DISEASES OF CHAMELEONS AT THE OKLAHOMA CITY ZOOLOGICAL PARK

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Introduction

Chameleons are highly specialized, arboreal reptiles best known for their color changing ability, independent turret-like eyes, and a projectile tongue. They are found almost exclusively in Africa and on the island of Madagascar. Chameleons as a group have diversified with species occupying a range of environmental conditions from hot, dry deserts to cool, moist montane areas. Many species, though locally abundant, may be threatened or endangered due to intensive habitat loss. In 1985 the Oklahoma City Zoo initiated a chameleon breeding program in order to learn more about the husbandry requirements needed to maintain chameleons in captivity. This is a summary of the diseases observed over a period of 7 years.

Sixteen species have been studied, some represented by only a few individuals. Successful breeding was accomplished in five species and include the Knsyna dwarf (Bradyopodium damaranum), Jackson's (Chamaeleo jacksoni), flap-necked (Chamaeleo dilepis), Oustelet's (Chamaeleo oustaleti), and sailfin (Chamaeleo montium) chameleons. Oviposition occurred in the tusked (Chamaeleo balteatus) and Will's (Chamaeleo willsii) chameleons without hatching success. Details of specific husbandry techniques have been described.

Chameleons have rather narrow but not necessarily obvious environmental requirements. As a group they are notoriously delicate and often present a challenge to the clinician. Some diseases of chameleons, such as dystocia, digital abscesses, and maladaptation or "fading chameleon syndrome" are seen with regular frequency and are likely to be a product of inappropriate captive environment.

Stress

Stress is an important factor in the development of illness in chameleons. Inappropriate temperature, humidity, caging and social conditions are all stressors. Subtle environmental deficiencies can result in failure to thrive. Chameleons tend to be solitary and individuals housed together must be watched closely for signs of stress. Males of most species will not tolerate being in the same enclosure with other males and may be stressed if kept within sight of other males of the same species (cages next to each other). The environment should always be carefully evaluated, specific to the species requirements, when one is presented with a sick chameleon. Unfortunately environmental factors that are important to many species are still being studied. Attempts are made to approximate environmental conditions, based on locality data, when available.
Vitamin supplementation

A relationship has been observed between vitamin D3 and vitamin A supplementation in chameleons kept indoors. Excessive vitamin A may interfere with D3 resulting in signs of hypovitaminosis D. Signs include gular edema and may progress to metabolic bone disease. This can be corrected in the early stages with large doses of vitamin D3. Chameleons have responded favorably to vitamin mixtures that contain 11,000 IU of vitamin D3 per gram, which is dusted on crickets prior to feeding. Many reptile vitamin supplements are excessively high in vitamin A and should not be used. Conversely some vitamin A is essential. Inadequate vitamin A may result in abscesses, matted eyes, and ataxia. The vitamin mixture used at the Oklahoma City Zoo contains 135 IU of vitamin A per gram. Specific requirements for vitamins A and D vary according to species or individual. The effect of high levels of Vitamin A on vitamin D is not observed in animals kept outdoors with access to natural sunlight.6

Physical Examination

Physical examination of chameleons should include careful inspection of the skin, oropharynx, appendages, and eyes as well as overall body condition. It should also include abdominal palpation. Many species are normally thin and some familiarity with a particular species is necessary to know what is normal weight. Chameleons will rapidly lose condition when ill.

Small volumes of blood can be sampled via the tail vein in larger species of chameleons. A blood sample can also be obtained from the heart. The heart can be visualized in the axilla by extending the a front leg forward. Microfilaremia is a common finding in wild caught individuals. Blood has not been drawn on healthy individuals routinely, therefore normal blood values have not been established.

With the exception of stomatitis and external abscesses, which are easily observed, a sick chameleon may present only general signs without revealing which organ system is involved. Enophthalmia, lethargy, and poor appetite are frequently the only presenting signs. Chameleons must be carefully observed on a daily basis so disease conditions can be detected early and therapy can be started.

Diseases

Tissue samples were taken from chameleons that died at the Oklahoma City Zoological Garden during the period 1987-1993. Neonates, below the age of 30 days were not included. The histopathological findings are summarized on Table 1. No pathology was observed in 65 percent of chameleons examined. The liver and the intestine were the most frequent organs where pathology was observed. Bacterial infections were responsible for roughly half of the cases that showed histopathological change. Bacterial cultures were taken in some cases and are summarized in Table 2.
Abscesses along the dental arcade, within the rostrum, and in the skin of the lips are common in chameleons. These are aggressively debrided and flushed with an antiseptic solution followed by systemic antibiotics. On one occasion a Parson's chameleon...
exsanguinated when an abscess in the palantine area eroded a large blood vessel. Caution is advised. Deep abscesses along the dental arcade, or in the feet may respond poorly to treatment and warrant a guarded response. Tissue samples from the oropharynx were not always submitted for histopathology. As a result stomatitis may be under represented on Table 1.

Chameleons that adjust to captivity can survive with disabilities. A Senegal chameleon (Chameleo senegalensis) is managing well in the collection with a front leg amputation. Also, an Oustelet's chameleon has learned to successfully site food items and eat following a unilateral enucleation.

One Meller's chameleon (Chameleo melleri) had encephalitis, possibly of viral origin. It exhibited ataxia and opisthotonos prior to death.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Bacteria Isolated from Chameleons</th>
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</thead>
<tbody>
<tr>
<td>Swabs were taken from gross lesions (abscesses), cloaca, or visceral organs at postmortem.</td>
<td></td>
</tr>
<tr>
<td>Organism</td>
<td>Culture Site</td>
</tr>
<tr>
<td>----------</td>
<td>--------------</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>Lung, liver, eye, foot, oral cavity</td>
</tr>
<tr>
<td>Proteus</td>
<td>Oral cavity, foot</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Fecal, cloaca, peritoneum</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>Oral cavity</td>
</tr>
<tr>
<td>Bacillus</td>
<td>Lingual abscess</td>
</tr>
<tr>
<td>Aeromonas</td>
<td>Oral cavity</td>
</tr>
<tr>
<td>Actinobacter</td>
<td>Lung</td>
</tr>
<tr>
<td>Serratia</td>
<td>Oral cavity</td>
</tr>
<tr>
<td>Providentia</td>
<td>Maxilla</td>
</tr>
</tbody>
</table>

Reproductive Problems

There were several deaths of gravid females around the time of oviposition. On a few occasions viable eggs were delivered by postmortem cesarean. Dystocia may be due to inappropriate nesting conditions which cause the female to delay ovipositing. The overall condition of the female during egg production can also be a contributing factor. Females should be robust before breeding. Individuals with marginal energy stores are candidates for dystocia.

A correlation has been observed between offspring survivability and breeding interval. Neonatal mortality increased greatly when Jackson's chameleons were allowed to breed back immediately after giving birth. Survivability of Jackson's chameleon hatchlings declined from...
70% to 30% and then 0% in three successive breedings. A similar effect on neonatal mortality was seen in egg laying flapneck chameleons allowed to breed following oviposition.

**Therapy**

Supportive care and appropriate environment are essential. Dehydration can appear rapidly, indicated by a sunken appearance to the eyes, and oral fluid therapy should be given early. Chameleons will stop eating if they are dehydrated. Hand feeding food items, or small amounts of baby food in more debilitated individuals may be necessary.

Parasites are frequently found in wild caught chameleons. Cestodes, nematodes, coccidia and flagellates have been observed. Subcutaneous filarids are not an uncommon necropsy finding. Microfilaremia has been observed, sometimes in high numbers, and has been treated with fenbendazole (100 mg/kg SID 10 days). Three new coccidia species have been identified from chameleons at the Oklahoma City Zoo.8

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Parasiticide Doses for Chameleons*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivermectin (Ivomec, MSD Agvet, Rahway, NJ 07065)</td>
<td>0.2 mg/kg oral or SQ</td>
</tr>
<tr>
<td>Mebendazole (Panacur, Pittman-Moore, Washington Crossing, NJ 08560)</td>
<td>20 mg/kg oral</td>
</tr>
<tr>
<td>Panacur (Fenbendazole, Hoechst, Somerville, NJ 08876)</td>
<td>30-100 mg/kg oral</td>
</tr>
<tr>
<td>Metronidazole (Sidmak Lab, E. Hanover, NJ 07936)</td>
<td>125-250 mg/kg oral</td>
</tr>
</tbody>
</table>

*Doses are offered only as guidelines. Studies of in vivo drug disposition have not been reported for chameleons. No toxic reactions have been observed using the dose listed with the exception of ivermectin. Two recently imported sailfin chameleons died shortly after receiving ivermectin. No other species, nor other individual sailfin chameleons have showed toxic reactions to this drug.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Antimicrobials Doses for Chameleons*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxin (Baytril, Haver, Shawnee, KS 66201)</td>
<td>5-15 mg/kg IM q 24 hrs x 10 - 14 d</td>
</tr>
<tr>
<td>Amikacin (Amiglide-V, Aveco, Fort Dodge Laboratories, Inc., Fort Dodge, IA 50501)</td>
<td>5 mg/kg IM q 72 hrs x 7 treatments</td>
</tr>
<tr>
<td>Carbenicillin (Geopen, Roerig, Roerig Division, Pfizer, Inc., 235 E. 42nd St., NY, NY 10017)</td>
<td>100 mg/kg IM q 24 hrs x 14 d</td>
</tr>
<tr>
<td>Chloramphenicol (Lyphomed, Division of Fujisawa USA, Inc., Deerfield, IL 60015-2548)</td>
<td>100 mg/kg IM/PO q 24 hrs x 10 d</td>
</tr>
</tbody>
</table>

*Pharmacokinetics have not been reported in chameleons for the agents listed. Frequently two drugs are used in combination, usually amikacin with enrofloxin or with carbenicillin.
Anesthesia

Isoflurane (Aerrane, Anaquest, Madison, Wisconsin 53713) (4% for induction) via small mask can be used for anesthesia. Induction is usually prolonged taking 30-45 minutes, or longer before a surgical plane is reached. Ketamine (Ketaset, Bristol, Aveco, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501) 15 mg/kg can be used to speed induction. Induction can be facilitated by placing the entire animal into a 1 gallon ziplock plastic bag and inflating the bag with 4% isoflurane in O2. This reduces stress from handling. There is a narrow range between pain sensation and respiratory arrest. Recovery from anesthesia can be prolonged.

Surgery

For celiotomies a paramidline approach is necessary to avoid the large ventral abdominal vein. Abscesses, as mentioned before should be removed entirely or debrided.

Conclusions

As habitats decline the need for comprehensive captive management of chameleons increases. This includes a need for greater understanding of the treatment and prevention of diseases.

LITERATURE CITED

SERUM AND TISSUE ENZYME ACTIVITIES IN THE YELLOW RAT SNAKE

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Samples of heart, liver, pancreas, skeletal muscle, kidney, lung, and intestine were collected from 6 yellow rat snakes, Elaphe obsoleta, and analyzed for clinically significant enzyme activities. Individual tissue samples were homogenized in distilled water, subjected to sonication, centrifuged, and the supernatants collected. The remaining pellets were then washed, centrifuged, and the supernatants collected, twice more. Pooled supernatants were analyzed using an automated serum chemistry analyzer for aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine kinase (CK), lactate dehydrogenase (LDH), alkaline phosphatase (AP), and gamma-glutamyl transferase (GGT). The general pattern of tissue enzyme activity distribution in these snakes was similar to the tissue enzyme activity distribution described in pigeons (Lumeij, JT, PhD dissertation, 1987). Heart tissue and skeletal muscle contained tremendous quantities of creatinine kinase activity (means: 1800 and 4000 IU/grams wet tissue (g tissue), respectively) compared to the other tissues (mean activities = 8-230 IU/g tissue). Liver tissue contained the greatest amount of LDH activity (mean = 195 IU/g tissue) of the tissues analyzed and low amounts of CK activity (mean activity = 8.9 IU/g tissue). Liver AST activity (mean = 93.6 IU/g tissue) was comparable to the AST activities found in skeletal muscle and kidney. Kidney tissue contained the greatest amount of ALT activity (mean = 227.1 IU/g tissue) of the tissues evaluated. Lung, pancreas, and intestine contained relatively low enzyme activities. No tissue contained appreciable quantities of GGT activity. Preliminary reference intervals for serum enzyme activities for the yellow rat snake, based on 23 samples from 7 individuals, are shown in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Range (IU/L)</th>
<th>Mean (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphatase</td>
<td>36-186</td>
<td>113.4</td>
</tr>
<tr>
<td>Lactic dehydrogenase</td>
<td>15-586</td>
<td>197.7</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>1-640</td>
<td>105.8</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>1-123</td>
<td>18.7</td>
</tr>
<tr>
<td>Gamma-glutamyl transferase</td>
<td>&lt;1-7</td>
<td>1.8</td>
</tr>
<tr>
<td>Creatinine kinase</td>
<td>27-2731</td>
<td>697</td>
</tr>
</tbody>
</table>

Reference intervals and mean concentrations for serum enzyme activities in healthy yellow rat snakes.
CAPTIVE REARING OF SEA TURTLES: HEAD STARTING KEMP'S RIDLEY, Lepidochelys kempii

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Kemp's ridley (Lepidochelys kempii) is a critically endangered species. It nests primarily near the village of Rancho Nuevo, Tamaulipas, Mexico, bordering the Gulf of Mexico. It also nests sporadically from Veracruz, Mexico to Padre Island, Texas, and recent nestings have been recorded for southwest Florida and the Carolinas. The species occurs in the Gulf of Mexico, along the eastern coast of North America to Nova Scotia, and in European Atlantic waters. It also has been reported from the Azores, Bermuda and Jamaica.

