Slow Agglutination Test (SAT) was found to be unsatisfactory. The Rose Bengal Test (RBT) and the Complement Fixation Test (CFT) were found to be satisfactory at the population level, if used in parallel and if the sample size is sufficient. We have developed an indirect ELISA for the detection of serum antibodies directed against the "smooth" Brucella lipopolysaccharide (S-LPS). In our study, the ELISA detected antibodies in all bacteriological positive wild boars and was used on poor quality sera that could not be analyzed by classical tests. Fifty-six sera (39%) were classified positive by the ELISA. According to the biology of the B. suis biotype 2 infection in suidae and to the excellent specificity of the ELISA in domestic pigs, our results suggest that the prevalence of the B. suis biotype 2 infection in wild boars is very high. The proposed ELISA should be evaluated in the context of B. suis infections in both domestic (or feral) pigs and wild boars. The contamination of cattle and domestic pigs by direct or indirect contact with infected wild boars or hares might be possible. Therefore, the implication of B. suis biotype 2 besides Yersinia enterocolitica 0:9 in the occurrence of (false?) positive serological reactions in brucellosis screening tests or eradication programs in cattle and in domestic pigs should be considered.


Rinderpest virus was first introduced into sub-Saharan Africa at the end of the 19th Century. Periodic outbreaks of the virus up until the 1950's had profound effects on ecosystem function; in particular, the virus kept ungulate numbers at low densities and
year. We collected samples from 10 of these bison: 5 females (4 card test positive, 1 card test negative) and 5 males (3 card test positive, 2 card test negative). Critical in our assessment of infection was a field sampling protocol that included collection of 18-20 lymph nodes and 12-15 other tissues, swabs and fluids from each carcass. This protocol was completed in 8 of the 10 bison collected thus far. The remaining 2 (card test negative males) had only blood samples taken. Multiple tissues from fetuses were also collected. One card-positive female had a retained placenta, necro-suppurative placentitis/metritis, and immunohistochemical evidence of Brucella bacteria in the uterus, placenta and exudate. Bacteriology and confirmatory serology are currently pending on all animals. Final results, including those from additional animals collected this year, will be reported.

ENZOOTIC BRUCELLOSIS (BRUCELLA SUIS BIOTYPE 2) IN WILD BOARS (SUS SCROFA) IN BELGIUM. J. Godfroid and F. Boelaert, National Veterinary Research Institute, Groeselenberg, 99, B-1180 Brussels, Belgium; C. Saegerman and X. Patigny, Veterinary Services, rue des Champs Elysées, 4, B-5590 Ciney, Belgium.

We describe the first case of enzootic Brucella suis biotype 2 brucellosis in wild boars (Sus scrofa) in Western Europe, where a potential Brucella suis biotype 2 reservoir in hares (Lepus capensis) exists. In 1994, 13 B. suis biotype 2 strains were isolated from 141 wild boars (9.2%) in the absence of visible pathological changes. The serological diagnosis of brucellosis is still problematic in suidae. In our study the Slow Agglutination Test (SAT) was found to be unsatisfactory. The Rose Bengal Test (RBT) and the Complement Fixation Test (CFT) were found to be satisfactory at the population level, if used in parallel and if the sample size is sufficient. We have developed an indirect ELISA for the detection of serum antibodies directed against the"smooth" Brucella lipopolysaccharide (S-LPS). In our study, the ELISA detected antibodies in all bacteriological positive wild boars and was used on poor quality sera that could not be analyzed by classical tests. Fifty-six sera (39%) were classified positive by the ELISA. According to the biology of the B. suis biotype 2 infection in suidae and to the excellent specificity of the ELISA in domestic pigs, our results suggest that the prevalence of the B. suis biotype 2 infection in wild boars is very high. The proposed ELISA should be evaluated in the context of B. suis infections in both domestic (or feral) pigs and wild boars. The contamination of cattle and domestic pigs by direct or indirect contact with infected wild boars or hares might be possible. Therefore, the implication of B. suis biotype 2 besides Yersinia enterocolitica 0:9 in the occurrence of (false?) positive serological reactions in brucellosis screening tests or eradication programs in cattle and in domestic pigs should be considered.


Rinderpest virus was first introduced into sub-Saharan Africa at the end of the 19th Century. Periodic outbreaks of the virus up until the 1950’s had profound effects on ecosystem function; in particular, the virus kept ungulate numbers at low densities and
allowed increased levels of recruitment in many plants species. The classic way to determine
the importance of a keystone species is to try and remove that species and monitor changes
in ecosystem function. The development of an vaccine for rinderpest allowed this
'experiment' to be undertaken. Vaccination of domestic cattle produced a significant
reduction in rinderpest prevalence in all species in the Serengeti ecosystem, this led to
significant increases in the wildebeest and buffalo populations. These in turn produced
significant increases in the density of some, but not all, predator species, particularly lions
and hyaenas. In this talk I will describe work on the history of rinderpest and rinderpest
vaccination in the Ngorongoro Conservation Area and in Serengeti. Some simple
mathematical models will be described that allow us to examine the ecological conditions
that preceded previous outbreaks. We will then examine how the spatial distribution of
different potential host species effects the long term efficacy of the rinderpest vaccination
program and the potential for future outbreaks.

EVIDENCE OF MYCOBACTERIUM PARATUBERCULOSIS IN THE FECES OF TULE
ELK FROM POINT REYES NATIONAL PARK. Walter Cook, Dept. of Vet. Science, Univ.
of Wyoming, 1174 Snowy Range Road, Laramie, WY 82070; Todd Cornish, Dept. of
Microbiology, Pathology, and Parasitology, College of Vet. Med., 4700 Hillsborough
Street, North Carolina State Univ., Raleigh, NC 27606; Bill Lasley, Dept. of Population Health and
Reproduction, School of Vet. Med., Univ. of Calif., Davis, CA 95616.

The tule elk (Cervus elaphus nannodes) of Point Reyes National Park, California were
reported to have paratuberculosis in 1981. However, definitive evidence is lacking that
Mycobacterium paratuberculosis has caused clinical disease in recent years, leading to
speculation about whether the herd is still infected. In July of 1993 100 fresh fecal samples
were collected by herding groups of elk away from bedding and feeding areas. Samples
were cultured on a modified BACTEC 12B radiometric medium for detection of M.
paratuberculosis. Four of 95 uncontaminated samples were positive and came from two
different areas of the Park. This study used a noninvasive technique to document the
presence of M. paratuberculosis in the elk of Point Reyes National Park. These findings
limit the management options available for this herd of tule elk.

Amphibians

A REVIEW OF CAUSES OF MORTALITY OF THE WYOMING TOAD (BUFO
HEMIOPHYRYS BAXTERI), 1989-1995. Sharon K. Taylor¹, Elizabeth S. Williams¹, and E.
Tom Thorne². ¹Department of Veterinary Sciences, University of Wyoming, 1174 Snowy
Range Road, Laramie, Wyoming 82070; ²Wyoming Game and Fish Department, Box 3313,
University Station, Laramie, Wyoming 82071.

The Wyoming toad (Bufo hemiophrlys baxteri) was listed as an endangered species in 1984
by the U.S. Fish and Wildlife Service. This toad is believed to be a glacial relict subspecies
of the Canadian toad (B. hemiophrys) and has historically only been known to have
allowed increased levels of recruitment in many plants species. The classic way to determine the importance of a keystone species is to try and remove that species and monitor changes in ecosystem function. The development of an vaccine for rinderpest allowed this 'experiment' to be undertaken. Vaccination of domestic cattle produced a significant reduction in rinderpest prevalence in all species in the Serengeti ecosystem, this led to significant increases in the wildebeest and buffalo populations. These in turn produced significant increases in the density of some, but not all, predator species, particularly lions and hyaenas. In this talk I will describe work on the history of rinderpest and rinderpest vaccination in the Ngorongoro Conservation Area and in Serengeti. Some simple mathematical models will be described that allow us to examine the ecological conditions that preceded previous outbreaks. We will then examine how the spatial distribution of different potential host species effects the long term efficacy of the rinderpest vaccination program and the potential for future outbreaks.

EVIDENCE OF MYCOBACTERIUM PARATUBERCULOSIS IN THE FECES OF TULE ELK FROM POINT REYES NATIONAL PARK. Walter Cook, Dept. of Vet. Science, Univ. of Wyoming, 1174 Snowy Range Road, Laramie, WY 82070; Todd Cornish, Dept. of Microbiology, Pathology, and Parasitology, College of Vet. Med., 4700 Hillsborough Street, North Carolina State Univ., Raleigh, NC 27606; Bill Lasley, Dept. of Population Health and Reproduction, School of Vet. Med., Univ. of Calif., Davis, CA 95616.