Since 1977, a binational Kemp's Ridley Recovery Program has been directed by the Kemp's Ridley Working Group, composed of representatives of Mexico's Instituto Nacional de la Pesca (INP) and the U. S. Fish and Wildlife Service (FWS), National Park Service (NPS), Texas Parks and Wildlife Department (TPWD) and National Marine Fisheries Service (NMFS). Gladys Porter Zoo, Brownsville, Texas also has participated in the program. Recently, Mexico's Instituto Nacional de Ecologia (INE) joined the working group.

Head starting of Kemp's ridleys is a subsidiary and experimental part of the Kemp's Ridley Recovery Program. It involves collecting eggs at Rancho Nuevo, Tamaulipas, Mexico, incubating them at Rancho Nuevo or Padre Island, Texas, exposing the hatchlings to either of these two beaches to "imprint" them, captive-rearing (9-11 months) and tagging the turtles in Galveston, Texas, and releasing the turtles into the Gulf of Mexico.

INP, with assistance from FWS and Gladys Porter Zoo, conducts egg collection, incubation and "imprinting" operations at Rancho Nuevo. NPS conducted incubation and "imprinting" operations at the Padre Island National Seashore near Corpus Christi, Texas until 1988, and continues to patrol beaches at the Seashore during the nesting season in hopes of finding head started nesters. The NMFS Southeast Fisheries Science Center's (SEFSC) Galveston Laboratory reared, tagged and released 22,596 Kemp's ridleys of the 1978-1992 year-classes, received as hatchlings from Padre Island and Rancho Nuevo.

All procedures, from collection of eggs through release, have been thoroughly and successfully developed. However, head starting of Kemp's ridleys and other sea turtles is highly controversial. The Recovery Plan for the Kemp's Ridley Sea Turtle (Lepidochelys kempii), published in 1992, calls for recovery actions needed to increase the Kemp's ridley population to a level of 10,000 females nesting during a season by the year 2020. This plan emphasizes that head starting is an experiment, not a recovery action.
Tag recoveries provide the basis for determining whether or not head starting achieves ultimate success, the major criteria of which are nesting and production of viable offspring.\textsuperscript{9,38,40} The standard method of tagging has been metal tags on the trailing edge of foreflippers, but additional external and internal tags have been used.\textsuperscript{11,12,13,19} In mark-recapture studies of harvested marine species, most tag returns are obtained from commercial or recreational fisheries targeting those species. Because Kemp's ridley is protected from exploitation by the U.S. Endangered Species Act, uncontrolled sources were relied upon for tag recoveries. Tag recoveries of head started ridleys have been based on foreflipper tags to date, because these external tags are easily recognized and reported by fishermen and the general public. Most flipper tag returns for which a recovery method was reported were from turtles found stranded (about 50\%) or caught incidentally by commercial shrimpers (about 25\%). The rest were reported by other fishermen, both commercial and recreational, and the general public.

To date, no nestings of head started Kemp's ridleys have been documented. Flipper tag recoveries have shown that head started ridleys become integrated into the wild population, by growing, surviving and becoming distributed throughout the range of the species.\textsuperscript{11,19,20} They have been found or captured among wild Kemp's ridleys in habitats typically occupied by wild Kemp's ridleys.

Published estimates of age at first maturity in wild Kemp's ridleys range from 6 to 15 yr or more.\textsuperscript{29,48,49} Head started Kemp's ridleys reared to maturity in captivity at Cayman Turtle Farm (1983), Ltd., Grand Cayman, B.W.I., nested successfully (produced viable offspring) for the first time at age 7,\textsuperscript{42,43,44} but Kemp's ridleys apparently grow faster in captivity than in the wild.\textsuperscript{6} Our analysis of growth, based on the Von Bertalanffy growth curve applied to length at age data from flipper tag recoveries, suggests that head started Kemp's ridleys reach size at maturity in the wild around age 8. However, if age to maturity is 15 yr or more, no year-class of head started ridleys would be old enough to have matured until now.

Sex ratios of head started Kemp's ridleys could influence the numbers of each sex surviving to maturity. Sample sex ratios based on turtles that died during head starting included both sexes. Year-classes 1978-1984 contained an estimated 32\% females.\textsuperscript{41} Pivotal incubation temperature for Kemp's ridley eggs (i.e., that temperature which produces a 1:1 female: male sex ratio) was not known until 1985, at which time NPS began to incubate the eggs at increased temperatures. As a result, the 1985-1988 year-classes were female-dominated, containing an estimated 83\% females.\textsuperscript{31,34} Year-classes 1989-1992, incubated at Rancho Nuevo, were estimated to contain more than 90\% females, so the eggs must have been incubated on that beach when temperatures were conducive to producing mostly females. Assuming that rates of mortality at sea are similar in the sexes, more females than males of the 1985-1992 year-classes would be expected to have survived, but the opposite would be expected for year-classes 1978-1984.\textsuperscript{5}

If any head started Kemp's ridleys have matured and nested, chances are remote that anyone saw them, even though Kemp's ridleys typically nest during daytime. Direct observation of a Kemp's ridley nesting at any location other than the primary nesting beach at Rancho Nuevo probably is a rare event. Even at Rancho Nuevo, more than half of the
nests found by beach patrollers are located without observing the turtles that laid them.\textsuperscript{28} It takes less than 1 hr for a Kemp's ridley to ascend the beach, nest and return to the water, so the window of opportunity for observing a nesting is short.\textsuperscript{29} If seen nesting, a head started turtle still may not be recognized as such. In some cases, when head started Kemp's ridleys in the wild lost their flipper tags but retained other tags or marks, even well qualified observers failed to recognize them as head started. All Kemp's ridley nesters at Rancho Nuevo observed with flipper tag scars but no tags have been assumed to be wild. All these factors work against documentation of nestings of head started Kemp's ridleys. Use of additional tags, including the external living tag (plastron tissue transplant to a carapace scute) and the internal magnetic wire tag and passive integrated transducer (PIT) tag, should increase recognition of head started Kemp's ridleys in the wild. However, it will take a greater effort than applied in the past to examine nesting ridleys for such tags in the future.

The greatest single source of sea turtle mortality caused by humans is incidental capture by shrimp trawls,\textsuperscript{16,18} and sea turtle strandings are correlated with shrimping.\textsuperscript{4} Strandings and incidental capture in shrimp trawls were the two major sources of tag returns from head started Kemp's ridleys. In 1989, a Blue Ribbon Panel of sea turtle experts concluded it was impossible to determine whether head started Kemp's ridleys are recruited into the natural breeding pool, because shrimp trawl-induced mortality rate was so high that few if any head started ridleys were expected to reach sexual maturity.\textsuperscript{38,40} This panel recommended the experiment be continued for 10 yr following installation of turtle excluder devices (TEDs) on all shrimping vessels in U.S. Gulf and Atlantic waters. In 1992, a second peer review panel\textsuperscript{9} evaluated the experimental design of head starting, and proposed the following two hypotheses:

1. Head starting can produce Kemp's ridley juveniles which are able to join the natural, wild population, find their way to nesting beaches and procreate (produce viable offspring); and

2. Head started Kemp's ridleys demonstrate equivalent or superior biological fitness (equal or better survival rates from egg to reproductive adult and equivalent or better fecundity) as compared to their wild counterparts.

Currently, emphasis is being placed on testing these hypotheses, and efforts to examine nesting Kemp's ridleys for evidence they were head started have been increased. The opportunity to test head starting of Kemp's ridleys under favorable conditions of TED use has existed only since 1989.

LITERATURE CITED


PROTOZOANS IN DENDROBATID FROGS: IDENTIFICATION, CLINICAL ASSESSMENT, AND INDICATIONS FOR TREATMENT

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Poison-dart frogs (Dendrobatidae) are small, brightly colored frogs indigenous to the Neotropics. Their common name attests to the toxic alkaloids which are secreted in the skin of certain species, and which are used by Amerindian hunters as poison for blowgun darts. The majority of the 200 or so unique alkaloids are of relatively low toxicity. In contrast, the batrachotoxins are among the most potent naturally occurring non-protein toxins. The poisons cause irreversible electrical depolarization of cells; they are effective upon entering the bloodstream and when digested, they are also irritating to porous skin. Other notable aspects of the dendrobatid biology include the fact that the majority are active only in daytime, eggs are laid on land, clutch size is small, and there is intensive care of the young. Currently approximately 135 species of dendrobatids are known, belonging to six genera, Aromobates, Colostethus, Dendrobates, Epipedobates, Minyobates, and Phyllobates; the latter is the only genus that secretes batrachotoxins.

In zoos and aquariums, increasing numbers of dendrobatids are being kept. This reflects not only their popularity as exhibit animals, but also the importance of captive breeding in the conservation of these threatened amphibians. The genera Dendrobates and Phyllobates are listed as threatened with extinction, and loss of rainforest habitat is a major factor contributing to their demise. Captive dendrobatids are also maintained for medical and behavioral research, and are popular as pets, particularly in Europe. Little is known of the health status of dendrobatids. In general, our knowledge of amphibian medicine is limited, with most of the scattered literature involving case histories or empirical treatments in a limited number of species.

Since 1982, the National Aquarium in Baltimore has exhibited poison-dart frogs, and pioneered a very successful breeding program. These efforts have been accompanied by monitoring of, and research on, the health of the frogs. We routinely provide clinical care for up to 24 species representing 4 genera. Of these species, 20 have been bred and reared successfully at the National Aquarium in Baltimore. Particular research efforts have been focused on identification of the commensal and parasitic protozoa of Dendrobates auratus, the black and green poison-dart frog, and Dendrobates pumilio, the strawberry poison-dart frog representing three groups of frogs:

1) wild-caught in Costa Rica - from virgin forest and secondary growth plantation;
2) wild-caught in Costa Rica and subsequently captive maintained;
3) captive-bred from parents collected in Costa Rica
Our aim is to identify the protozoan fauna, determine the relationships with disease, and develop safe, effective treatments for use when indicated.

To determine the identity of the protozoa, and aspects of their ecology such as prevalence and density of infection, the research frogs are sacrificed humanely by pithing or an overdose of tricaine methanesulfonate (MS 222, Finquel, TMS). Particular attention has been paid to the protozoa in the blood and in the intestine as these are the host tissues most readily sampled in colony frogs. The protozoans are being identified by light microscopy, scanning and transmission electron microscopy, and in histological section. In addition, video tape recordings are being made of live protozoa, to assist in identification.

The examination of freshly collected fecal material provides a non-invasive diagnostic tool in the health assessment of these small frogs. Both direct, and floatation methods are used. To avoid contamination with free living organisms, we place a well fed frog into an isolette containing a wet paper towel for substrate. Occasionally, moss is also analyzed from terrariums that do not house animals, but which have otherwise been maintained in an identical manner. This helps us to better understand which protozoans and metazoans live in the terrariums, and may be acquired by the frogs.

The clinical assessment and treatment of protozoans in dendrobatids requires a better understanding of which organisms are potentially pathogenic. The mere presence of these protozoans may not imply disease, but rather represent a component of normal flora. A quarantine program is essential to every preventative medicine program as it allows close scrutiny of potential problems and the collection of baseline data. Maladaptation and the inability to compete in newly created social groups are commonly seen, often resulting in secondary disease. Historically, we have had good success at regaining normal homeostasis using a regime that includes: assist feedings, identification and reduction of stress, fenbendazole dosed at 100 mg/kg repeated in 10 days, followed by or concurrent with metronidazole at 10 mg/kg SID for 5 days. Other infections should be treated appropriately. The metazoans and protozoans are usually not eliminated by these antiparaciticides, but are suppressed. Coccidia are rarely seen on the fecal examination, but should be considered in thin, depressed and anorectic animals passing loose, mal-colored stool. Treatment with trimethoprim-sulfa has resulted in limited success.

All wild caught frogs thus far examined have carried protozoans, with the greatest diversity being in the digestive tract. Opalinids, ciliates, and flagellates including trichomonads and diplomonads have been identified. These organisms were maintained in frogs held in captivity. In the minority of wild-caught animals, the blood supports the hemoflagellate Trypanosoma, but at low density. The rarity of the hemoflagellates and the difficulty in obtaining repetitive blood samples from specific individuals means it is hard to determine the effects of captive maintenance on the infections. We have, however, made no association between moribund frogs and the presence of this protozoan.

To date, it appears that the spectrum of commensal and parasitic protozoans in dendrobatids is similar to that from other anurans. The presence of dense and diverse populations of protozoa in the large intestines of apparently healthy wild caught, and captive
maintained frogs, suggests that these organisms are commensals rather than parasites. Morphological features of certain protozoa suggest that they represent new species. Our future work will include: describing these new species with the aid of their maintenance in culture, describing ecological aspects of the infections, and performing detailed examinations of protozoal fauna in captive bred frogs for comparison. Once we gain an appreciation of these organisms in "normal" animals, we will be better prepared to interpret the changes that occur when illness exists. Ultimately, we believe that the results of our investigations will allow more effective care and treatment of dendrobatids, which should in turn enhance the preservation of these fascinating and threatened frogs.