The tule elk (Cervus elaphus nannodes) of Point Reyes National Park, California were reported to have paratuberculosis in 1981. However, definitive evidence is lacking that Mycobacterium paratuberculosis has caused clinical disease in recent years, leading to speculation about whether the herd is still infected. In July of 1993 100 fresh fecal samples were collected by herding groups of elk away from bedding and feeding areas. Samples were cultured on a modified BACTEC 12B radiometric medium for detection of M. paratuberculosis. Four of 95 uncontaminated samples were positive and came from two different areas of the Park. This study used a noninvasive technique to document the presence of M. paratuberculosis in the elk of Point Reyes National Park. These findings limit the management options available for this herd of tule elk.

Amphibians

A REVIEW OF CAUSES OF MORTALITY OF THE WYOMING TOAD (BUFO HEMIOPHRYHS BAXTERI), 1989-1995. Sharon K. Taylor1, Elizabeth S. Williams1, and E. Tom Thorne2. 1Department of Veterinary Sciences, University of Wyoming, 1174 Snowy Range Road, Laramie, Wyoming 82070; 2Wyoming Game and Fish Department, Box 3313, University Station, Laramie, Wyoming 82071.

The Wyoming toad (Bufo hemiophrys baxteri) was listed as an endangered species in 1984 by the U.S. Fish and Wildlife Service. This toad is believed to be a glacial relict subspecies of the Canadian toad (B. hemiophrys) and has historically only been known to have
allowed increased levels of recruitment in many plant species. The classic way to determine the importance of a keystone species is to try and remove that species and monitor changes in ecosystem function. The development of a vaccine for rinderpest allowed this 'experiment' to be undertaken. Vaccination of domestic cattle produced a significant reduction in rinderpest prevalence in all species in the Serengeti ecosystem, this led to significant increases in the wildebeest and buffalo populations. These in turn produced significant increases in the density of some, but not all, predator species, particularly lions and hyaenas. In this talk I will describe work on the history of rinderpest and rinderpest vaccination in the Ngorongoro Conservation Area and in Serengeti. Some simple mathematical models will be described that allow us to examine the ecological conditions that preceded previous outbreaks. We will then examine how the spatial distribution of different potential host species affects the long term efficacy of the rinderpest vaccination program and the potential for future outbreaks.

**EVIDENCE OF MYCOBACTERIUM PARATUBERCULOSIS IN THE FECES OF TULE ELK FROM POINT REYES NATIONAL PARK.** Walter Cook, Dept. of Vet. Science, Univ. of Wyoming, 1174 Snowy Range Road, Laramie, WY 82070; Todd Cornish, Dept. of Microbiology, Pathology, and Parasitology, College of Vet. Med., 4700 Hillsborough Street, North Carolina State Univ., Raleigh, NC 27606; Bill Lasley, Dept. of Population Health and Reproduction, School of Vet. Med., Univ. of Calif., Davis, CA 95616.

The tule elk (*Cervus elaphus nannodes*) of Point Reyes National Park, California were reported to have paratuberculosis in 1981. However, definitive evidence is lacking that *Mycobacterium paratuberculosis* has caused clinical disease in recent years, leading to speculation about whether the herd is still infected. In July of 1993 100 fresh fecal samples were collected by herding groups of elk away from bedding and feeding areas. Samples were cultured on a modified BACTEC 12B radiometric medium for detection of *M. paratuberculosis*. Four of 95 uncontaminated samples were positive and came from two different areas of the Park. This study used a noninvasive technique to document the presence of *M. paratuberculosis* in the elk of Point Reyes National Park. These findings limit the management options available for this herd of tule elk.

**Amphibians**

**A REVIEW OF CAUSES OF MORTALITY OF THE WYOMING TOAD (**Bufo hemiophrys baxteri**), 1989-1995.** Sharon K. Taylor¹, Elizabeth S. Williams¹, and E. Tom Thorne². ¹Department of Veterinary Sciences, University of Wyoming, 1174 Snowy Range Road, Laramie, Wyoming 82070; ²Wyoming Game and Fish Department, Box 3313, University Station, Laramie, Wyoming 82071.

The Wyoming toad (*Bufo hemiophrys baxteri*) was listed as an endangered species in 1984 by the U.S. Fish and Wildlife Service. This toad is believed to be a glacial relict subspecies of the Canadian toad (*B. hemiophrys*) and has historically only been known to have
inhabited the Laramie Basin of Albany County, Wyoming. In the mid 1970's, Wyoming toad populations rapidly declined. We examined carcasses of 278 Wyoming toads that died from January 1989 through May 1995 and were submitted to the Wyoming State Veterinary Laboratory for postmortem evaluation. Gross and histologic evaluations, bacteriology, mycology, electron microscopy, and parasitology were conducted on toads from the free-ranging population and from the five captive populations. Causes of mortality were consistent in all populations and included: mycotic dermatitis, septicemic edema syndrome, peritonitis, hepatitis, intestinal rupture, intestinal obstruction, muscle dysplasia, muscle degeneration, and hibernation. Developmental anomalies included ocular defects and polydacty. Information collected on the causes of mortality will provide an important tool in managing the captive Wyoming toad population and assist in reintroduction decisions.
POSTERS

SURVEILLANCE OF WILD ANIMAL DISEASES IN FRANCE: THE SAGIR NETWORK. François Lamarque, Office National de la Chasse, Ferme de Saint Benoist, 78610 Auffargis, France; Jacques Barrat, Centre National d'Etudes Vétérinaires et Alimentaires, Domaine de Pixérécourt, B.P. 9, 54220 Malzéville, France.

The SAGIR network was created in 1986 by the Office National de la Chasse (ONC), a governmental agency as a national system of surveillance of wildlife diseases. SAGIR is organized as a cooperative venture among ONC, the "Centre National d'Etudes Vétérinaires et Alimentaires" (CNEVA) in Nancy, the toxicology laboratory of the National Veterinary School in Lyon (ENVL), the "Departemental" Veterinary Laboratories (LVD) and the "Departemental" Federations of hunters (FDC), the latter two forming the basic unit of the whole system. As a warning system covering almost the whole french territory, SAGIR worked perfectly for the last eight years. It showed, for instance, the existence of VHD and EBHS in France as well as the effect of some pesticides (e.g. chlorophacinone) on game species. It has contributed efficiently to the monitoring of the 1992 Hog cholera outbreak in the east of France. By the numerous laboratory tests made in this framework, SAGIR also obtained some reliable data on wildlife pathology and allowed significant improvements in the diagnosis and prevention of some wildlife diseases. However, a few limiting factors still occurring at each stage of the network, are preventing SAGIR from becoming a real epidemiologic-surveillance system. The main difficulties to reach this goal are the loss of information, the insufficient quantity and diversity as well as the heterogeneity of data collected. It is expected that the reactivation programme currently implemented will improve again this precious tool.

BASELINE COAGULATION ASSAY VALUES FOR NORTHERN ELEPHANT SEALS (MIROUNGA ANGUSTIROSTRIS) AND THE DIAGNOSIS OF A CASE OF DISSEMINATED INTRAVASCULAR COAGULATION (DIC) IN THIS SPECIES. B.E. Royal, University of Illinois, College of Veterinary Medicine, 2001 S. Lincoln, Urbana, Illinois 61801; I. Strubel, University of Illinois, College of Veterinary Medicine, 2001 S. Lincoln, Urbana, Illinois 6180; F.M.D. Gulland, The Marine Mammal Center, Golden Gate National Recreation Center, Sausalito, California, 94965; L. Werner, Department of Veterinary Pathology, Microbiology and Immunology, University of California, Davis, California 95616; and S. O'Neill, Department of Veterinary Pathology, Microbiology and Immunology, University of California, Davis, California 95616

The current population of northern elephant seals (Mirounga angustirostris) is descended from the approximately 20-100 individuals that survived the near-extinction of the species in the late nineteenth century. A decrease in genetic variation can increase susceptibility to infectious diseases. Little is known about normal hematologic values or the response to pathogens in this species compared to that of other phocids. Coagulation assays were performed on 20 healthy juvenile northern elephant seals to establish baseline
POSTERS

SURVEILLANCE OF WILD ANIMAL DISEASES IN FRANCE: THE SAGIR NETWORK. François Lamarque, Office National de la Chasse, Ferme de Saint Benoist, 78610 Auffargis, France; Jacques Barrat, Centre National d'Etudes Vétérinaires et Alimentaires, Domaine de Pixérécourt, B.P. 9, 54220 Malzéville, France.