ACKNOWLEDGEMENT

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THE USE OF POLY VINYL CHLORIDE GLUES AND THEIR POTENTIAL TOXICITY TO AMPHIBIANS

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National Aquarium in Baltimore, 501 E. Pratt St., Baltimore, Maryland 21202, USA

Maintaining amphibians in good health requires a proper environment. Aquaria are frequently used to house both aquatic and terrestrial species. Plants, bark, moss, rock, pumps, filters, lights, and other materials, such as poly vinyl chloride (PVC) pipe-work, are often utilized in the creation of these mini-habitats. Ponds, streams, and misting devices commonly serve to provide a high humidity. This is essential to most amphibians as their skin is semi-permeable, allowing both respiratory and osmoregulatory function to occur. Environmental toxins, even at low levels, may therefore result in devastating effects including the failure to thrive, the inability to reproduce, and the loss of life.

The use of PVC pipe-work is popular in the building of aquatic filtration and water supply systems. Elaborate designs may be constructed quickly with relatively little investment of time and labor. Joining PVC pipe-work is a two step process: the application of PVC primer/cleaner containing methyl ethyl ketone and tetrahydrofuran, followed by the application of a PVC solvent cement containing cyclohexanone and tetrahydrofuran. When improperly cured, the release of these volatile chemicals may result in toxicity to animals housed within the system.

In October of 1992, 75 terrariums containing a total of 211 dendrobatid frogs were exposed to high levels of methyl ethyl ketone, tetrahydrofuran, and cyclohexanone at the National Aquarium in Baltimore following the installation of a three tier PVC pipe misting system. Each line was 8.23 M long and contained 30 T joints with misting nozzles. The misting system delivered carbon filtered freshwater as follows: "on" for 5 minutes every hour during an 8 hours period, "off" for 5 hours. Normally semi-active or secretive, the majority of animals were found scaling the glass of their enclosures. Rostral abrasions were found in approximately 30 animals as a result of pushing their snouts against the lid of their enclosure. With such a marked increase in activity, many of the frogs lost weight despite heavy feeding. No deaths occurred immediately following the exposure. All aquaria were rinsed thoroughly with freshwater, and over a one to two month period the animals behavior returned to normal.

Diagnosis of toxicity was made by the characteristic ketone odor and analysis of the mist for PVC solvents. Tests were made on two occasions; following the initial exposure of the frogs, and after flushing with forced air for 7 days followed by cool water for 7 days and then hot water for 3 days. Water samples were collected in 50 ml serum vials, leaving no air space, and were immediately acidified with 2.5 mls of 1 N Hydrochloric acid. Analysis was carried out in a Varian 3400 gas chromatograph with a Durabond D.B. Wax 30 m column and a Tekmar 7000 head space analyzer. Reference standards were used for the identification of unknown peaks.
The results of the first test confirmed high levels of tetrahydrofuran (25 ppm), methyl ethyl ketone (2 ppm), and cyclohexanone (16 ppm). These chemicals were non-detectable once curing had been achieved. Following the first exposure to these toxins and subsequent attempt to cure the joints, all but the final three misting ports were capped. Terrariums containing *Dendrobates auratus*, *Dendrobates azureus*, and *Phyllobates vittatus* were placed under the functional nozzles to serve as a biological indicator. Although subjective, the high concentration of these chemicals in the mist corresponded well with the chemical odor in the water and the hyperactivity observed in the frogs. A final flush with domestic hot water for 3 days was necessary before odor was no longer detectable and frogs displayed no observable reaction.

Exposure of amphibians to toxic substances can have serious consequences. In many countries, the decline of certain species has been linked to environmental pollutants and destruction of habitats.\(^1\) Clinical signs of toxicity are often non-specific and include: anorexia, ascites, skin ulcerations and hemorrhage, neurological dysfunction, secondary infection, and sudden death.

The solvents contained in PVC cleaners and glues have been implicated in the toxicity of fish when proper curing has not occurred (Reimschuessel pers. comm.). Tetrahydrofuran is a strong irritant to the skin and mucous membranes, and may cause renal damage. Methyl ethyl ketone is also an irritant to the skin and has been associated with central nervous system depression in experimental animals.\(^2,4\) Given that the skin of most amphibians has osmoregulatory and respiratory function, damage to this organ may have serious consequences.

The long term effects of exposure to these chemicals are difficult to assess. Cyclohexanone, for instance, like many solvents has been shown to be a teratogen in animal models.\(^3\) Approximately two months after the initial exposure, several animals died for no apparent reason. Only one was fresh enough for histopathological evaluation, however, no evidence of toxicity was found.

The proper curing of PVC primer and glue is necessary to prevent exposure to potential toxins. The amount of time needed to accomplish this will be directly related to the complexity of the system and the water flow through it. No adverse reactions were found in animals placed under our original test model which consisted of 3 joints/nozzles and was allowed to air dry for two weeks prior to use. Because these solvents are volatile, they readily dissipate from systems using high, constant flow rates that create agitation. Carbon filtration, commonly found in aquatic filters, may also help to remove these chemicals. Sealed water lines that remain static for periods of time create the greatest potential for solvent contamination.

Several precautions may be taken to avoid accidental exposure to the solvents found in PVC glues (table 1). In all cases, materials should be tested prior to use and avoided if mal-odor of any kind is detected. While expensive, chemical analysis may be carried-out. In cases where concern remains, there is no substitute for the use of sentinel animals when bringing a new system on-line.
ACKNOWLEDGEMENTS

I wish to thank Mr. Todd Daviau and his employer, Scios-Nova, Baltimore, Maryland for carrying out analysis of the water samples. Mr. John Cronnin of the Center of Marine Biotechnology, University of Maryland, Baltimore County, was also instrumental in coordination of sample collection and analysis.

REFERENCES

5. Reimschuessel R. 1993. Personal communication, University of Maryland, Laboratory of Aquatic Toxicology.

Table 1

Suggested Protocol When Using PVC Glues in an Aquatic System

* Minimize the number of glued joints when designing a system.
* Use the minimal amount of PVC primer/cleaner and PVC solvent cement necessary to make the bond.
* Allow air-curing to occur for a minimum of 14 days. In large, complicated systems this may require forced-air.
* Flush the pipe-work with domestic hot water for 7 days.
* Fill the pipe with water, and let it stand for 24 hours. Collect the effluent and determine if mal-odor is present. If so, continue to flush with air and water.
* Connect pipe-work to the system and monitor for mal-odor. If glue joints are used in the final connection, allow curing to occur.
* Incorporate activated carbon into the system for a period of time following initiation of the system.
* On large, complex systems consider chemical analysis of effluent after 48-72 hours of operation.
* Use sentinel animals as biological indicators, whenever possible.
CAPTIVE AMPHIBIAN CULTURE: SOME OBSERVATIONS AND SUGGESTIONS FOR FUTURE VETERINARY INVESTIGATIONS

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With current concerns over observed global declines of numerous populations of amphibians and the renewed commitment of the zoo community to conservation, veterinary practitioners can and must play an integral role in developing techniques to enhance maintenance and effective propagation of these creatures in a captive environment. In my view, amphibians present some of the greatest challenges and opportunities for a veterinarian in a zoo setting. What follows are some observations I have made over some 14 years direct involvement with zoos and some suggestions I would like to make for future research into the husbandry of amphibians in general.

Contrary to popular conception, amphibians are one of the oldest and most speciose groups of vertebrates in existence today, with some 4,000 known species\(^2\) and dozens of new taxa being found yearly. Their reproductive strategies and behaviors are the most diverse of any group of vertebrates.\(^1\) Their diversity of form and lifestyle show them to be a dynamic class as a whole and also demonstrate their worthiness of scientific investigation and as subjects of zoo display and husbandry. However, this very diversity leads to many problems in perception, husbandry, and propagation.

Herpetology as a science has a peculiar dichotomy shared by no other field of vertebrate biology. Herpetologists are forced to work with two groups of animals that are no more closely related to each other than pelicans are to hedgehogs. Consequently, herpetologists tend to find themselves working predominantly with either reptiles or amphibians, although there is considerable variation of orientation through time. This orientation of interest has enormous impact and consequence on the value given one or the other group.

When I entered the zoo business many years ago, it became quite evident to me that most people working with these animals had a tendency to view them as nothing more than precursors to or degenerate reptiles. Although amphibians represent nearly 40% of the beasts that make up the subjects of herpetology, it was and is rare to see them account for more than 5% of any zoo herpetological collection. The reasons for this are varied, but it is my opinion that the major reason behind such neglect is that most folks working with amphibians in zoos are primarily interested in reptiles, specifically snakes, and amphibians are usually nothing more than a second thought as a part of the collection. Consequently, the vast majority of herpetaria, structures ideally built to house both amphibians and reptiles, have physical parameters that accommodate the needs of reptiles almost exclusively, and if certain amphibians are able to tolerate these parameters, well, that is fine but it is not a major concern of keepers and curators. The "snake" mentality is quite strong, regardless of protestations otherwise. This is a major error of thinking and results directly, in my view, in the dismal success of husbandry and propagation efforts for amphibians in this country.
Although reptile husbandry is certainly decades behind mammals or birds, it is light years ahead of amphibian husbandry at this time. It is time to stop thinking of amphibians as nothing more than not-quite-reptiles. Recognize amphibians as a class of animals with their own particular needs which have nothing or very little to do with those of other vertebrates. Start to establish baseline data for what is normal and abnormal for all aspects of their captive husbandry, be it physical, physiological, behavioral, reproductive, etc. Recognize that these animals are exquisitely sensitive to their environments and are superbly adapted to same. Accommodate their needs insofar as is possible. Does it make any sense to maintain a tiger salamander (Ambystoma tigrinum), whose preferred body temperature may be 68°F, in the same environment as a corn snake (Elaphe guttata), whose preferred body temperature is 82°F? Of course not, but I have seen similar scenarios in virtually every herpetarium in this country I have visited. The fact that a salamander can survive, and for quite a number of years, at such temperatures does not mean that it should be maintained under such conditions.

In my experience, a second factor to keep in my mind is to never make any assumptions about these animals. From what I have seen, there are no "rules of thumb" for amphibian husbandry. Every species may very well have its own set of specific conditions under which it does best. Remember that amphibians have the most diverse reproductive strategies known for any class of vertebrates. As such, their raison detre' for existence is to reproduce or, to put it another way, they live to breed. Amphibians are basically cannon fodder for the rest of the vertebrate world and that fact colors every aspect of their existence. Although they are enormously successful as a group, their success has come from species adapting to specific and, frequently, narrow parameters so as to ensure the survival of the next, immediate generation. This living close to the biological edge may be the major factor in recently observed declines in many species of amphibians.

So, what can veterinarians do to enhance survivability and reproductive success of captive caecilians, salamanders, frogs, and toads? In my view, again, you can pretty much pick your own starting point. As noted, baseline physiological and physical data is virtually non-existent. Start doing blood workups at the least. Develop new techniques in radiography. My friend and colleague, Bill Bryant, recently told me of some developments in amphibian radiography using outdated mammography machines that I find extremely interesting and promising. Develop and investigate antibiotic therapies for known syndromes. Amphibians tolerate some drug regimes quite well, others extremely poorly, but the efficacy of virtually any therapy is virtually unknown at this time. For example, how do you treat an advanced Rhabdias infection in a 2 gm poison dart frog without killing it but eliminating the nematode infestation? Beats me, but I believe it can be done. As poison dart frogs on the whole are suffering enormous declines due to tropical deforestation, are quite prone to lungworm infections, are quite popular in zoos, just solving this one problem alone could have considerable impact on captive husbandry and conservation programs throughout the country. Defining and diagnosing particular disease entities and developing effective treatments are particularly important at this time. Except for perhaps the disease called "red-leg", there are very few widely known or recognized diseases in amphibians, other than parasites. The etiology of red-leg itself, which is essentially a stress-induced septicemia caused by a variety of pathogens, is still very poorly known.
One important change that you as veterinarians can make in your respective institutions is to insist that the physical environment that your amphibians occupy is close to that which they would experience in the wild. At the very least, this means that your amphibians must not be maintained at the same temperatures as for reptiles in your collections. To quote my late colleague, Joe Laszlo, "they like it cool, mon." The majority of amphibians have preferred body temperatures of 70°F and below. It is my belief that heat-induced sterility is a major cause of reproductive failure in many, if not most, zoo amphibian collections. Do not assume that because you have leaf frogs from the jungles of Malaysia, that they operate best at air temperatures of 85°F. Temperatures in the microhabitats that tropical frogs occupy are much lower than ambient temperatures.

Now, let's move on to some more conservation-oriented topics. As we all well know, the most pressing problem for zoos trying to enhance reproduction of endangered taxa is space, or, rather, the lack thereof. Although amphibians certainly do not have space requirements like those of large mammals, given their lack of appropriate representation in collections, spaces are limited for exhibition and propagation because of that lack. A survey presented to the AAZPA Amphibian Advisory Group a few years ago indicated that at this time there are only enough spaces and commitment to effectively maintain six species of amphibians in North American zoos under SSP guidelines (H. Quinn, pers. comm.). That certainly is not very promising for the future of captive propagation of amphibians for the long term. However, I believe there is a solution, one that has extremely exciting prospects, and it fits perfectly into the interests of the group assembled here today.

That solution is cryopreservation or, more accurately, cryopreservation of amphibian sperm and ova for later in vitro fertilization. As noted earlier, most amphibians' life cycles center around reproduction. As a consequence of this life style, most taxa produce large clutches, up to several thousand eggs per breeding for certain species. The offspring from these breedings are generally free-living larvae not dependent on maternal or paternal care and these offspring are easily raised in captivity with nearly 100% survival rates, much different from the 5% or less that is the norm in the wild.

Techniques need to be explored and developed in non-invasively harvesting both sperm and ova, properly cryopreserving those products, defrosting and mixing, determining the best mix of sperm and eggs to ensure maximum fertility, proper storage, length of storage and its effects on viability, etc. The frozen zoo is a very real possibility with amphibians. Instead of the six taxa mentioned earlier, establishing a viable frozen zoo for amphibians opens the possibility for preserving and conserving hundreds of species without any measurable increase in space requirements other than perhaps a large cabinet-sized area in your respective clinics or hospitals. The frozen zoo could also solve some other problems in amphibian culture.

At this time, there are very few long-term propagation programs for any species of amphibian in the United States. The longest I am aware of is for a certain type of poison dart frog at an institution at which I used to work. That program has been continuing for approximately 17 years. Most amphibian breeding programs typically last for two to five years and then seem to fizzle out. Why this happens is unclear and is another area worthy
of investigation, but the fact remains that sustained captive breeding of most amphibians in this country is not happening at this time. Cryopreservation solves that problem by always maintaining a stock that can be activated at virtually any time.

In addition, most of the captive-bred stocks of certain amphibians, such as the tomato frog (*Dyscophus antongili*) or ornate horned frog (*Ceratophrys ornata*), originate from very few founders. One successful captive breeding can suddenly make available hundreds or thousands of offspring. Inbreeding and other undesirable genetic effects are very real problems with amphibian husbandry because of this "founder effect". The frozen zoo could potentially maintain enough genetic material to eliminate or drastically reduce these deleterious side effects.

In summary, the contribution that veterinary practitioners can make to conservation of captive amphibians is great. Virtually all aspects of amphibian husbandry are little known and little explored and someone desiring to investigate new horizons of exotic animal husbandry could do no better than to start with amphibians.

LITERATURE CITED

Captive birds frequently develop malnutrition when hospitalized for medical or surgical problems. Factors contributing to the development of malnutrition include stress related anorexia, physical inability to eat, malabsorption, and hypermetabolism associated with sepsis and trauma. As with other species, malnutrition can have a negative net effect on cell-mediated and humoral immune functions, wound healing, and physiological processes associated with the cardiopulmonary and gastrointestinal systems. Few controlled studies have been conducted to assess the nutritional requirements of normal or physically impaired wild birds. Since most nutritional studies have been carried out in non-avian species, caution must be exercised in extrapolating these findings to birds. To partially overcome this problem, metabolic scaling formulas can be used to calculate basic caloric and nutrient requirements for hospitalized birds. Starvation in critically ill or injured human patients often results in increased metabolic rate and nutrient requirements (hypermetabolism), in contrast to decreased metabolic rate and nutrient requirements associated with uncomplicated starvation. Because it is circumstantially suggested that hypermetabolism occurs in critically ill or injured birds, a variety of intensive clinical management techniques and products are required to reverse the downward spiral of malnutrition and the subsequent severe emaciation. This armamentarium includes a variety of commercially available and individually formulated enteral feeding products (Tables I & II); the use of intraosseous cannulas; venous access devices; and enterostomy tubes for fluid and nutrient administration.
Table I - Sources of Products and Devices for Nutritional Support

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
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<tr>
<td>CliniCare® Liquid TEN Diets</td>
<td>Pet Ag. Inc., Elgin, IL 60120</td>
</tr>
<tr>
<td>Fortison®, Fortical®</td>
<td>Sherwood Medical, St. Louis, MO</td>
</tr>
<tr>
<td>Isocal®, Traumacal®</td>
<td>Mead Johnson, Evansville, IN 47721</td>
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<tr>
<td>Pulmocare®, Osmolite®</td>
<td>Ross Laboratories, Columbus, OH 43216</td>
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<tr>
<td>Reabilan®</td>
<td>O’Brien Pharmaceuticals, Parsippany, NY 13815-0231</td>
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<tr>
<td>Venous Access Devices</td>
<td>Norfolk Medical Products, Access Technologies, Skokie, IL</td>
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<tr>
<td>Vivonex TEN®*</td>
<td>Norwich Eaton Pharmaceuticals, Norwich, NY 13815-0231</td>
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*TEN = Total Enteral Nutrition

Table II - Nutrients per 100 Kcal Energy of Various Nutritional Support Products

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<tr>
<th>Product</th>
<th>Protein,g</th>
<th>Fat,g</th>
<th>Carbohydrate,g</th>
<th>H₂O/100 ml</th>
<th>Kcal/ml</th>
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<tr>
<td>CliniCare® Feline TEN Diet</td>
<td>7.0</td>
<td>4.6</td>
<td>5.7</td>
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<td>0.92</td>
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<td>CliniCare® Canine TEN Diet</td>
<td>5.0</td>
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<td>0.98</td>
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<td>Isocal®</td>
<td>3.4</td>
<td>4.4</td>
<td>13.3</td>
<td>84</td>
<td>1</td>
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<tr>
<td>Traumacal®</td>
<td>5.5</td>
<td>4.5</td>
<td>9.5</td>
<td>52</td>
<td>1.5</td>
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<tr>
<td>Pulmocare®</td>
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<td>6.1</td>
<td>7.0</td>
<td>52</td>
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<tr>
<td>Emeraid II® (50:50 with water)</td>
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<td>1.3</td>
<td>16.8</td>
<td>≈ 33</td>
<td>1.9</td>
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<tr>
<td>Isocal® HCN</td>
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<td>5.1</td>
<td>10.0</td>
<td>35.5</td>
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PASSIVE TRANSFER OF IMMUNITY AND THE DEVELOPMENT OF ANTIBODY RESPONSES IN THE BLUE & GOLD MACAW

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Although much is known about the avian immune system, knowledge comes primarily from studies performed in gallinaceous species. The purpose of this study is to 1) measure the degree of passive transfer of antigen-specific IgG from blue & gold macaw hens to chicks via the egg and 2) measure the ability of blue & gold macaw chicks to mount an antigen-specific IgG response from age 2 through 10 weeks. Bovine Serum Albumin (BSA) was chosen as the antigen for this study because it has been shown to be both safe and highly antigenic in chickens. Anti-BSA IgG titers were measured in all test serum samples with an enzyme-linked immunosorbent assay which utilizes anti-Blue & Gold macaw IgG monoclonal antibodies.

A passive transfer study was designed as follows: Adult breeding blue & gold macaw hens were immunized with 200μg Bovine Serum Albumin every 21 days from April through September, 1992. Anti-BSA IgG titers were measured in serum from these hens at the time of each immunization. Eggs laid by these hens were incubator hatched to eliminate the possibility of antibody transfer through crop secretions. Anti-BSA IgG titers were measured twice weekly in the chicks from 14 through 28 days of age, then weekly through 42 days of age. In a second study, 10 chicks, 2 from each of 5 clutches laid by non-immunized hens were pulled from the nest at 14 days of age. They were divided into 2 study groups consisting of five chicks each, one chick from each clutch. The first study group was immunized with 200μg BSA at 2 and 6 weeks of age. The second study group was immunized with 200μg BSA at 6 and 10 weeks of age. Anti-BSA IgG titers were measured weekly in all chicks until 4 weeks post-booster.

In the passive transfer study, results showed that immunized breeding hens maintained strong anti-BSA titers throughout the breeding season. Peak antibody titer is reached 14 days following immunization. Seven chicks hatched by 3 immunized hens were included in the study. A direct calculation of percent of anti-BSA antibody transferred from hen to chick cannot be made because the hens were not bled on the days of egg laying. However, at 14 days of age the chicks had serum anti-BSA titers of roughly 1% of the hen's at her most recent bleed. By 21 days of age the chicks had lost approximately 50% of their 14 day
anti-BSA titer. By 42 days of age the anti-BSA titers in the chicks were virtually undetectable. Results of the second study showed that 9 of 10 chicks (from non-immunized hens) mounted strong anti-BSA responses. Chicks at 6 weeks of age mounted significantly higher anti-BSA IgG responses than those at 2 weeks of age. Chicks in both age groups mounted primary and secondary responses. However, the magnitude of these responses were significantly lower than those mounted by adults.

This study shows that adult blue & gold hens can mount a strong class-specific IgG response to the protein BSA. These circulating serum antibodies are transferred to the chick through the egg at levels which are detectable through 42 days of age. Two to 10 week old chicks mount weaker anti-BSA IgG responses than adults. Chicks at 6 weeks of age mount stronger anti-BSA responses than chicks at 2 weeks. Information gained from this study will aid in the development of vaccination programs for psittacine collections, as well as provide tools for future studies of both the humoral and cellular immune responses of psittacine birds.
HEMOSIDEROSIS IN A WILD CAUGHT BIRD OF PARADISE AND OTHER SPECIES IN PAPUA NEW GUINEA

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One factor which has limited the captive propagation of birds of paradise has been premature death associated with liver disease characterized by abnormal accumulation of iron in the liver. This condition has been documented in birds of paradise and other species in several zoos around the world. Occurrence of this abnormal finding has been limited to captive birds. Proposed causes of this condition include high iron diets, genetic disorder, vitamin/mineral imbalance, intestinal mucosal defect, or toxic insult due to pesticides.

Since 1984 the Zoological Society of San Diego has participated in four collecting expeditions to Papua New Guinea, where a wide variety of avifauna has been trapped for captive propagation from areas being deforested. Some of the species being collected have included birds of paradise which the Society has historically propagated with varying degrees of success.

In August-September, 1988 necropsy examinations were performed on fourteen birds that died during the capture and post-capture acclimatization period in Madang Province. These included a single bird of paradise. Tissues were placed in 10% buffered formalin for histological examination. Hepatocellular hemosiderosis was a prominent finding in an immature King Bird of Paradise (Cicinnurus regius). This is the first known bird of paradise species directly from the wild demonstrating liver lesions compatible with those documented historically in captive specimens. Hemosiderosis was also present in a White-Eared Catbird (Ailuroedus buccoides) and a Metallic Starling (Aplonis metallica).
TREATMENT OF IRON STORAGE DISEASE IN A BALI MYNAH

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A 4-year-old, captive hatched, female Bali mynah (Leucopsar rothschildi) was diagnosed as having iron storage disease based on a liver biopsy. The liver biopsy was stained with Pearl's iron stain and showed a marked amount of stainable iron. At the time of diagnosis, the serum iron level was 493 micrograms/dl.

The bird was treated with a combination of phlebotomy and an iron chelator, deferoxamine mesylate. Blood volume equal to one percent of the bird's body weight was removed followed 48 hours later by treatment with deferoxamine at a dose of 40 mg/kg IM once a day for seven days.

The bird was treated for two cycles. Each cycle consisted of phlebotomy and seven days of deferoxamine mesylate therapy repeated every other week for a total of 5 treatments. A liver biopsy was performed at the end of each treatment cycle and stained for iron.

Serum iron levels and liver iron load based on iron stain decreased with each treatment cycle. At the end of the second cycle, the serum iron level was 118 micrograms/dl and a liver biopsy showed minimal stainable iron.
MEDICAL ASPECTS OF BALI MYNAHS (*Leucopsar rothschildi*)

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The Bali mynah, *Leucopsar rothschildi*, (also known as the Rothschild's mynah or Bali starling) is the only endemic vertebrate animal species in Bali. Its current range is approximately 65 square kilometers in the Bali Barat National Park in Northwestern Bali. The Bali mynah is currently considered to be critically endangered and on the verge of extinction. It is listed in Appendix 1 of the 1973 Convention on International Trade in Endangered Species of Wild Fauna and Flora. The purpose of this paper is to review the captive medical management of the Bali mynah.

1) Husbandry

For captive management purposes, individual enclosures are recommended for breeding pairs. This prevents dominant pairs from inhibiting other pairs from breeding and enables parenthood of offspring to be definitively established. Some degree of visual isolation of breeding pairs from each other is recommended. Minimal contact with humans is desirable. Genetically unimportant birds should be used for display, while breeding pairs should be maintained off exhibit if possible. Large enclosures are not necessary for breeding success. Breeding has occurred in 2 meter x 2 meter x 4 meter enclosures. It is very important to have enough vegetation to provide cover, perching, and stimulation. Nest boxes should be introduced to the enclosure in the early spring in North America. The nest box should have a small opening which allows the birds to have a sense of security and privacy. It should be placed high in the enclosure, and fresh nesting material should be provided throughout the breeding season. Both parents build the nest, which is an important part of courtship and pair bonding. The clutch size is usually 3 eggs, although 4 or 5 eggs may be laid occasionally. The eggs are bright blue. The incubation period is approximately 14 days. Incubation and rearing are shared by both parents. The young fledge at 21 to 24 days of age. Once the young fledge, it is often best to remove them because adults may attack and injure them. This is presumably an attempt to drive them to occupy new territory and leave space and food for the next clutch.
2) Handrearing

Handrearing chicks has been successful; however, it is thought to decrease breeding potential and suitability for release into the wild. The St. Louis Zoological Park has developed a "hands-off" approach to handrearing that appears to be successful in avoiding raising imprinted birds. Handrearing should be used only as a last resort where a particular pair has repeatedly been unsuccessful at rearing offspring.

3) Nutrition

The Bali Mynah is primarily omnivorous. Preferred food items in the wild include seasonal fruits of native trees/shrubs, a variety of insects, and even small reptiles. Surprisingly, one of its favorite foods is the fruit of the introduced Lantana from South America.

Bali mynahs are sensitive to excess dietary iron, thus diets should be formulated to contain iron concentrations within the range recommended for other avian species (44-111 mg/kg dry matter). A 100 gram bird requires 25-50 kcal daily to meet maintenance energy requirements, depending on activity level. An example diet which meets known nutrient needs (based upon domestic poultry species as physiological models) for an individual includes:

- 25 grams Softbill Fare (Reliable Protein Products, Rancho Mirage, CA 92270)
- 25 grams produce salad (50% fruit, 25% mixed vegetables, 20% minced leafy greens, 4.6% powdered complete poultry maintenance ration, 0.2% ground oyster shell, 0.1% dicalcium phosphate, and 0.1% dry powdered vitamin E supplement).