The SAGIR network was created in 1986 by the Office National de la Chasse (ONC), a governmental agency as a national system of surveillance of wildlife diseases. SAGIR is organized as a cooperative venture among ONC, the "Centre National d'Etudes Vétérinaires et Alimentaires" (CNEVA) in Nancy, the toxicology laboratory of the National Veterinary School in Lyon (ENVL), the "Departemental" Veterinary Laboratories (LVD) and the "Departemental" Federations of hunters (FDC), the latter two forming the basic unit of the whole system. As a warning system covering almost the whole french territory, SAGIR worked perfectly for the last eight years. It showed, for instance, the existence of VHD and EBHS in France as well as the effect of some pesticides (e.g. chlorophacinone) on game species. It has contributed efficiently to the monitoring of the 1992 Hog cholera outbreak in the east of France. By the numerous laboratory tests made in this framework, SAGIR also obtained some reliable data on wildlife pathology and allowed significant improvements in the diagnosis and prevention of some wildlife diseases. However, a few limiting factors still occurring at each stage of the network, are preventing SAGIR from becoming a real epidemiologic-surveillance system. The main difficulties to reach this goal are the loss of information, the insufficient quantity and diversity as well as the heterogeneity of data collected. It is expected that the reactivation programme currently implemented will improve again this precious tool.

BASELINE COAGULATION ASSAY VALUES FOR NORTHERN ELEPHANT SEALS (MIROUNGA ANGUSTIROSTRIS) AND THE DIAGNOSIS OF A CASE OF DISSEMINATED INTRAVASCULAR COAGULATION (DIC) IN THIS SPECIES. B.E. Royal, University of Illinois, College of Veterinary Medicine, 2001 S. Lincoln, Urbana, Illinois 61801; I. Strubel, University of Illinois, College of Veterinary Medicine, 2001 S. Lincoln, Urbana, Illinois 6180; F.M.D. Gulland, The Marine Mammal Center, Golden Gate National Recreation Center, Sausalito, California, 94965; L. Werner, Department of Veterinary Pathology, Microbiology and Immunology, University of California, Davis, California 95616; and S. O'Neill, Department of Veterinary Pathology, Microbiology and Immunology, University of California, Davis, California 95616

The current population of northern elephant seals (Mirounga angustirostris) is descended from the approximately 20-100 individuals that survived the near-extinction of the species in the late nineteenth century. A decrease in genetic variation can increase susceptibility to infectious diseases. Little is known about normal hematologic values or the response to pathogens in this species compared to that of other phocids. Coagulation assays were performed on 20 healthy juvenile northern elephant seals to establish baseline
parameters for this species. The ranges were as follows: prothrombin time 10.3-13.7 seconds, activated partial thromboplastin time (APTT) 17.6-28 seconds, citrated fibrinogen 50-162 mg/dl, 56° fibrinogen 100-300 mg/dl, antithrombin III 70-120%, platelets 167-859 x 10^3, activated clotting time 55-70 seconds. Both monoclonal and polyclonal fibrinogen degradation products were negative to trace. These data were then used to diagnose a case of disseminated intravascular coagulation (DIC) in a live elephant seal. DIC is an important process commonly associated with mortality in elephant seals. Diagnosis of DIC in the live animal is important due to the poor prognosis and difficulties involved in treating animals in this condition.

TEMPORAL RELATIONSHIP OF VIREMIA, α AND β INTERFERON PRODUCTION, AND CIRCULATING ANTIBODIES IN ORBIVIRUS-INFECTED WHITE-TAILED DEER.
Charlotte F. Quist,1,2 Elizabeth W. Howerth,2 and David E. Stallknecht,1. 1Southeastern Cooperative Wildlife Disease Study and 2Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA.

Epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) cause the most important infectious disease complex of white-tailed deer (WTD). While the resultant clinical syndrome and lesion are well-described, little is known about the defense mechanisms of infected animals. The objective of this study was to elucidate the host defense responses seen in orbivirus-infected WTD. In each of two trials, eight WTD were infected with EHDV-2, then challenged at day 28 post-inoculation (PI) with homologous (EHDV-2; first trial) or heterologous virus (BTV; second trial). Every two days, animals were examined and blood was collected for CBCs, α and β interferon production (IFN), viral isolation, and antibody production. Surviving animals were euthanized and necropsied at day 56 PI. All animals became viremic by day 4 PI after initial EHDV-2 and BTV-10 infection. Some EHDV-2-infected deer remained viremic until day 56 PI. Generally, viremia peaked at day 6 PI, coinciding with peak α and β IFN levels. Circulating antibodies developed by day 10 PI. In contrast to short-term viremias previously reported in EHDV-infected WTD these prolonged viremias suggest WTD may play an important role in the maintenance of orbiviruses and are potential sources of infection for livestock.


We conducted a retrospective survey of parasitological results reported in necropsy records for 48 gray wolves (Canis lupus) submitted to the National Wildlife Health Center from 1988-1994. Twenty-five females, eight of which were immature, and 23 adult males were collected from Wisconsin (N=17), Minnesota (N=23), Michigan (N=3), USA and Ontario, Canada (N=5). Ninety-four percent of the wolves had parasites. The most common
parameters for this species. The ranges were as follows: prothrombin time 10.3-13.7 seconds, activated partial thromboplastin time (APTT) 17.6-28 seconds, citrated fibrinogen 50-162 mg/dl, fibrinogen 100-300 mg/dl, antithrombin III 70-120%, platelets 167-859 x 10^9, activated clotting time 55-70 seconds. Both monoclonal and polyclonal fibrinogen degradation products were negative to trace. These data were then used to diagnose a case of disseminated intravascular coagulation (DIC) in a live elephant seal. DIC is an important process commonly associated with mortality in elephant seals. Diagnosis of DIC in the live animal is important due to the poor prognosis and difficulties involved in treating animals in this condition.

TEMPORAL RELATIONSHIP OF VIREMIA, α AND β INTERFERON PRODUCTION, AND CIRCULATING ANTIBODIES IN ORBIVIRUS-INFECTED WHITE-TAILED DEER. Charlotte F. Quist, Elizabeth W. Howerth, and David E. Stallknecht. Southeastern Cooperative Wildlife Disease Study and Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA.

Epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) cause the most important infectious disease complex of white-tailed deer (WTD). While the resultant clinical syndrome and lesion are well-described, little is known about the defense mechanisms of infected animals. The objective of this study was to elucidate the host defense responses seen in orbivirus-infected WTD. In each of two trials, eight WTD were infected with EHDV-2, then challenged at day 28 post-inoculation (PI) with homologous (EHDV-2; first trial) or heterologous virus (BTV; second trial). Every two days, animals were examined and blood was collected for CBCs, α and β interferon production (IFN), viral isolation, and antibody production. Surviving animals were euthanized and necropsied at day 56 PI. All animals became viremic by day 4 PI after initial EHDV-2 and BTV-10 infection. Some EHDV-2-infected deer remained viremic until day 56 PI. Generally, viremia peaked at day 6 PI, coinciding with peak α and β IFN levels. Circulating antibodies developed by day 10 PI. In contrast to short-term viremias previously reported in EHDV-infected WTD these prolonged viremias suggest WTD may play an important role in the maintenance of orbiviruses and are potential sources of infection for livestock.


We conducted a retrospective survey of parasitological results reported in necropsy records for 48 gray wolves (Canis lupus) submitted to the National Wildlife Health Center from 1988-1994. Twenty-five females, eight of which were immature, and 23 adult males were collected from Wisconsin (N=17), Minnesota (N=23), Michigan (N=3), USA and Ontario, Canada (N=5). Ninety-four percent of the wolves had parasites. The most common
parameters for this species. The ranges were as follows: prothrombin time 10.3-13.7 seconds, activated partial thromboplastin time (APTT) 17.6-28 seconds, citrated fibrinogen 50-162 mg/dl, fibrinogen 100-300 mg/dl, antithrombin III 70-120%, platelets 167-859 x 10^9, activated clotting time 55-70 seconds. Both monoclonal and polyclonal fibrinogen degradation products were negative to trace. These data were then used to diagnose a case of disseminated intravascular coagulation (DIC) in a live elephant seal. DIC is an important process commonly associated with mortality in elephant seals. Diagnosis of DIC in the live animal is important due to the poor prognosis and difficulties involved in treating animals in this condition.

TEMPORAL RELATIONSHIP OF VIREMIA, α AND β INTERFERON PRODUCTION, AND CIRCULATING ANTIBODIES IN ORBIVIRUS-INFECTED WHITE-TAILED DEER. Charlotte F. Quist,1 Elizabeth W. Howerth,2 and David E. Stallknecht,1. 1Southeastern Cooperative Wildlife Disease Study and 2Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA.

Epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) cause the most important infectious disease complex of white-tailed deer (WTD). While the resultant clinical syndrome and lesion are well-described, little is known about the defense mechanisms of infected animals. The objective of this study was to elucidate the host defense responses seen in orbivirus-infected WTD. In each of two trials, eight WTD were infected with EHDV-2, then challenged at day 28 post-inoculation (PI) with homologous (EHDV-2; first trial) or heterologous virus (BTV; second trial). Every two days, animals were examined and blood was collected for CBCs, α and β interferon production (IFN), viral isolation, and antibody production. Surviving animals were euthanized and necropsied at day 56 PI. All animals became viremic by day 4 PI after initial EHDV-2 and BTV-10 infection. Some EHDV-2-infected deer remained viremic until day 56 PI. Generally, viremia peaked at day 6 PI, coinciding with peak α and β IFN levels. Circulating antibodies developed by day 10 PI. In contrast to short-term viremias previously reported in EHDV-infected WTD these prolonged viremias suggest WTD may play an important role in the maintenance of orbiviruses and are potential sources of infection for livestock.


We conducted a retrospective survey of parasitological results reported in necropsy records for 48 gray wolves (Canis lupus) submitted to the National Wildlife Health Center from 1988-1994. Twenty-five females, eight of which were immature, and 23 adult males were collected from Wisconsin (N=17), Minnesota (N=23), Michigan (N=3), USA and Ontario, Canada (N=5). Ninety-four percent of the wolves had parasites. The most common
nematode was *Oslerus olseri* in the lung and trachea from 25% of wolves. *Uncinaria stenocephala* and *Ancylostoma caninum* were found in 17% and 10% of the wolves, respectively. Two genera of cestodes, *Taenia* spp. (44%) and *Echinococcus granulosus* (27%) were reported. The trematode, *Alaria* sp. was recovered from 35% of the carcasses. Three ectoparasites were common; *Trichodectes canis* (33%) *Dermacentor* spp. (15%) and *Ixodes* spp. (10%). Oocysts and/or sporocysts of *Sarcocystis* spp. were reported from 10% of wolves with an additional 6% having sarcocysts in cardiac or striated muscle. Intensity of infections was not described in necropsy reports.

**CALIFORNIA'S OFFICE OF OIL SPILL PREVENTION AND RESPONSE: WILDLIFE REHABILITATION, VETERINARY, AND BIOMEDICAL PROGRAMS.**

David A. Jessup, Jonna A.K. Mazet, Office of Oil Spill Prevention and Response, California Department of Fish and Game, 1701 Nimbus Road, Suite D, Rancho Cordova, CA. 95670

Catastrophic oil spills and toxic substance spills can cause considerable environmental damage and loss of animal life. In 1990 the legislature of California enacted SB 2040 (Lempert, Keene, Seastrand) which placed a $.04 per barrel tax on oil transported or processed in California, the proceeds of which are to be used to prevent spills, to respond to spills, to clean up those which occur where no responsible party is identified (orphan spills), to rehabilitate affected wildlife habitat, and to care for wildlife affected by oil. To meet the mandated response capabilities for oiled wildlife OSPR has developed a veterinary team, built and outfitted a pair of mobile veterinary laboratories and washing and care trailers, and a Mobile Oily Bird Care and Rehabilitation Trailer (MOBCART). Four small trailers containing supplies needed in the first 24 hours of an oil spill have been built, stocked and deployed to strategic locations along the California coast. This response equipment is capable of reaching any location in California within 24 hours.

The legislation (SB 2040) states "The administrator shall establish rescue and rehabilitation stations for sea birds, sea otters, and other marine mammals." To comply with this charge OSPR is building a facility at University of California Santa Cruz (next to Long Marine Laboratory) for veterinary care, rehabilitation and research on oiled marine wildlife. When completed in the winter of 1996 the facility will have cost approximately $5 million dollars and will be capable of caring for 125 sea otters, and be flexible enough to care for other species of marine animals and house ongoing research projects. To further meet the above goal a second piece of legislation SB 775 (Watson) was passed by the California Legislature allowing OSPR to use the interest from the $50 million dollar emergency response fund (a total of approximately $7 million dollars over a 3 year period) to establish an Oiled Wildlife Care Network for the entire California coast in conjunction with existing scientific, educational and wildlife rehabilitation facilities. Current plans call for these additional centers to be developed in San Diego, Orange County, Los Angeles, Santa Barbara, the San Francisco Bay Area and Humboldt County.

Under the Oiled Wildlife Care Network and recent changes in Title 14 of the California
nematode was Oslerus olseri in the lung and trachea from 25% of wolves. Uncinaria stenocephala and Ancylostoma caninum were found in 17% and 10% of the wolves, respectively. Two genera of cestodes, Taenia spp. (44%) and Echinococcus granulosus (27%) were reported. The trematode, Alaria sp. was recovered from 35% of the carcasses. Three ectoparasites were common; Trichodectes canis (33%) Dermacentor spp. (15%) and Ixodes spp. (10%). Oocysts and/or sporocysts of Sarcocystis spp. were reported from 10% of wolves with an additional 6% having sarcocysts in cardiac or striated muscle. Intensity of infections was not described in necropsy reports.

CALIFORNIA'S OFFICE OF OIL SPILL PREVENTION AND RESPONSE: WILDLIFE REHABILITATION, VETERINARY, AND BIOMEDICAL PROGRAMS. David A. Jessup, Jonna A.K. Mazet, Office of Oil Spill Prevention and Response, California Department of Fish and Game, 1701 Nimbus Road, Suite D, Rancho Cordova, CA. 95670

Catastrophic oil spills and toxic substance spills can cause considerable environmental damage and loss of animal life. In 1990 the legislature of California enacted SB 2040 (Lempert, Keene, Seastrand) which placed a $.04 per barrel tax on oil transported or processed in California, the proceeds of which are to be used to prevent spills, to respond to spills, to clean up those which occur where no responsible party is identified (orphan spills), to rehabilitate affected wildlife habitat, and to care for wildlife affected by oil. To meet the mandated response capabilities for oiled wildlife OSPR has developed a veterinary team, built and outfitted a pair of mobile veterinary laboratories and washing and care trailers, and a Mobile Oily Bird Care and Rehabilitation Trailer (MOBCART). Four small trailers containing supplies needed in the first 24 hours of an oil spill have been built, stocked and deployed to strategic locations along the California coast. This response equipment is capable of reaching any location in California within 24 hours.

The legislation (SB 2040) states "The administrator shall establish rescue and rehabilitation stations for sea birds, sea otters, and other marine mammals." To comply with this charge OSPR is building a facility at University of California Santa Cruz (next to Long Marine Laboratory) for veterinary care, rehabilitation and research on oiled marine wildlife. When completed in the winter of 1996 the facility will have cost approximately $5 million dollars and will be capable of caring for 125 sea otters, and be flexible enough to care for other species of marine animals and house ongoing research projects. To further meet the above goal a second piece of legislation SB 775 (Watson) was passed by the California Legislature allowing OSPR to use the interest from the $50 million dollar emergency response fund (a total of approximately $7 million dollars over a 3 year period) to establish an Oiled Wildlife Care Network for the entire California coast in conjunction with existing scientific, educational and wildlife rehabilitation facilities. Current plans call for these additional centers to be developed in San Diego, Orange County, Los Angeles, Santa Barbara, the San Francisco Bay Area and Humboldt County.

Under the Oiled Wildlife Care Network and recent changes in Title 14 of the California
Code minimum veterinary care standards, minimum wildlife rehabilitation facility standards, and minimum training required for all persons working on oiled wildlife in California are prescribed. Participants in the Network will share pertinent information, improve and standardize treatment protocols and cooperate in research.

SB 2040 also says "The administrator shall conduct studies and evaluations necessary for improving .... oil spill wildlife rehabilitation..." And further states "The administrator shall evaluate potential adverse impacts on the environment and public health including, but not limited to, adverse toxic impacts on water quality, fisheries, and wildlife with consideration to bioaccumulation and synergistic impacts...." Currently OSPR is funding research at several California Universities and at Hubbs/Sea World Research Institute. Research programs address the effects of oil on various organ systems in sea otters (using mink as a model), immediate detection of trace amounts of oil in the fur and feathers of live animals, characterizing the potential effects of oil on the immune response of sea otters, characterizing the immune response of harbor seals including differentiating the effects of the rehabilitation process from exposure to oil and other health hazards, establishing baseline health information for pinnipeds, updating information on the status of marine mammal populations and delineating populations at greatest risk of exposure to oil, and establishing baseline health information on key marine bird species and population status.