Diet components represent nutritionally balanced rations, each individually formulated to meet (at least) minimal avian nutrient requirements. This diet provides approximately 70 kcal of metabolizable energy, with a nutrient composition (dry matter basis) of: 26% crude protein, 10% crude fat, 1.0% Ca, 0.6% P, 26,000 IU/kg vitamin A, 1200 IU/kg vitamin D3, and 200 mg/kg vitamin E. Iron content of this diet is calculated at less than or equal to 150 mg/kg. Twenty-five grams of Bird of Paradise pellets (Zeigler Bros., Inc., Gardners, PA 17324) could substitute for the Softbill Fare, but the extra minerals in the salad mixture should be deleted. A small quantity of insects can be added to the diet for behavioral enrichment. Iron content of a variety of food items is listed in Table 2.

4) Anesthesia

Isoflurane is the anesthetic agent of choice. Ketamine HCl (Ketaset, 100 mg/ml, Aveco Co., Inc., 800 5th St. NW, Ft Dodge, Iowa 50501) at a total dose of 4 mg IM in the pectoral muscle in adult birds was utilized by one of the authors (TMN) on several Bali mynahs in Indonesia for surgical sexing. No side effects were noted and recoveries were fairly rapid (approximately 2 hours). The birds were recovered in a small cardboard box.
5) Sexing

The preferred methods of identifying the sex in Bali mynahs are direct visualization via laparoscopy or DNA sexing from blood (Zoogen Incorporated, 1105 Kennedy Place, Suite 4, Davis, California 95616). Zoogen has perfected their technique for Bali mynahs.

6) Infectious Disease

(A) Parasites

(i) Atoxoplasmosis

*Atoxoplasma* sp. is a coccidian parasite of passerine birds with a prolonged life cycle that has stages involving the intestinal submucosa and the reticuloendothelial system. Atoxoplasmosis was first reported in Bali mynahs at the National Zoo in 1989. Retrospective studies revealed the organism had been present in the National Zoo's collection since 1975. During the past 3 years, atoxoplasmosis has been documented to be a problem in Bali mynahs at several US and Indonesian zoos, and probably exists in many others. It is currently thought to be the most detrimental disease to Bali mynah captive propagation in the US and Indonesia. Birds at 8 out of 9 US zoos screened for the organism have been positive. *Atoxoplasma* (synonyms: *Isospora serini*, *Lankesterella garnhami*) has been previously described in sparrows (*Passer domesticus*), canaries (*Serinus canarius* Linnaeus), evening grosbeaks (*Hesperiphona vespertina*), rose breasted grosbeaks (*Pheucticus ludovicianus*), common mynahs (*Acridotheres tristis*), Indian hill mynahs (*Gracula religiosa*), and Bali mynahs. Two studies suggest that *Atoxoplasma* is species specific. *Atoxoplasma* oocysts were transmitted from sparrow to sparrow, but not from sparrow to canary. These birds were evaluated via weekly blood films for 7 weeks post-inoculation and then necropsied. Oocysts from canaries could not be transmitted to cockatiels. Tissues from evening grosbeaks naturally infected with *Atoxoplasma* were transmitted by intracoelomic injection to uninfected grackles, cowbirds, swamp and song sparrows, but not to ducks. Further research into the species specificity of *Atoxoplasma* is needed.

*Atoxoplasma* is spread when infected birds shed oocysts in their droppings and these are consumed by other birds via contaminated food or water. The sporozoites are released in the intestinal lumen and invade intestinal epithelial cells, lymphocytes and macrophages and then disseminate throughout the body. Clinical signs of atoxoplasmosis are attributed to the systemic schizonts which cause the most damage rather than to the gamonts in the intestinal tract. Infected adult birds may intermittently shed large numbers of oocysts for prolonged periods. This is unlike most other coccidial infections where an immunity develops and shedding stops within months, unless the bird is reinfected through environmental contamination.

The clinical history in Bali mynahs usually consists of acute death in fledgling birds most often between 3 and 8 weeks of age. Adults are usually asymptomatic, while immature birds...
have high morbidity and mortality.\textsuperscript{12,27} Adult birds may develop clinical signs if stressed or exposed to a large number of oocysts.\textsuperscript{12,16} Clinical signs are most often nonspecific and may include diarrhea, weight loss, lethargy, decreased appetite, and ruffled feathers.\textsuperscript{12}

Antemortem diagnosis is difficult. Physical examination may reveal an enlarged liver and dilated loops of bowel.\textsuperscript{12} Fecal examination may be positive or negative. In a recent study, 6 out of 8 Bali mynahs, that reared chicks which had pathology attributed to toxoplasmosis, were shedding oocysts in the feces.\textsuperscript{27} Fledgling birds with toxoplasmosis diagnosed antemortem or at postmortem are less likely to be shedding oocysts in the feces. Fecal samples should be collected over several days due to possible intermittent shedding of the oocysts. Collection of feces in 2.5\% potassium dichromate with periodic aeration allows the organism to sporulate in approximately 96 hours and enhances identification.\textsuperscript{2} The oocysts have the typical Isospora spp. morphology (i.e. 2 sporocysts, each containing 4 sporozoites).

\textit{Atoxoplasma} organisms can be found on blood smears in mononuclear cells. Buffy coat smears concentrate the white blood cells, thus making it more likely to find the organism when compared to routine blood smears. In canaries known to have toxoplasmosis, organisms were found in only 5 out of 8 birds on buffy coat smears. Furthermore, only 1 to 8 cells out of 500 counted contained the organism.\textsuperscript{12}

Liver biopsy impression smears appear to be the most accurate diagnostic test for juvenile birds.\textsuperscript{27} In one study, 6 out of 7 young Bali mynahs had macrophages with organisms present on liver impression smears.\textsuperscript{27} Organisms were least numerous in the oldest birds. Adult birds were not biopsied in this study.

Wright stained impression smears of affected organs are more beneficial diagnostically than histopathology.\textsuperscript{2} It is often difficult to visualize organisms on histopathology unless the infection is severe. The organism may be seen in lymphocytes or macrophages. It has a pale basophilic cytoplasm with a small central nucleus. The parasite forms a reddish cytoplasmic inclusion in the mononuclear cells which accompany the inflammatory reaction to the parasite.\textsuperscript{12}

The most common gross pathological findings are an enlarged liver and spleen with pinpoint foci of necrosis, an edematous and hemorrhagic pancreas, and a fluid filled intestinal tract.\textsuperscript{12,27} Histopathology usually reveals hepatic and splenic necrosis with a marked mononuclear cell infiltration.\textsuperscript{12,27} The myocardium, thyroid glands, pancreas, adrenal glands, kidneys, and lungs may also be involved.\textsuperscript{26,27}

Several drugs have been used unsuccessfully to treat toxoplasmosis in canaries. These include tetracycline\textsuperscript{28}, sulfa drugs\textsuperscript{7}, spiramycin\textsuperscript{12}, and a combination of primaquine, chloroquine and amprolium.\textsuperscript{12} Oocyst shedding decreased with the use of these drugs; however, intracellular stages were unaffected.\textsuperscript{12} A recent report in psittacine birds described the successful treatment of \textit{Sarcocystis}, another invasive coccidian species, with a combination of trimethoprim-sulfa and pymethrinine.\textsuperscript{25} This regimen has been used successfully in human toxplasmosis cases. Preliminary results in Bali mynahs utilizing this treatment regimen for toxoplasmosis are encouraging.
(ii) Toxoplasmosis

Toxoplasmosis has been diagnosed in other mynah species, but has not been reported in Bali mynahs. The clinical picture described for toxoplasmosis is similar to atoxoplasmosis.\textsuperscript{5,6,26} Toxoplasmosis induced a multifocal granulomatous hepatitis, splenitis, pneuminitis, air sacculitis, and focal myocarditis.\textsuperscript{5} The primary breeding center for Bali mynahs in Indonesia (Surabaya Zoo) has a large feral cat population on the zoo grounds. Thus, toxoplasmosis could be a potential problem in Bali mynahs.

(iii) Plasmodium

\textit{Plasmodium} has been reported in other mynah species causing a hepatosplenomegaly and pulmonary congestion.\textsuperscript{6}

(iv) Cestodiasis

Cestode ova and proglottids are frequently found in feces from Bali mynahs housed in the US and Indonesia. Praziquantel (Droncit) at 25 mg/kg was found to be safe and effective in treating Bali mynahs for tapeworms.\textsuperscript{20}

(v) Nematodiasis

\textit{Capillaria} spp. and Ascarids have been associated with captive Bali mynah morbidity and mortality.\textsuperscript{6,27} \textit{Tetrameres} spp. have been reported to cause red nodular lesions in the glands of the proventriculus in other mynah species.\textsuperscript{6,26}

(B) Viral

(i) Pox virus

Pox virus has been documented in a Bali mynah at one US zoological institution to date. The virus was also diagnosed in 6 of 15 Bali mynahs housed in a large indoor flight cage. Free-flying birds including starlings (\textit{Sturnus vulgaris}), sparrows (\textit{Passer domesticus}), and pigeons (\textit{Columbia livia}), had access to the aviary. Proliferative lesions around the eyes and at the comissures of the beak were observed. The disease was experimentally transmitted to starlings by injecting infected tissues into the wing web. The starlings developed lesions consistent with pox virus at the injection site, on the eyelids, in the oropharynx, and on self-inflicted traumatized areas of the face and limbs. Typical eosinophilic intracytoplasmic inclusions were seen on histopathological examination. It was thought the most likely source of pox viral infection in the Bali mynahs was from the free flying starlings.\textsuperscript{19}

(ii) Paramyxoviruses

Information on Newcastles Disease virus (NDV) in Bali mynahs is not available, however, other mynah species appear to be important reservoirs of NDV.\textsuperscript{6,11,26} Viscerotropic velogenic and NDV have been isolated from a variety of mynah species imported from
Southeast Asia into the United States. Clinical signs and pathological lesions of naturally developing ND in mynahs are not described. Other paramyxoviruses (PMV) are common in mynahs. In a 5 year period, the National Veterinary Services Laboratories, Ames, Iowa, made 102 isolates of PMV from cloacal swabs and tissues of various mynah species. The birds had originated from Southeast Asia and were held in quarantine centers prior to entry into the US. Of the 102 isolates, 57 were PMV-2 and 42 were PMV-3. These viruses could not be transmitted to susceptible chickens and turkeys.11

(iii) Avian Influenza

Other mynah species are known to harbor influenza virus type A. The virus causes a hemorrhagic tracheitis, pneumonia, airsacculitis, and hemorrhagic enteritis.26 It is likely that Bali mynahs are also susceptible to influenza virus, but this has not been documented.

(C) Bacterial

Bacterial species reported to cause clinical disease in other mynah species include Pasteurella spp. (Avian cholera)33, Yersinia pseudotuberculosis6, Salmonella typhimurium6, Klebsiella pneumonia6, Escherichia coli6, Erysipelothrix insidiosa13, and Nocardiya spp.12 Coxiella burnetti (cause of Q fever)40, Campylobacter spp.26, and Salmonella spp.31 have been isolated from other mynah species in the wild. Refer to Table 1 for bacterial problems seen in the US Bali mynah population.

(D) Chlamydia

One case of Chlamydia psittaci infection in the US Bali mynah population has been reported to the SSP Veterinary Advisor.

(E) Fungal

Aspergillosis is the most serious fungal disease in mynahs6,35 and has been documented in the US Bali mynah population. One case of aspergillosis occurred concurrently with toxoplasmosis in a juvenile Bali mynah. Aspergillosis may be systemic or may involve only the syrinx in mynahs.6

7) Metabolic/Nutritional Diseases

(A) Hemochromatosis

Hemochromatosis or iron storage disease is caused by excessive iron accumulation that incites an inflammatory response in various body organs, especially the liver. Eventually this process may lead to fibrosis and end stage liver disease. It is important to differentiate hemochromatosis from hemosiderosis, the latter being defined as the accumulation of iron in parenchymal organs without inflammation or fibrosis.22 Hemochromatosis is common in several mynah species, 6,8,9,10,13,22,23,26Ramphastids,38,39 birds of paradise1, and tanagers.17
In humans, hemochromatosis can be secondary (the body accumulates iron in response to a specific cause such as certain anemias or chronic ingestion of excess iron in the diet) or idiopathic (an apparently heritable condition of abnormal iron deposition from an iron-balanced diet). The exact cause of the disease in avian species is unknown, but appears to be multifactorial. High dietary iron has been shown to be one of the factors causing hemochromatosis in birds. Lowering dietary iron levels has decreased the incidence of the problem, but it still occurs. Several commercially prepared low iron diets are now available. Numerous factors affect iron absorption including reproductive status (egg laying hens require more iron for mobilization to the yolk), iron status of the animal, health status, conditions in the gastrointestinal tract (i.e. pH), quality and chemical form of iron, iron levels in soil, and the proportion of other dietary components. Dietary components which potentially increase iron absorption include ascorbic acid, sugars (lactose, glucose, fructose), and some amino acids. Components of the diet that decrease iron absorption due to competition for binding sites include Co, Zn, Cd, Cu, and Mn. Ceruloplasmin is a copper containing enzyme involved in iron transport. In copper deficiency, movement of stored iron from the liver is decreased, thus, causing increased iron storage to occur.