All of this research is designed to improve our ability to care for oiled marine wildlife, and to improve our ability to determine both the immediate and the sublethal effects of oil pollution on marine animal populations. This will enable Federal and State trustee agencies to complete comprehensive wildlife injury assessments as part of the Natural Resource Damage Assessment (NRDA) process. Settlements with responsible parties will enable trustee agencies to undertake restoration of injured wildlife resources. In summary, progressive programs for response, rehabilitation, and research for oiled wildlife are conducted by the Veterinary Services Unit of the California Department of Fish and Game-Office of Oil Spill Prevention and Response.


Medical records of 649 reptile and 98 amphibian cases presented to Willowbrook Wildlife Center (WWC) in DuPage Co., IL between 1979 and 1994 were reviewed. Information collected included signalment, township where found, weight, body condition, status (injured, diseased or displaced), presumed cause of injury, primary diagnosis, treatment provided, case outcome, and release month and site. This report describes various risk factors to native reptiles and amphibians identified by this review, and summarizes the veterinary management and rehabilitation of these species. In addition, an overview of strategies incorporated into the WWC educational program used to prevent and lessen human impact on extant populations in this highly urban landscape is presented.
OCCURRENCE of AVIAN POX IN WISCONSIN WILD TURKEYS (MELEAGRIS GALLOPAVO SILVESTRIS). Keny Beheler-Amass¹, Steven Schmidt², Sarah Hurley¹, Melvin Prantner², and Barb Bodenstein¹. ¹Wildlife Health Program, Bureau of Wildlife Management, Wisconsin Dept of Natural Resources, Madison WI 53707; ²Wisconsin Animal Health Laboratory, Department of Agriculture, Trade, and Consumer Protection, Madison WI 53705.

Wild turkey restoration programs based on translocating wild caught adult birds from well established flocks into new areas of suitable habitat have successfully occurred in many states since the 1950's. Wisconsin (WI) began a turkey restoration program in 1976 by introducing 334 adult wild-caught Missouri turkeys over an 8 year period. By 1993, about 130,000 wild turkeys were living in southern WI's hill and valley mixed forest and agricultural areas. Avian pox infections in wild turkeys have been reported from 11 southeastern states, but had not been reported from Missouri. WI wildlife biologists inspected reintroduced wild turkeys from 1977 to 1983 for various avian pathogens, and did not observe avian pox lesions on any bird. This is the first report of avian pox in WI wild turkeys. Avian pox was diagnosed in WI birds from 1990 to 1994, and the first case was diagnosed one year after the annual fall and spring turkey hunts began. Eleven cases of avian pox have been diagnosed since 1990, with 64% (7 of 11) of cases from fall hunter killed birds. 45% (5 of 11) of the total cases were submitted from two Adjacent WI counties in 1993. Adult turkeys accounted for 91% (10 of 11) of the cases, and this may be due to hunting restrictions on juvenile birds. Quantification of the effects of avian pox on the WI wild turkey population is difficult to assess without further investigations. Less than 10% of the WI population is harvested in the fall either sex hunt, yet this is when we diagnosed the majority of avian pox infections. It is not known if avian pox is contributing to WI juvenile turkey morbidity and mortality during late summer and early fall, when turkey populations are highest. Although WI wild turkey populations fluctuate mainly in response to severity of the winters and available food, other factors including avian pox may seasonally affect the birds. Avian pox may play an important seasonal role in WI wild turkey population dynamics.


Sarcoptic mange, a dermal infection caused by the mange mite Sarcoptes scabiei variety canis, is a severe disease of red fox (Vulpes vulpes) and coyote (Canis latrans). Sarcoptic mange has been reported from timber wolf (C. lupus lycaon) populations in Alberta, Canada and from red wolves (C. rufus) raised in captivity. Sarcoptic mange has not previously been
OCCURRENCE OF AVIAN POX IN WISCONSIN WILD TURKEYS (MELEAGRIS GALLOPANO SILVESTRIS). Keny Beheler-Amass¹, Steven Schmidt², Sarah Hurley¹, Melvin Prantner², and Barb Bodenstein¹. ¹Wildlife Health Program, Bureau of Wildlife Management, Wisconsin Dept of Natural Resources, Madison WI 53707; ²Wisconsin Animal Health Laboratory, Department of Agriculture, Trade, and Consumer Protection, Madison WI 53705.

Wild turkey restoration programs based on translocating wild caught adult birds from well established flocks into new areas of suitable habitat have successfully occurred in many states since the 1950's. Wisconsin (WI) began a turkey restoration program in 1976 by introducing 334 adult wild-caught Missouri turkeys over an 8 year period. By 1993, about 130,000 wild turkeys were living in southern WI's hill and valley mixed forest and agricultural areas. Avian pox infections in wild turkeys have been reported from 11 southeastern states, but had not been reported from Missouri. WI wildlife biologists inspected reintroduced wild turkeys from 1977 to 1983 for various avian pathogens, and did not observe avian pox lesions on any bird. This is the first report of avian pox in WI wild turkeys. Avian pox was diagnosed in WI birds from 1990 to 1994, and the first case was diagnosed one year after the annual fall and spring turkey hunts began. Eleven cases of avian pox have been diagnosed since 1990, with 64% (7 of 11) of cases from fall hunter killed birds. 45% (5 of 11) of the total cases were submitted from two Adjacent WI counties in 1993. Adult turkeys accounted for 91% (10 of 11) of the cases, and this may be due to hunting restrictions on juvenile birds. Quantification of the effects of avian pox in the WI wild turkey population is difficult to assess without further investigations. Less than 10% of the WI population is harvested in the fall either sex hunt, yet this is when we diagnosed the majority of avian pox infections. It is not known if avian pox is contributing to WI juvenile turkey morbidity and mortality during late summer and early fall, when turkey populations are highest. Although WI wild turkey populations fluctuate mainly in response to severity of the winters and available food, other factors including avian pox may seasonally affect the birds. Avian pox may play an important seasonal role in WI wild turkey population dynamics.


Sarcoptic mange, a dermal infection caused by the mange mite Sarcoptes scabiei variety canis, is a severe disease of red fox (Vulpes vulpes) and coyote (Canis latrans). Sarcoptic mange has been reported from timber wolf (C. lupus lycaon) populations in Alberta, Canada and from red wolves (C. rufus) raised in captivity. Sarcoptic mange has not previously been
reported as occurring in the Great Lakes States, Minnesota, Wisconsin, and Michigan, timber wolf populations. An epizootic of sarcoptic mange occurred in these timber wolf, red fox, and coyote populations from 1991 to 1994. Wolf field collected skin scrapes and/or biopsies revealed S. scabiei mites in 7 of 14 (50%) samples. The sarcoptic mite was also found in 13 of 46 (28%) field collected fecal samples. *Trichodectes canis*, the dog biting louse, was also occasionally found on *Sarcoptes* infested wolves. Adult wolf mortality was affected by *Sarcoptes* infestation, especially during the winter months of November through February. Morbidity and mortality information concerning *Sarcoptes* infestation on juvenile wolves is not known, but Wisconsin experienced a decline in wolf pup production during this same time period.

**MONOCLONAL ANTIBODY COMPETITIVE ELISA FOR CRANE ANTIBODIES AGAINST GRUID HERPESVIRUS TYPE 1 (GHV-1).** Geoffrey J. Letchworth and Jared R. Fishel, Animal Health and Biomedical Sciences, University of Wisconsin-Madison, 1655 Linden Drive, Madison, Wisconsin 53706; and Wallace Hansen, National Wildlife Health Research Center, 6006 Schroeder Road, Madison, Wisconsin 53711.