A liver biopsy (histopathology) is the method of choice to confirm hemochromatosis. This may not be possible if the bird is in late stages of the disease, because of the potential risks of anesthesia and surgery. A thorough history, physical exam, whole body radiographs, serum liver enzymes, albumin, and serum bile acid levels may aid in a presumptive diagnosis of hemochromatosis. Serum iron levels do not correlate with hepatic histopathology in birds or humans with hemochromatosis, but can be utilized to monitor therapy. In a recent case report serum iron levels aided in diagnosis of hemochromatosis and monitoring the efficacy of therapy in a greater Indian hill mynah. Total serum iron levels and whole body radiographs were most useful in monitoring the efficacy of therapy. Total iron binding capacity (TIBC), percent iron saturation, serum ferritin, and serum iron are utilized to diagnose human hemochromatosis. Although normal values have been reported in domestic poultry and a few exotic avian species, further research is still needed in avian species to correlate TIBC and other diagnostic tests with hepatic histopathology.

For therapy to be successful, early diagnosis of hemochromatosis is essential. Therapy consists of lowering the iron levels in the diet to below 150 ppm. In more severe cases periodic phlebotomy has been successful. A treatment regimen utilizing an iron chelating agent, deferoxamine mesylate in combination with phlebotomy will be discussed in detail by M. Loomis in another talk at this conference. Other recommendations for therapy and monitoring treatment include: aspiration of enough ascitic fluid to relieve dyspnea, serial whole body radiographs, assessment of body weight after each treatment, and monitoring of serum iron, TIBC, aspartate aminotransferase (AST), albumin, and packed cell volume (PCV).

8) Neoplasia

Hepatocellular carcinoma and lymphosarcoma have been diagnosed in other mynah species with hemochromatosis. A hepatoma and a pancreatic adenocarcinoma have been...
reported in other mynah species. Neoplastic conditions in Bali mynahs which have been reported to the SSP Veterinary Advisor are listed in Table 1.

9) Traumatic

Trauma from conspecifics or predators is a very common cause of injury and mortality in Bali mynahs.

10) Miscellaneous Problems

A significant percentage of the Bali mynah population exhibits chronic cervical feather picking behavior. This can lead to serious injury to the skin in this region and may extend to other areas of the body. The cause of this condition is currently unknown. Hyperkeratosis of skin on legs is a common finding in older captive Bali mynahs. The condition can become severe enough to entrap a leg band. This condition appears to be induced by captive confinement.

11) Preventive Medicine

Routine fecal examinations and deworming programs should be developed. All birds should be screened for toxoplasmosis and treated (experimental) if positive (Table 3 and 4). Complete postmortem examinations should be performed following the necropsy protocol (Table 5). Annual physical examinations and blood work (CBC and a chemistry panel with liver parameters being most important), and serum banking should be performed. A preshipment and quarantine workup should take place. Blood work, fecal examination and appropriate deworming, fecal aerobic bacterial culture including *Salmonella*, and toxoplasmosis diagnostics and treatment should be a part of the workup. The latex agglutination test and cloacal culture (collect over 3-5 days) should be utilized for chlamydial screening.

LITERATURE CITED


Table 1. Summary of Bali mynah necropsies submitted to the SSP.

A. Necropsies performed without SSP protocol (N=30)

<table>
<thead>
<tr>
<th>Final diagnosis</th>
<th>No. of cases</th>
<th>Age or age range</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Metabolic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Hemochromatosis</td>
<td>14</td>
<td>6 mo. to 17 yrs</td>
</tr>
<tr>
<td>b. Visceral (renal) gout</td>
<td>1</td>
<td>3 wks</td>
</tr>
<tr>
<td>II. Neoplastic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Ovarian adenocarcinoma</td>
<td>2</td>
<td>7 yrs</td>
</tr>
<tr>
<td>b. Ovarian tumor (unidentified)</td>
<td>1</td>
<td>7 yrs</td>
</tr>
<tr>
<td>c. Renal tubular adenoma</td>
<td>1</td>
<td>18 yrs</td>
</tr>
<tr>
<td>d. Intracoelomic lipoma</td>
<td>1</td>
<td>18 yrs</td>
</tr>
<tr>
<td>III. Parasitic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Cestode obstruction</td>
<td>1</td>
<td>adult</td>
</tr>
<tr>
<td>b. Atoxoplasmosis</td>
<td>1</td>
<td>adult</td>
</tr>
<tr>
<td>c. Hepatic amebiasis</td>
<td>1</td>
<td>8 yrs</td>
</tr>
<tr>
<td>IV. Bacterial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Acute hepatitis (E. coli)</td>
<td>2</td>
<td>3 d</td>
</tr>
<tr>
<td>b. Salmonella septicemia</td>
<td>3</td>
<td>3 wks</td>
</tr>
<tr>
<td>V. Viral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Pox virus</td>
<td>1</td>
<td>1.25 yrs</td>
</tr>
<tr>
<td>VI. Miscellaneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Chronic egg binding</td>
<td>1</td>
<td>adult</td>
</tr>
<tr>
<td>with peritonitis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. Postmortems utilizing the SSP protocol (N=18)

<table>
<thead>
<tr>
<th>Final diagnosis</th>
<th>No. of cases</th>
<th>Age or age range</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Metabolic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Hemochromatosis</td>
<td>5</td>
<td>6 mo. to 17 yrs</td>
</tr>
<tr>
<td>b. Hepatic lipidosis</td>
<td>1</td>
<td>2 yrs</td>
</tr>
<tr>
<td>II. Toxicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Renal gout-drug overdose</td>
<td>1</td>
<td>1 mo.</td>
</tr>
<tr>
<td>III. Parasitic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Atoxoplasmosis</td>
<td>8</td>
<td>18 d to 4 mo.</td>
</tr>
<tr>
<td>IV. Fungal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Aspergillosis</td>
<td>1</td>
<td>1 mo.</td>
</tr>
<tr>
<td>(also had atoxoplasmosis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. Bacterial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Septicemia</td>
<td>2</td>
<td>3 d</td>
</tr>
<tr>
<td>VI. Miscellaneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Granulomatous hepatitis</td>
<td>1</td>
<td>adult</td>
</tr>
<tr>
<td>b. Renal tubular necrosis</td>
<td>1</td>
<td>adult</td>
</tr>
</tbody>
</table>
Table 2. Iron content (dry matter basis) of typical foods used in diets for zoo birds.¹

<table>
<thead>
<tr>
<th>Food</th>
<th>Iron (mg/kg)</th>
<th>Food</th>
<th>Iron (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meat Products</strong></td>
<td></td>
<td><strong>Whole Fish</strong></td>
<td></td>
</tr>
<tr>
<td>Horsemeat</td>
<td>179.0</td>
<td>Capelin</td>
<td>143.7</td>
</tr>
<tr>
<td>Chicken flesh</td>
<td>48.1</td>
<td>Herring</td>
<td>44.5</td>
</tr>
<tr>
<td>Whole quail</td>
<td>114.6</td>
<td>Talapia</td>
<td>61.8</td>
</tr>
<tr>
<td>Whole chicken</td>
<td>146.6</td>
<td>Goldfish</td>
<td>54.5</td>
</tr>
<tr>
<td>Veal</td>
<td>73.7</td>
<td>Brook trout</td>
<td>78.3</td>
</tr>
<tr>
<td>Adult rat</td>
<td>171.2</td>
<td>Smelt</td>
<td>38.1</td>
</tr>
<tr>
<td>Adult mouse</td>
<td>239.0</td>
<td>Mackerel</td>
<td>90.0</td>
</tr>
<tr>
<td>Mouse pup</td>
<td>125.5-217.0</td>
<td>Sardines</td>
<td>60.0</td>
</tr>
<tr>
<td>Heart, beef</td>
<td>125.0</td>
<td><strong>Insects</strong></td>
<td></td>
</tr>
<tr>
<td>Heart, chicken</td>
<td>126.9</td>
<td>Crickets</td>
<td>32.0</td>
</tr>
<tr>
<td>Liver, beef</td>
<td>216.7</td>
<td>Crickets dusted</td>
<td>41.0</td>
</tr>
<tr>
<td>Liver, chicken</td>
<td>207.9</td>
<td>with limestone</td>
<td></td>
</tr>
<tr>
<td><strong>Eggs</strong></td>
<td></td>
<td><strong>Fruits</strong></td>
<td></td>
</tr>
<tr>
<td>Chicken, hard-cooked w/o shell</td>
<td>81.9</td>
<td>Apple</td>
<td>18.8</td>
</tr>
<tr>
<td><strong>Vegetables</strong></td>
<td></td>
<td>Banana</td>
<td>28.8</td>
</tr>
<tr>
<td>Beans, green</td>
<td>80.0</td>
<td>Cantelope</td>
<td>44.4</td>
</tr>
<tr>
<td>Broccoli</td>
<td>100.0</td>
<td>Figs</td>
<td>50.0</td>
</tr>
<tr>
<td>Carrots</td>
<td>58.3</td>
<td>Grapes</td>
<td>22.2</td>
</tr>
<tr>
<td>Corn</td>
<td>18.9</td>
<td>Mango</td>
<td>22.2</td>
</tr>
<tr>
<td>Kale</td>
<td>150.0</td>
<td>Papaya</td>
<td>27.3</td>
</tr>
<tr>
<td>Peas</td>
<td>86.4</td>
<td>Pear</td>
<td>17.6</td>
</tr>
<tr>
<td>Potatoes</td>
<td>30.0</td>
<td>Orange</td>
<td>28.6</td>
</tr>
<tr>
<td>Spinach</td>
<td>310.0</td>
<td>Raisins</td>
<td>42.6</td>
</tr>
<tr>
<td>Yam</td>
<td>23.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Seeds</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Millet</td>
<td>79.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut</td>
<td>23.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pumpkin</td>
<td>161.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sesame</td>
<td>153.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunflower</td>
<td>74.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Iron content of selected feeds (mg/kg on a dry matter basis). 4

<table>
<thead>
<tr>
<th>Feed</th>
<th>Iron Content (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey chow/biscuit</td>
<td>105-433</td>
</tr>
<tr>
<td>Dog food</td>
<td>390</td>
</tr>
<tr>
<td>Bird of Paradise</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Softbilled bird (Lo-Fe)</td>
<td>145</td>
</tr>
<tr>
<td>Bird of Prey</td>
<td>191</td>
</tr>
</tbody>
</table>

Table 4. Protocol for Evaluation of a Therapeutic Regimen to Combat Atoxoplasmosis in the Bali Mynah, *Leucopsar rothschildi*

1) Fecal Collection

Collect feces in 3% potassium dichromate for 3 days for the initial atoxoplasmosis evaluation and 1 to 10 days prior to starting treatment (if atoxoplasmosis has already been diagnosed). Place approximately 1 part feces to 10 parts potassium dichromate in the vial. The total amount of feces collected in the vial should be weighed in grams or milligrams so that quantification of the oocysts can be made pre- and post-treatment. After the 3 day collection period, the sample should be aerated once daily for 5 minutes for 4 days. A small aquarium pump can be utilized for sample aeration. After the 30 day treatment, feces should be collected at approximately 10 to 20 days post-treatment and then, if possible, every 4 months for 1 year following the above protocol. These samples should be sent to Dr. Greiner shortly after the 4 days of aeration. Atoxoplasmosis is shed intermittently and may be missed on fecal examination even if collected over several days. If the results of the first fecal collection are negative and the clinical history is suggestive of atoxoplasmosis (acute chick and fledgling mortality), then a second screening 1 month after the initial testing is appropriate if the clinical history is suggestive of atoxoplasmosis (acute chick and fledgling mortality). Please contact Dr. Greiner prior to sample collection so that he can be ready to process them.

2) Buffy coat smears

Two blood samples should be taken 3 to 5 days apart coinciding with the fecal collection for initial screening and pre- and post-treatment. The buffy coat from hematocrit tubes should be smeared onto several slides and fixed in absolute methanol. Buffy coat smears should be collected every 4 months in conjunction with the fecal sample collection. If the pre-treatment buffy coat samples are negative then taking the post-treatment buffy coat samples is not necessary. The above samples should be sent by regular mail to the following address:
Dr. Ellis Greiner  
Department of Infectious Diseases  
IFAS 633, College of Veterinary Medicine  
University of Florida  
Gainesville, Florida  32611  
Phone: 904-392-4700, ext. 5861 or 5867  
Fax: 904-392-8351

Please notify Dr. Greiner in advance so that he will be expecting samples in the mail.

3) Liver biopsy and impression smears

This is an optional diagnostic procedure and should only be performed by veterinarians experienced and comfortable with the technique. The liver biopsies should be performed 1 to 4 weeks prior to treatment, and if positive for atoxoplasmosis, then a second biopsy should be taken 1 to 2 months post-treatment. Two to 4 impression smears should be made and fixed. A small piece of liver tissue should be placed in 10% formalin.

These samples should be sent to the following address:

Dr. Terry M. Norton  
Riverbanks Zoological Park  
500 Wildlife Parkway  
Columbia, SC 29210

Steps 1 and 2 should be utilized prior to shipment of Bali mynahs to other facilities and, if positive, then the birds should be treated prior to shipment. Stressful situations such as transferring a bird to a new facility can cause atoxoplasmosis to become clinical in an asymptomatic carrier bird.

If all the samples are negative and the bird(s) have not had a history of producing chicks that die from atoxoplasmosis, then the treatment should not be instituted and the second set of samples do not need to be collected.

The most common clinical presentation for atoxoplasmosis is acute death in fledgling birds and the most accurate diagnostic test is to follow the Bali mynah necropsy protocol. The parents of these birds are carriers and should not be paired up with non-carrier birds until they have undergone the treatment regimen.