Gruid Herpesvirus type 1 (GHV-1), also known as Inclusion Body Disease of Cranes Virus, causes fatal disease in captive cranes worldwide. Diagnosis depends upon virus isolation from acutely infected birds and virus neutralization assays for antibodies in convalescent animals. The potential for persistent infections with stress-triggered shedding from captive cranes demands a rapid field assay. We developed a competitive ELISA based on a murine monoclonal antibody directed against GHV-1. Monoclonal antibodies were made by conventional techniques against GHV-1 purified from infected duck embryo cells. Hybridomas reacting in an ELISA with GHV-1 but not uninfected duck embryo cells were further screened by radioimmunoprecipitation, in situ immunohistochemistry, and competitive ELISA with neutralizing and non-neutralizing crane sera. Monoclonal antibody 2C11 immunoprecipitated 59, 61, and 110 kDa proteins from infected but not uninfected cells, stained glutaraldehyde-fixed GHV-1 plaques but not surrounding uninfected duck cells in vitro, and bound to GHV-1 treated with non-neutralizing crane antibody but not to GHV-1 treated with neutralizing antibody. An ELISA using antibody 2C11 is being tested for sensitivity in relation to the neutralization test.
reported as occurring in the Great Lakes States, Minnesota, Wisconsin, and Michigan, timber wolf populations. An epizootic of sarcoptic mange occurred in these timber wolf, red fox, and coyote populations from 1991 to 1994. Wolf field collected skin scrapes and/or biopsies revealed *S. scabiei* mites in 7 of 14 (50%) samples. The sarcoptic mite was also found in 13 of 46 (28%) field collected fecal samples. *Trichodectes canis*, the dog biting louse, was also occasionally found on *Sarcoptes* infested wolves. Adult wolf mortality was affected by Sarcoptes infestation, especially during the winter months of November through February. Morbidity and mortality information concerning *Sarcoptes* infestation on juvenile wolves is not known, but Wisconsin experienced a decline in wolf pup production during this same time period.

**MONOCLONAL ANTIBODY COMPETITIVE ELISA FOR CRANE ANTIBODIES AGAINST GRUID HERPESVIRUS TYPE 1 (GHV-1).** Geoffrey J. Letchworth and Jared R. Fishel, Animal Health and Biomedical Sciences, University of Wisconsin-Madison, 1655 Linden Drive, Madison, Wisconsin 53706; and Wallace Hansen, National Wildlife Health Research Center, 6006 Schroeder Road, Madison, Wisconsin 53711.

Gruid Herpesvirus type 1 (GHV-1), also known as Inclusion Body Disease of Cranes Virus, causes fatal disease in captive cranes worldwide. Diagnosis depends upon virus isolation from acutely infected birds and virus neutralization assays for antibodies in convalescent animals. The potential for persistent infections with stress-triggered shedding from captive cranes demands a rapid field assay. We developed a competitive ELISA based on a murine monoclonal antibody directed against GHV-1. Monoclonal antibodies were made by conventional techniques against GHV-1 purified from infected duck embryo cells. Hybridomas reacting in an ELISA with GHV-1 but not uninfected duck embryo cells were further screened by radioimmunoprecipitation, in situ immunohistochemistry, and competitive ELISA with neutralizing and non-neutralizing crane sera. Monoclonal antibody 2C11 immunoprecipitated 59, 61, and 110 kDa proteins from infected but not uninfected cells, stained glutaraldehyde-fixed GHV-1 plaques but not surrounding uninfected duck cells in vitro, and bound to GHV-1 treated with non-neutralizing crane antibody but not to GHV-1 treated with neutralizing antibody. An ELISA using antibody 2C11 is being tested for sensitivity in relation to the neutralization test.
HYPOPHOSPHATEMIA IN FREE-RANGING MOOSE (Alces alces)

J.M. Arnemo*
Department of Arctic Veterinary Medicine, Norwegian College of Veterinary Medicine, N-9005 Tromsø Norway

T. Soveri
Helsinki Zoo, FIN-00570 Helsinki, Finland

In the two southernmost counties in Norway, a region heavily affected by acid rain, osteoporosis has been recognized as an increasing problem in free-ranging moose (Alces alces). Fifteen adult moose (Group I) immobilized in early fall in this region were severely hypophosphatemic [mean (SD) serum concentration of inorganic phosphorous was 0.8 (0.4) mmol/L] compared to the reference range [1.8-1.9 mmol/L; 95% confidence interval] established for Norwegian moose (n=211). A corresponding hypophosphatemia [0.9 (0.4) mmol/L] were found in 44 adult moose (Group II) sampled during March in a region of central Finland with aluminum-rich soil. There was no statistical difference between Groups I and II or between males (n=22) and females (n=22) in Group II (p>0.05; two sample t-test). No evidence of skeletal abnormalities have been found in Finnish moose. In Group I lactating females (n=8) had a significantly lower mean serum concentration of inorganic phosphorous than non-lactating females (n=5) (p<0.05, two-sample t-test). The actual values were 0.5 (0.3) and 1.0 (0.5) mmol/L, respectively. Acidification of the soil is known to reduce the uptake of phosphorous and increase the uptake of aluminum in plants. Studies in sheep and pigs have demonstrated that a high level of aluminum in the diet reduces the serum level of inorganic phosphate. The classical pathological lesions in phosphorous deficient animals are osteomalacia in adults and rickets in growing individuals. However, under certain circumstances osteoporosis is found. Based on the present study we hypothesize that acid rain and/or aluminum-rich soil may cause phosphorous deficiency, hypophosphatemia and osteoporosis in free-ranging moose.
A NEW SURGICAL APPROACH FOR IMPLANTATION OF HEART RATE TRANSMITTERS IN WILD HOOFSTOCK

Margaret A. Wild
Colorado Division of Wildlife, 317 W. Prospect Rd., Fort Collins, Colorado 80526, USA

Donald L. Piernatte
Professor Emeritus, Department of Clinical Sciences, Colorado State University, Fort Collins, Colorado 80523, USA

Dan L. Baker
Colorado Division of Wildlife, 317 W. Prospect Rd., Fort Collins, Colorado 80526; William R. Lance, Wildlife Pharmaceuticals, 1401 Duff Dr., Suite 600, Fort Collins, Colorado 80524, USA

R. Bruce Heath
Professor Emeritus, Department of Clinical Sciences, Colorado State University, Fort Collins, Colorado 80523, USA

We required a safe, reliable, and unobtrusive system to remotely monitor heart rate over an extended period in bighorn sheep (*Ovis canadensis*). Because we found no documentation of a method that fully met these criteria, we developed a new surgical technique. Three domestic goats and then five bighorn sheep received Telonics model HR400 heart rate transmitters placed subcutaneously on the dorsolateral thorax using aseptic technique. With the animals under gas anesthesia, we made a 15 cm paramedian skin incision about 12 cm lateral to dorsal midline and caudal to the scapular cartilage. Fat was elevated and the latissimus dorsi muscle incised parallel to the muscle fibers. Deep fascia was incised parallel to the ventral lateral border of the longissimus dorsi to allow for its elevation. The transmitter was placed in the naturally occurring shelf between the ribs and longissimus dorsi muscle and secured by reattaching the split fascial plane over it. The positive electrode was sutured to muscle fascia just ventral to the transmitter. The negative electrode was passed through a subcutaneously placed trocar to a second incision on the ventral lateral thorax at the level of the antebrachiohumoral joint and sutured to superficial muscle. Each incision was closed in three layers. No morbidity or mortality has occurred. All transmitters are functioning well with the exception of one goat's transmitter, which produced spurious signals, likely due to unsuitable electrode placement. Preliminary evidence suggest that this surgical technique offers a promising alternative for implantation of heart rate transmitters in hoofstock.
IMMOBILIZATION PROTOCOL FOR FREE-RANGING GRAY WOLVES (Canis Lupus) TRANSLOCATED TO YELLOWSTONE NATIONAL PARK AND CENTRAL IDAHO

Terry J. Kreeger*, MS, DVM, PhD
International Wildlife Veterinary Services, Inc., Cedar, MN 55011, USA

David L Hunter, DVM
Idaho Department of Fish and Game, Caldwell, ID 83605 USA

Mark R. Johnson, MS, DVM
Center for Resources, National Park Service, Yellowstone National Park, WY 82190, USA

Chemical immobilization was determined to be the best means of capturing gray wolves for reintroduction into Yellowstone National Park and central Idaho. Two Bell Jet Rangers were used in the operation. Each helicopter carried a crew of three: pilot, shooter, and assistant. Shooters were equipped with two Palmer .22-cal. dart guns (Palmer Chemical Company, Douglasville, Ga.) per helicopter with extra charge adapters for quick reloading. Brown and green blanks were used to power the darts with green most commonly used. The standard drug dose was 250 mg tiletamine and 250 mg zolazepam (Telazol®, Fort Dodge Laboratories, Inc., Fort Dodge, Ia.) dissolved in 0.75 ml (75 mg) xylazine, 1.0 ml water for injection, and 1.0 ml propylene glycol to prevent freezing. This solution was placed into 3-ml Palmer Cap-Chur darts equipped with a 0.5-inch barbed needles. This solution was considered viable for 48 hours after which time the mannitol used in the preparation began to precipitate and the solution discarded. This dose was used on all wolves regardless of size or sex. The majority of wolves were immobilized in less than 5 min after a single dart injection, although some larger animals required boosters of 200 mg ketamine to induce complete anesthesia. Wolves were also given 200 mg ketamine boosters to maintain anesthesia if the return to base camp was prolonged or if additional time was required for marking and sampling. Overall, an average of 5 darts per wolf were expended. A total of 30 wolves were darted (16m, 14f). One female wolf died as a result of a misplaced shot using a dart other than the Palmer Cap-Chur dart. Another male wolf struck with a dart in the dorsal thorax experienced a partial pneumothorax, but recovered after surgical intervention. Wolves anesthetized again for processing on subsequent days were anesthetized with 500 mg ketamine and 100 mg xylazine which had been prepared in a concentrated solution (200 mg ketamine and 40 mg xylazine per ml). Wolves were returned to temporary holding pens and either given 0.15 mg/kg yohimbine to antagonize the xylazine or allowed to recover slowly without the administration of yohimbine. Wolves prepared for transport to the U.S. were anesthetized with 500 mg Telazol® and 100 mg xylazine and placed into aluminum transport containers. This drug combination produced several hours of anesthesia and the wolves did not require additional sedatives during the flight. Due to legal delays, wolves were not released into Idaho immediately and had to be re-anesthetized because they had to be removed from the shipping containers for transport via helicopter into the wilderness. These wolves were immobilized with 500 mg ketamine and 100 mg ketamine and given 0.15 mg/kg yohimbine upon arrival at the release site where they recovered quickly. After arrival in YNP, one wolf in an acclimation pen was anesthetized.
for veterinary care. The wolf was physically restrained with a net and anesthetized with 4 mg/kg ketamine and 2 mg/kg xylazine. This wolf was confined to a den box for recovery prior to be returned to the pen. There were no adverse effects on any wolf despite the prolonged transport and repeated immobilizations.
JUST WHEN YOU THOUGHT IT WAS SAFE TO WALK IN THE WOODS: WHEN MOUNTAIN LIONS ATTACK PEOPLE

David A. Jessup
California Department of Fish and Game, 1701 Nimbus Rd., Suite "D", Rancho Cordova, CA 95670, USA

Bradd C. Bai
California Veterinary Diagnostic Laboratory System, University of California, Davis, CA 95616, USA

A recent (Beier, 1991) review of mountain lion (Felis concolor) attacks on people in North America over the last 100 years revealed 9 attacks causing 10 human deaths and 44 nonfatal attacks. This included attacks on two children in a California park in the late 1980's that resulted in serious injuries. Since that review two additional fatal attacks have occurred in California, both on women walking or jogging in rural areas. Another child was attacked while hiking with his family, an adult female on horseback was attacked and bitten while riding in a State park, an adult male mountain biker riding in a National Forest was attacked and mauled, and a rabid mountain lion attacked and injured three adults. Necropsy of one lion responsible for a human death revealed various mild pathologic changes considered to be incidental. This animal was in good body condition. The rabid mountain lion was in poor body condition, had a focally extensive nonsuppurative encephalitis, intraneuronal Negri bodies, and was positive for Rabies by FA on brain impression smears. Although mountain lion attacks are rare, they appear to be increasing in California. Fatal and nonfatal attacks have occurred in other western States recently.

Several theories have been advanced to explain this. It is clear that development of, and human activity in, mountain lion habitat is increasing. In some areas prime prey species such as black-tailed deer are decreasing and mountain lions are exploiting alternative prey. Others believe that the virtual elimination of mountain lion hunting in the 1970's, which was made permanent by State proposition in the 1990, has resulted in increasing mountain lion numbers and the loss of fear of humans. Most indirect measures of mountain lion density in California, including frequency of sightings, livestock depredation, and public safety incidents also suggest that mountain lion numbers are increasing. There are estimated to be from 4000 to 6000 adult mountain lions (not including kittens and subadults) in California. Field studies of using radio telemetry documented population densities higher than previously estimated from observation of tracks and other signs of the presence of lions.

Observations suggest that yearling and underweight lions, rather than large males are most likely to attack human beings, and that children and joggers are likely targets of attack. Several human behaviors appear to have potential to encourage attacks, others have to potential to discourage eminent attack. Mountain lion postures and behaviors often preceed aggression, or show lack of fear or submission. People working, living or recreating in mountain lion habitats and wildlife professionals working with them, need a through understanding of mountain lion behavior.
EXPERIMENTAL EVALUATION OF RESPONSES TO A MULTIVALENT Pasteurella haemolytica TOXOID-BACTERIN IN CAPTIVE BIGHORN SHEEP (Ovis canadensis)

M. W. Miller*
Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, Colorado 80526-2097, USA

J. A. Conlon
Fort Dodge Laboratories, 800 Fifth Street Southwest, Fort Dodge, Iowa 50501, USA

H. J. McNeill
Ayerst Veterinary Laboratories, 131 Malcolm Road, Guelph, Ontario N1K 1A8, Canada

J. M. Bulgin and A. C. S. Ward
University of Idaho, Caine Veterinary Teaching and Research Center, 1020 East Homedale Road, Caldwell, Idaho 83605-8098, USA

We examined effects of an experimental Pasteurella haemolytica toxoid-bacterin (A1, A2, T10) on humoral immune responses and P. haemolytica carriage/shedding rates in bighorn sheep (Ovis canadensis) in a randomized, complete block experiment with a repeated measures structure. Thirty captive bighorns were divided into trios on the basis of age, sex, and previous history of pneumatic pasteurellosis; one bighorn from each trio was randomly assigned to receive 0, 1, or 2 doses of toxoid-bacterin. Because our experiment is ongoing, data and analyses reported here are preliminary. Mild, transient lameness in most vaccinated bighorns 1 day after initial vaccination was the only adverse effect observed. We identified 32 distinguishable biogroup variants among 266 P. haemolytica isolates from bighorns, but carriage and shedding rates did not differ among treatment groups ($P > 0.53$). In contrast, bighorns receiving 1 or 2 vaccine doses showed marked elevations in P. haemolytica cytotoxic neutralizing antibody titers 1 wk after vaccination ($P = 0.0001$); mean responses peaked at 2 wks and titers remained elevated at least 6 wks after vaccination ($P = 0.0001$). Titers of agglutinating antibody to P. haemolytica serotype A1 capsular antigen were also elevated in vaccinated bighorns beginning 1 wk after vaccination ($P < 0.0014$) and showed similar response patterns. Preliminary data suggest this experimental P. haemolytica toxoid-bacterin is safe and may stimulate protective immunity in bighorn sheep. Based on our findings, further evaluation of this vaccine as a tool in preventing and managing pasteurellosis in bighorn sheep appears warranted.
ACCLIMATION AND HUSBANDRY OF GRAY WOLVES RELEASED IN YELLOWSTONE NATIONAL PARK

Mark R. Johnson•, Mike K. Phillips, Doug W. Smith, W.F. Brewster
Center for Resources, National Park Service, P.O. Box 168, Yellowstone National Park, Wyoming, USA

In January, 1995, the National Park Service in conjunction with the U.S. Fish and Wildlife Service and the Canadian government translocated 14 gray wolves (*Canis lupus*) from Hinton, Alberta, Canada, to Yellowstone National Park, Wyoming. These animals originated from 4 packs in Canada. Once at Yellowstone we placed two of the packs in separate pens and placed in the third pen the adult male from the third pack and adult female and her female pup from the fourth pack. To predispose the wolves to restricting movements to Yellowstone, each group was confined in an acclimation pen for 10 to 11 weeks. We constructed the acclimation pens from panels made of 9-gauge chainlink that measured 10 foot by 10 foot with a 2 foot 45° overhang. We assembled panels into circular enclosures covering about 1 acre in partially forested areas that were not visible from the road. Twice a week we fed wolves road-killed deer (*Odocoileus* spp.), elk (*Cervus elaphus*), moose (*Alces alces*), or bison (*Bison bison*) at a rate of 7 kg per wolf per day. The wolves completely consumed the meat provided. Human disturbance was minimized throughout acclimation. During the first two weeks, wolves paced excitedly about the pen and chewed the chainlink fence which caused superficial lacerations to their lips and gums. However, once the wolves became settled they paced less, ignored the chainlink, and routinely vocalized. Intrapack aggression was only observed during feedings and was probably displacement behavior caused by our presence. Although we detected proestrus bleeding in 2 adult females and observed breeding behavior in all three pens we did not observe wolves copulating. We released wolves during late March because warm weather prompted the emergence of grizzly bears from dens and reduced snow cover which complicated transport of food to the acclimation pens and reduced the availability of water.
PROTOCOL FOR ADDRESSING DISEASE ASPECTS IN GRAY WOLF REINTRODUCTIONS

Mark R. Johnson, MS, DVM
Center for Resources, National Park Service, Yellowstone National Park, WY 82190, USA