It is very difficult to rule out atoxoplasmosis even with all of the above diagnostic testing. Treatment may be considered on birds that are negative on the above diagnostic tests if the clinical history is suggestive of atoxoplasmosis.

Birds can be returned to a breeding situation if negative on the first post-treatment workup if fecal samples from the pair can be collected for post-treatment monitoring. It is recommended that breeding pairs of birds be placed in exhibits that can be easily cleaned.
or at least broken down on an annual basis. Larger exhibits which are less easily cleaned and less controlled should be reserved for nonbreeding birds. The species specificity of the Bali mynah Atoxoplasma is currently unknown. Atoxoplasma oocysts were transmitted from sparrow to sparrow, but not from sparrow to canary in one study. Atoxoplasma oocysts from canaries could not be transmitted to cockatiels. Tissues from evening grosbeaks naturally infected with atoxoplasmosis were transmitted by intra-coelomic injection to uninfected grackles, cowbirds, swamp and song sparrows, but not to ducks. Further research is needed in this area, but it seems reasonable that at least other sturnidae would potentially be susceptible to Bali mynah atoxoplasmosis.

The initial results of the treatment regimen have been promising but it is still too early to know whether the carrier state in treated adult birds is completely resolved.
Table 5. Therapeutic Trials for the treatment of Atoxoplamosis in the Bali mynah Utilizing a Combination of Pyrimethamine and Trimethoprim sulfadiazine.

1) The pre and post-treatment diagnostic protocol to evaluate efficacy of various therapeutic agents against Atoxoplasmosis should be followed.

2) Experimental treatment regimen (both drugs used concurrently)

   a) 0.75 mg/kg pyrimethamine (Daraprim, 25 mg tablets Burroughs Wellcome Co., Research Triangle Park, NC 27709) given orally in a preferred food item (mealworms and grapes have worked well) once daily for 30 days. It is recommended to mix the Daraprim in peanut oil (rather than water), so there is less chance of evaporation and to allow the drug to go into solution better.

   b) 60 mg/kg trimethoprim-sulfadiazine given orally in a preferred food item 2 times daily for 30 days.

   c) Refer to the following article for details on therapy.


   d) The Atoxoplasma oocysts can remain in the environment (especially soil) for years. It is very important to remove the old soil and plants, disinfect the exhibit, and replacing it with new substrate and plants (or develop a new enclosure with cement floors and drains) before putting the treated birds back in their exhibit.

   e) The Kansas City Zoo and Riverbanks Zoo were able to medicate their birds in meal worms and grapes. Preliminary results from Kansas City Zoo were promising for clearing Atoxoplasma from the adult mynahs. Riverbanks Zoo was unsuccessful in clearing their Bali mynahs and fairy blue birds with dosages used at KCZ, thus the dosage recommendations have been increased. It is recommended to add a vitamin supplement to the diet which contains folic acid during the treatment period, so that a deficiency does not occur in the treated birds.
Table 6. Bali Mynah (*Leucopsar rothschildi*) Necropy Protocol

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Kenneth S. Latimer, DVM, PhD
Veterinary Pathologist
University of Georgia

A thorough necropsy should be performed on bali mynahs which die at institutions housing this species. In addition to the institution's regular necropsy protocol, the following protocol should be performed.

1) Collect a small section of all major tissues (heart, lung airsacs (on a piece of paper towel), thymus, bursa, crop, proventriculus, multiple sections of intestine, pancreas, kidney, adrenal gland, gonad, oviduct, muscle, bone marrow, and brain) in 10% formalin. Three impression smears should be made of the liver and spleen and fixed. Very small chicks should be opened up and placed in 10% formalin. The University of Georgia, Department of Pathology will perform histopathology and cytology. Dr. Ellis Greiner, a parasitologist, will evaluate slides for atoxoplasmosis. Please send all samples to Dr. Norton at the above address. The results should be available in 2 to 4 weeks. Please contact Dr. Norton if you have not received results within this time frame.

2) Include a description of the gross findings, body weight, in grams at the time of death, important clinical history (including current diet).

3) Anaerobic, aerobic, and fungal cultures of various lesions should be taken when indicated. Please send a copy of the results to Dr. Norton.

4) Two sections of liver, spleen, kidney, and any tissue with obvious gross lesions should be frozen at -30 to -70 degrees C (if possible) for future virus isolation, mycobacterial, bacterial, or fungal culture.

5) The remaining liver (a minimum of 1 gram) should be placed in 10% buffered formalin. Tissue iron levels will be performed by Dr. Ellen Dierenfield from the New York Zoological Park. The sample should be sent to Dr. Norton. The results from this diagnostic test may not be available for several months.

The above protocol has been established in order to standardize necropsies performed on Bali mynahs. There is no charge for any of the above work. A yearly mortality report will be submitted to the Bali Mynah propagation group along with recommendations for improved captive management of the species.
SCINTIGRAPHY AS A TOOL IN AVIAN ORTHOPEDIC DIAGNOSIS

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The purpose of this study was 1) to compare the sensitivity of scintigraphy, or the bone scan, with survey radiography in the diagnosis of orthopedic pathology in birds and 2) to compare image quality following brachial vein and medial metatarsal vein injection for administration of the radioisotope. Survey radiography is the traditional diagnostic tool for evaluation of the avian skeleton. Due to the low cortical density of avian bones, survey radiography is often not sensitive enough to detect subtle lesions, especially in regions such as the spine and pelvis. Twelve birds (primarily raptors and waterfowl) exhibiting signs of decreased ability to fly or paresis of the thoracic or pelvic limbs were evaluated radiographically for evidence of orthopedic pathology. Scintigraphy was performed on each bird utilizing 5-10 millicuries of diphosphonate-labelled $^{99m}$technetium injected intravenously into either the medial metatarsal vein or the brachial vein of a clinically normal limb. Time to imaging was 3 to 4 hours post-injection and scintillation counts were collected for 60 seconds for each area of interest. A second scan was performed on each bird within 3 to 7 days of the first, utilizing the alternate vein. Results show that 100% of the lesions detected by survey radiography were also detected by scintigraphy. However, in several birds lesions detected by scintigraphy were not detected by survey radiography. These included lesions in the long bones, skull, spine and pelvic girdle and included both occult fractures and early osteomyelitis. Injection of the technetium into the brachial vein was more sensitive at detecting lesions and gave "cleaner" images than injection into the medial metatarsal vein. This was presumed to be due to loss of technetium through the avian renal portal system following medial metatarsal vein injection. Medial metatarsal vein injection resulted in high uptake of technetium in the liver and kidney, which particularly obscured evaluation of the thoracic vertebrae and synsacrum. Although scintigraphy is not practical as a routine diagnostic tool at many zoological institutions, its use should be considered in the non-flighted or paretic bird for which a diagnosis is not made by survey radiography.
AN OVERVIEW OF FELINE LENTIVIRUS INFECTION IN NONDOMESTIC CATS

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Feline immunodeficiency virus (FIV) is a lentivirus which causes immunosuppressive disease in domestic cats. Antibodies to FIV have been detected in many species of nondomestic cats.1-4 In addition, lentiviruses similar to FIV have been isolated from several FIV-antibody positive Florida panthers (Felis concolor coryi),4 an African lion (Panthera leo),5 and a Pallas' cat (F. manul).2 The pathogenic potential of lentivirus infections in nondomestic cats is unknown.

Nondomestic cats from 12 zoo populations and free-ranging lions from Kruger National Park in South Africa were tested by ELISA and immunoblots for antibodies reactive against FIV. Positive animals were identified in 3 zoos and in the free-ranging population of lions. Similar to findings in reports by other researchers,4 the 12 Kruger Park lions had a high prevalence of infection, with 8 (67%) positive tests and 2 (17%) equivocal tests. In the zoo populations, a total of 233 cats were tested, and 6 cats (2.6%) had antibodies to FIV. The positive cats included 4 African lions, 1 Pallas' cat (from which a lentivirus has been isolated), and 1 Siberian tiger (P. tigris altaica). In addition, sera from 2 snow leopards (P. uncia) had reactivity against a single protein (about 32 kilodaltons in size, similar to FIV p32 endonuclease) on immunoblots and faint reactivity on ELISA tests, and a puma (F. concolor) was positive by ELISA but negative by immunoblot. These results were interpreted as negative; however, minimal reactivity such as this could suggest the presence of a highly divergent feline lentivirus.

Repeated attempts in our laboratory to isolate a lentivirus from peripheral blood samples from FIV-antibody-positive nondomestic cats, including 3 African lions, a Siberian tiger, 2 snow leopards, and a jaguar (P. onca) have been unsuccessful; however, a lentivirus has been isolated from a young, male Pallas' cat.2 Antigenically, this virus (FIV-Pallas) is closely related to domestic cat FIV isolates, but it has unique biological and molecular characteristics. FIV-Pallas infects Crandell feline kidney cells (CrFK, a domestic cat cell line), resulting in formation of large syncytia and eventual lysis of the monolayer. In contrast, some domestic cat FIV isolates are unable to infect these cells, while other isolates establish chronic, nonlytic infections in CrFK cells. At the molecular level, FIV-Pallas cross-hybridizes poorly with FIV-Petaluma (the prototype domestic cat FIV isolate) on Southern or northern blots.2 A region of the polymerase gene has been cloned and sequenced. The nucleic acid identity between FIV-Petaluma and FIV-Pallas for this highly conserved region is approximately 77% (M. Barr, et al., in preparation).

LITERATURE CITED

of Feline Immunodeficiency Virus Researchers, p.38.


TOXOPLASMOSIS IN BLACK-FOOTED FERRETS (*Mustela nigripes*) AT THE LOUISVILLE ZOO

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An outbreak of toxoplasmosis occurred in a population of 22 adult and 31 kit black-footed ferrets maintained under quarantine conditions at the Louisville Zoological Garden (LZG).

Clinical signs included complete or partial anorexia (19 adults), lethargy (19 adults), corneal edema (4 adults), ataxia (3 adults), and death (2 adults, 6 kits). Later clinical problems included glaucoma (2 adults) and cataracts (3 adults). Ten ferrets that were tested had high antibody titers (≥1:2048) to *Toxoplasma gondii* both in the latex agglutination test and in the modified direct agglutination test. Eight fold rising titers were demonstrated.

One adult and 6 kits were necropsied. Predominant microscopic lesions were multifocal hepatitis, splenitis, pneumonitis, myocarditis, nephritis, and nonsuppurative encephalitis. *Toxoplasma gondii* organisms were found in histological sections stained with hematoxylin and eosin. The diagnosis was confirmed by immunohistochemical staining with anti-*Toxoplasma gondii* serum and ultrastructural examination. Although the source of *Toxoplasma gondii* for ferrets was not identified, frozen uncooked rabbit was the most likely source.

Treatment with trimethoprim-sulfadiazine (sulfamethoxazole and trimethoprim oral suspension USP, Biocraft Laboratories, Inc., Elmwood Park, NJ 07407) (30 mg/kg PO BID for 5 days) followed by sulfadimethoxine (Albon oral suspension 5%, Hoffmann-LaRoche, Nutley, NJ 07110) (30 mg/kg PO BID for 10 days) may have improved the ferrets' health.

ACKNOWLEDGEMENTS

The authors would like to thank Virginia Crossett, Guy Graves, and Joanne Luyster for their technical and husbandry expertise. We also thank Sam Vaughn, DVM for his help in necropsy and treatment.
CRYPTOCOCCOSIS IN TREE SHREWS (*Tupaia tana* and *Tupaia minor*) AND ELEPHANT SHREWS (*Macroselides proboscides*)

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Fungal infections due to *Cryptococcus neoformans* (*C. neoformans*) occurred in 5 large tree shrews (*Tupaia tana*), 3 lesser tree shrews (*Tupaia minor*), and 5 short-eared elephant shrews (*Macroselides proboscides*) during a 24 month period in 1991-1993 at the National Zoological Park (NZP). Cryptococcosis directly or indirectly contributed to the death of 11 animals. One large tree shrew died from cagemate induced trauma and had a localized lung infection which was considered an incidental finding. One lesser tree shrew administered antifungal therapy apparently recovered.

Two animals died without any prior clinical signs. In two others, debility due to cryptococcosis may have played a role in their deaths from intraspecific trauma. Clinical signs noted in the other shrews included dyspnea (*n=4*), neurologic disease (*n=3*), or both (*n=1*). Two elephant shrews and one lesser tree shrew exhibited clinical signs shortly following parturition.

Definitive antemortem diagnosis was a challenge due to the patients' size. Diagnostics included physical examination, hematology, radiographs, and a serum cryptococcal antigen assay. In a large tree shrew, tracheal washing (TW) through a sterile endotracheal tube identified the fungus. A TW, positive for *C. neoformans*, was obtained from a severely compromised elephant shrew which died shortly following the procedure.

Most cases were diagnosed on postmortem examination. The respiratory (*n=11*) and/or central nervous (*n=8*) systems were most commonly affected. In seven cases, more than one organ system was involved, whereas five cases were localized. Gross lesions usually occurred in the lungs and consisted of scattered to coalescing, gelatinous to firm nodules measuring up to 1 cm. Mediastinal and mandibular lymphadenopathy was also common. Histologically, lesions in all organs characteristically consisted of infiltrates of numerous pleomorphic yeasts accompanied by minimal to mild nonsuppurative inflammation. The fungi were surrounded by mucinous capsules and/or clear spaces, and often demonstrated narrow-based budding. In most cases, identification of the organism as *C. neoformans* was confirmed through cultures taken antemortem or at necropsy.