Steve Fritts, MS, PhD
U.S. Fish and Wildlife Service, Ecological Services, Helena, MT 59601, USA

When wolves or other animals are moved from one location to another, the potential exists for transporting infectious diseases with them. Diseases carried by translocated wolves may impact not only the wolves, but be harmful to other fauna in the new ecosystem. A few zoonotic diseases associated with wolves may even impact people, examples being rabies and Echinococcus spp., although the health risks to humans are very minimal. Also, specific procedures for addressing disease concerns with translocated animals may be required by state and/or federal law. Accordingly, we developed a protocol for the wolf reintroduction program for Yellowstone National Park, Wyoming and central Idaho to address these disease aspects. The peer-reviewed protocol provides for monitoring the health of captured wolves and examining for infectious diseases; collecting and examining biological samples for genetics, physiology, and diseases; and identifying, preventing, or treating diseases of concern that could potentially be carried by wolves. The recommendations included in this protocol are consistent with those of the IUCN Canid, Hyena, and Aardwolf Conservation and Assessment Plan. Licensed veterinarians provide and oversee veterinary care, physical examinations, sampling, and prophylactic treatments. Donor wolf populations are selected from areas free of significant diseases that are untreatable and difficult to diagnose, such as rabies and tuberculosis. General disease screening is conducted utilizing physical examinations for signs of infectious diseases (including ectoparasites), hematology and serum chemistry, serology, and examination of fecals for internal parasites. Propylactic use of anthelmintics, such as Ivermectin and praziquantel, is used to prevent transmission of a broad spectrum of parasites. In addition, vaccinations are given against rabies and common canine diseases, such as canine parvovirus and canine distemper. Although, no vaccinations are labeled for use in gray wolves; captive gray, red, and Mexican wolves are routinely and safely vaccinated with the commercial vaccines for domestic dogs recommended in this protocol. A pyrethrin dust is given to remove ectoparasites that are not blood-sucking. Wolves with signs of illness or infectious disease cannot pass inspection for international or interstate transport. Therefore, wolves captured in Canada with signs of illness or infectious disease would be isolated, and treated, if possible, or released back into the donor population. All translocation personnel prevent direct and indirect transmission of diseases from dogs to translocated wolves. Transport containers are disinfected after each use. Wolves that die during any stage of the reintroduction are thoroughly necropsied for infectious and noninfectious diseases as well as cause of death. Only wolves without clinical signs of illness or infectious disease are released into the new ecosystems. Because source populations are carefully selected and wolves are given effective prophylactic treatments, results from disease sampling do no influence selection of translocated individuals.
ECOLOGICAL ASPECTS OF A POPULATION CRASH OF BUFFALO (Syncerus caffer) 
IN THE WILLEM PRETORIUS GAME RESERVE, SOUTH AFRICA AND THE USE OF 
Psoroptes pienaari INFESTATION AS AN INDICATOR OF HABITAT STRESS 

P.J. Nel*
Orange Free State Department of Agriculture and Environmental Affairs (Nature Conservation Division), P.O. Box 517, Bloemfontein, 9300 

The Willem Pretorius Game Reserve in South Africa, maintained an unnaturally large population of buffalo for several years. During 1992, this management error coincided with a severe drought and extreme cold spells which led to a population crash in which ± 37% of the buffalo population consisting of ± 144 individuals died. This die-off was preceded by an outbreak of clinical Psoroptes pienaari infestation in most of the individuals in the population. Deaths occurred between the last week of July and the second week of September. Both density-dependent and density-independent factors were involved in the die-off. Inanition was the primary density-dependent factor responsible for lowering the temperature threshold tolerance. Most animals died during severe cold spells, with minimum temperatures reaching -11°C. In most cases the direct cause of death was hypothermia and thus density-independent in its action. Habitat utilization and competition (with mammal and insect herbivores) are other factors which all acted synergistically to aggravate the situation. Very young and very old animals were affected most severely. Deaths stopped occurring two weeks after the first spring rains in early September. As the condition of the buffalo improved, the skin condition due to P. pienaari infestation also improved. It was concluded that dermatitis and alopecia due to P. pienaari infestation in affected buffalo herds can be used as an indicator of habit stress. The die-off caused a significant change in the buffalo population structure. The events which led to the population crash, the factors contributing towards it as well as the results are discussed.
DETERMINING ECOSYSTEM HEALTH: SI/MAB TECHNIQUES FOR LONG-TERM MONITORING OF BIOLOGICAL DIVERSITY

A. Alonso Aguirre*, Francisco G. Dallmeier and James Comiskey
Smithsonian Institution, SI/MAB Biodiversity Program, 1100 Jefferson Drive SW Suite 3123, Washington D.C. 20560, USA (Dr. Aguirre's current address: P.O. Box 1522, Fort Collins, CO 80522, USA)

The Smithsonian's Man and Biosphere Biological Diversity Program (SI/MAB) focuses on problems associated with maintaining global biodiversity, emphasizing the practical application of research to achieve sustainable resource management. SI/MAB systematic methodology for designing and implementing long-term measuring and monitoring projects has been tested and refined at eight research sites - primarily biosphere reserves and conservation units in developing countries of Latin America. SI/MAB methodology is based on the establishment and maintenance of permanent inventory plots in tropical forests. Monitoring these forests for plants, invertebrates, and vertebrates is necessary before deciphering the effects of losses and changes caused by rampant deforestation. SI/MAB provides training for professionals to ensure that teams are in place to conduct on-going inventories and monitoring, disseminate information, and participate in the decision-making process. As part of its international courses, SI/MAB has recently incorporated monitoring of parasites and diseases. As forests are cleared for crop production and cattle ranching, wildlife concentrate in smaller forest stands being more susceptible to epidemics, malnutrition, and environmental pollutants. In addition, veterinary support is utilized in the application of humane and safe chemical immobilization techniques to assist inventorying and monitoring wildlife. Other challenges for veterinary research in the conservation of biodiversity include the development of integrated actions to stabilize animal populations within their habitats. When optimal population densities exceed carrying capacity, these will require of population or fertility control, or in some instances, removal of exotic introduced species. SI/MAB will be expanding its monitoring and training projects, attempting to increase its global network to 300 plots by the turn of the century, focusing on Latin America, Africa, and Asia. This effort will represent the world's largest grouping of biological diversity monitoring plots in a diverse range of forest habitats. This network will be linked by the SI/MAB protocol following consistent methodology, data management, and dissemination of information through workshops and publications. SI/MAB attempts to increase our knowledge of basic ecological functions for practical field applications. The sustainable use of natural resources is possible if reliable data about changes in ecosystems and their impacts on biological diversity are linked to conservation programs and integrated resource management.
RECOMBINANT MYXOMA VIRUSES CONTAINING REPRODUCTIVE TRACT ANTIGENS: CAN THEY BE USED TO CONTROL THE WILD RABBIT IN AUSTRALIA?

Anthony J. Robinson*, Ronald J. Jackson, Peter J. Kerr, and Michael K. Holland

Cooperative Research Centre for the Biological Control of Vertebrate Pest Populations, CSIRO Division of Wildlife and Ecology, PO Box 84, Lyneham, ACT 2602, Australia

The European rabbit (Oryctolagus cuniculus) was introduced into Australia in the 19th century as a game animal. Since that time it has spread to all areas in Australia and is a major pest species competing for sparse vegetation with native and production animals and causing widespread soil erosion particularly in more arid areas. The rabbit also helps maintain foxes that prey upon endangered and valued native fauna. Myxoma virus, a virus of the Brazilian cottontail rabbit (Sylvilagus brasiliensis), was introduced in 1950 and this reduced rabbit numbers from an estimated 600 million to half that figure. The introduction of the European rabbit flea (Spilopsylus cuniculi) in 1968 aided the spread of the virus in temperate areas but rabbits are still a major problem, particularly in the more arid zones. Additional methods of control are being sought and one of these is virally vectored immunocontraception. This is a concept whereby a gene encoding an antigen specific to an animal’s reproductive system is inserted into a virus and, during infection, stimulates the formation of antibodies to that antigen such that the animal is rendered infertile. Recombinant myxoma viruses expressing influenza virus haemagglutinin (HA) have been constructed and these are able to stimulate a high antibody response to the HA. A number of sperm antigen genes have been isolated and these are being inserted into myxoma virus to test their immunogenicity. Provided the immune response generated in rabbits to these antigens is sufficient to induce infertility and the antigens can be shown to be host specific, application will be made to the appropriate regulatory authorities for testing in contained field trials. Ultimately, the decision on whether or not to deploy such viruses in the field will depend upon an extensive public process where the benefits of the technology are weighed against the perceived risks.