The lesser tree shrew, that apparently recovered from cryptococcosis following antifungal treatment, was housed with a sibling that died from the disease. This animal was presented in lateral recumbency unable to right itself. Its mentation was markedly depressed, and the severity of neurologic signs progressed very rapidly during the next 12 hours. Due to the animal's compromised state and a history of cryptococcosis exposure, antifungal therapy was initiated before diagnostic tests were performed. The shrew was solely treated with fluconazole (Diflucan, Roerig Division, Pfizer Incorporated, New York, NY) (25 mg/kg PO BID). Improvement was noted at five days, and no discernable neurologic disease was
detectable by 21 days. Serum collected 49 and 63 days post-therapy showed high cryptococcal antigen titers (>1:256), but the shrew remained free of apparent neurologic disease.

The source of infection for these animals is uncertain, but it is quite commonly found in soil and organic material. The tree shrews were wild caught or first generation offspring of wild caught parents, whereas the elephant shrews were captive born. All animals were housed at NZP's small mammal house or the zoological research facility. The elephant shrews were exhibited on a sand/gravel substrate with desert plants in the enclosure. Four of the five elephant shrews cohabitated with both a painted quail (Excalfactoria chinensis) and an African plated lizard (Gerrhosaurus validus), whereas the fifth animal was housed only with the lizard. The proliferation of C. neoformans in the arid environment was probably enhanced by the abundant creatine rich fecal material, which is required for the growth of this organism. The tree shrews were maintained in cages with tree limbs for climbing, wooden nest boxes, and a leaf/pine bark mulch substrate. Five of the tree shrews were housed with a crested wood partridge (Rollulus roulroul) for at least one month.

Attempts to reduce the incidence of cryptococcosis in the shrew populations have included replacing the mulch substrate from the zoological research facility's exhibits with sterilized wood shavings, removing the birds and lizard from the small mammal house enclosures, and high incidence cages have been completely broken down, disinfected, and replaced with new materials.

Although animals with cryptococcosis are incapable of direct transmission of the disease, the organism poses a potential threat to humans caring for these animals. Keepers cleaning exhibits are advised to wear appropriate face masks and minimize aerosolization of the organism by wetting the substrate prior to excavation.
LYMPHOTROPIC AND IMMUNOSUPPRESSIVE RETROVIRUSES OF NONHUMAN PRIMATES: A REVIEW AND UPDATE

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Introduction

Nonhuman primates, from tree shrews to gorillas, are hosts to a variety of pathogenic and nonpathogenic retroviruses. Of interest in recent years has been the recognition of simian retroviruses related to the lymphotropic and immunosuppressive viruses of humans. Concern has been expressed about the zoonotic potential of these agents and the possible threat to zoo collections of rare or endangered primates. This brief paper is designed to update the zoo community on recent findings concerning the lymphotropic and immunosuppressive retroviruses of nonhuman primates (GALV, STLV, SRV or Type D retrovirus, SIV) and HIV (see Table 1). (For more information see Lowenstine and Lerche 1988 and 1993, Letvin and King 1990).

Oncornaviruses: Type C

Gibbon Ape Leukemia virus (GALV)

This type C oncornavirus was first identified in Hylobates lar, the white-handed gibbon. Its presence in a collection is often associated with lymphoid and myelogenous malignancies. The lesions seen also included an osteo-proliferative lesion associated with areas of marrow infiltration. Seropositive/virus culture negative animals generally remain healthy, but little work has been done to determine by newer technologies (e.g. polymerase chain reaction) whether or not these animals are truly virus negative. Both H. lar and H. concolor have been identified as hosts. Siamang (H. (Symphalangus) syndactylus) and other gibbon species have been incompletely studied, although lymphomas have been identified in siamang of unknown virus status (Lowenstine, unpublished). Virus positive, antibody negative animals can act as carriers. The mode of transmission is unknown. No zoonotic potential is known and transmissibility to other species of primates has not been studied. Testing for GALV is not readily available, but should be considered if new exhibits or breeding groups are being formed.

Simian T cell lymphotropic viruses (STLV-1)

The first isolation of a member of this group was from Japanese macaques (M. fuscata) from an area endemic for human T-cell leukemia virus (HTLV-1). Subsequent studies showed the human and macaque agents to be antigenically and genetically related but distinct. Genetic analysis revealed that the human and simian agents diverged many years ago. Additional studies have demonstrated the agent to be present in many Old World monkeys and apes including guenons, patas, talapoins, macaques, mangabeys, baboons, siamang, chimpanzees, gorillas (Table 2). New World species have not been adequately investigated.
Evidence for clinical disease associated with these viruses is limited. Persistent lymphocytosis (5.6-9.4 x 10^3/μm^3) and abnormal circulating T cells, including large lymphoblasts and small or medium-sized lymphocytes with convoluted nuclei, have been reported in African green monkeys (Cercopithecus aethiops). It is uncertain if this is a preleukemic state. Lymphocytic hepatitis and anemia have also been reported.

T-cell lymphomas and leukemias associated with STLV infection have been reported in several species of primates including one report in a gorilla (G. g. gorilla), two in African green monkeys (C. aethiops), one in a sooty mangabey (Cercocebus atys), one in an olive baboon (Papio anubis), and several in hamadryas baboons (P. hamadryas). One of the African green monkeys and the sooty mangabey were also infected with SIV, but the SIV status of the other animals was not given. Most of these animal have had generalized lymphadenopathy and splenomegaly, although the gorilla had a retropharyngeal mass and cervical node enlargement. A report of possible association of STLV with lymphomas in macaques has also been published but is controversial.

A chronic wasting syndrome was described in three captive lowland gorillas (Gorilla gorilla gorilla) seropositive for STLV. The animals had chronic diarrhea, sometimes associated with shigellosis or balantidiasis, and loss of 17-37% body weight over the 3 to 8 year course of illness. Recurrent anemia and lymphopenia, and severe hemosiderosis of liver and spleen at postmortem, were also common features. Lymphoid depletion was noted in two of the three. STLV was implicated, but not proven as a cause of this syndrome.

A strong presumptive diagnosis of STLV infection can be made with serology, but definitive diagnosis relies on isolation of virus. Screening is by ELISA using HTLV-1 as the antigen. Confirmation is by Western blotting. The mode of transmission of STLV is unknown, but sexual route and maternal transmission to offspring in milk are postulated. There is no evidence to support direct transmission of STLV to humans. However, macaques and African primates known to carry STLV may carry other zoonotic agents and precautions should be taken to minimize exposure.4,16,17 Like most retroviruses, STLV is inactivated by 70% ethanol, 10% household bleach, formalin, and most lipolytic detergents.

Oncornaviruses: Type D
Simian retrovirus type D (SRV or "Type D")

Simian exogenous type D retrovirus infection is found almost exclusively in macaques (genus Macaca) and is the etiology of most naturally occurring cases and epizootics of the simian acquired immunodeficiency syndrome (SAIDS). Antibodies directed against type D virus specific proteins have also been found in wild and long term captive talapoins (Miopithecus talapoin) and in African green monkeys, but type D virus has not yet been isolated and these two species may have non-specific binding reactions yielding false positive serologies. Limited transmission studies have shown African green monkeys and squirrel monkeys (Saimiri sciureus) to be resistant to intravenous inoculation.
Evidence for type D retrovirus infection has been found in zoologic collections of macaques, as well as in vivaria and in the wild. Species reported to be infected are listed in table 3. Seropositivity within any one population of macaques may be 0 to 70%, but in most population studies a seroprevalence of about 30% is common.

Disease caused by Type D infection may be sporadic, enzootic or epizootic. Sporadic disease occurs in individually housed chronic carrier animals. Devastating epizootics, with mortality as high as 85%, can occur in groups of naive animals into which virus positive animals have been introduced. Enzootic disease is seen in large breeding groups, often following an epizootic. Adults are apparently healthy but there is high infant and juvenile mortality of up to 40% of each birth cohort.

Infection with type D viruses can have a variety of clinical outcomes. There are no pathognomonic lesions of type D infection with the possible exception of noma (necrotizing stomatitis with osteomyelitis) and retroperitoneal fibromatosis. Acute deaths (less than 3 months after infections) occur in which anemia, neutropenia and profound immunosuppression with overwhelming opportunistic infections are the key lesions. In more chronic infections generalized lymphadenopathy and splenomegaly are common followed by a more insidious onset of immunosuppression or fibroproliferative disorders and death in 6 months to 2 years. Anemia may be the only sign in chronically infected cynomolgus monkeys (Lerche, unpublished). Transient disease may occur after which a strong antibody response apparently clears the virus. The animal is antibody positive, virus isolation negative. Persistence of latent infection may occur. A fourth outcome is the development of an inapparent carrier state. These animals are clinically healthy, antibody negative, and may shed the virus for years before eventually succumbing to amyloidosis, fibrosarcoma or immunosuppression and opportunistic infection.

Since not all populations of the same species of macaques are equally infected, and since inapparent healthy carriers occur, it is imperative that adequate screening of both resident and newly acquired animals be undertaken prior to formation of any new groups. Both serologic and virologic screening are necessary to detect healthy carriers as virus positive seronegative carriers occur. Seropositive animals may have recovered fully from infection, but latently infected (negative by virus isolation) seropositive M. fascicularis have later undergone recrudescent infection and spread the disease (Lerche, unpublished).

Type D virus infections can be eliminated from a group of macaques using a test and removal system. Virus or antibody positive animals must be removed. Testing and removal must be repeated monthly as new infections may show up as late as 6 months after initial testing. It is possible to derive virus free stock from an infected population. Vaccines have been shown to be effective in protecting against IV challenge and could be employed in situations where test and removal is not feasible.

Simian type D viruses do not have significant zoonotic potential. There have, however been reports in the literature of renal transplantation patients with antibodies against type D viruses. A recent report has documented the presence of an SRV-like virus in the bone marrow and lymphocytes of an AIDS patient with an unusual syncytial variant of B-cell
lymphoma. Although the risk of transmission of type D virus is slight, other primate born zoonoses dictate the use of adequate precautions (see Muchmore 1987 and Richardson 1987).

**Lentiviruses**

**Simian immunodeficiency viruses (SIV's)**

The simian immunodeficiency viruses, which are genetically related to the human AIDS viruses, are endemic in many populations of African Old World monkeys, especially the guenons (genus *Cercopithecus*) (Table 4). An isolate has also been made from a chimpanzee in Gabon, but natural infection does not seem to be widespread in chimps. African monkeys in the wild, in zoos and vivaria have been shown to be antibody and/or virus positive (Table 4). Antibody or virus positive captive, but not free-ranging, macaques have been identified in several primate research centers. SIV isolations have been made from macaques which are thought to have acquired the infection in captivity.

The diagnosis of SIV infection relies on accurate serology. Immunoblotting or immune precipitation with electrophoresis are more accurate than ELISA, although the later, using HIV-2 as the antigen, is useful for screening large collections. HIV-2 ELISA, however, may not detect animals infected with the more distantly related strains such as SIV<sub>agm</sub>, SIV<sub>mod</sub>, SIV<sub>syk</sub>, and SIV<sub>cmz</sub>. Newly acquired animals should be individually housed during quarantine. Because infection may precede seroconversion by several weeks, sera should be tested repeatedly (at least at acquisition and again in 3 months). Recent data suggests that even this may not be sufficient and virus isolation or sophisticated gene amplification techniques may be needed to certify an animal free from SIV.

The entire host range and pathogenicity of these viruses has not yet been elucidated. Generally the infection is endemic, life-long and clinically inapparent in most African primates. The mode of spread is uncertain but sexual or mother/infant transmission is postulated. Evidence for pathogenicity in African species is limited to reports of lesions or opportunistic infections similar to those found in experimentally infected macaques. In a breeding colony of sooty mangabeys the following cases were seen: disseminated cytomegalovirus infections in two infants; noma in one juvenile; disseminated amoebiasis in an 11 year old (yo) female, lymphocytosis and giant cell pneumonia in a 23 yo, disseminated giant cell disease and lymphoma in a 10 yo female, and T cell leukemia in a 23 yo female. All the adults were dually infected with SIV and STLV. Zoo-housed older adult mandrills that were SIV positive have died with disseminated amoebiasis or atypical mycobacteriosis (Drs William Torgerson and Kent Osborn, personal communication). One African green monkey dually infected with SIV and STLV-1 developed T-cell lymphoma. More long-term studies of African nonhuman primates infected with SIV alone or in combination with STLV are needed to establish the spectrum of lesions associated with these viruses. It seems however, from the limited data, that African primates may live their entire life span without adverse effects from these infections.
In contrast, these viruses cause devastating AIDS-like disease when introduced into macaques. Naturally occurring cases in macaques are thought to have resulted from inadvertent inoculation of blood or tissue products or direct interspecific aggression from African monkeys, most likely sooty mangabeys, or other infected macaques. Once infected animals occur in a group, the infection may spread horizontally in macaques causing epizootics similar to those seen with SRV infection. Transmission across genital mucosa has been demonstrated experimentally, and asexual transmission, probably by biting or being bitten, can occur. Transmission between African and Asian primates apparently occurred in vivaria settings in the 1960's leading to a devastating epizootic in stump-tailed macaques. This is another reason not to house African and Asian Old World primates in direct contact with one another (in addition to simian hemorrhagic fever and the varicella-like herpesviruses).

Experimental inoculation of rhesus and retrospective examination of clinical and pathologic findings in "naturally infected" macaques have allowed the identification of conditions or lesions associated with SIV infections. Most infected macaques succumb within 2 years and may die as acutely as 6 weeks post infection. Occasional chronically infected antibody positive latently infected macaques have been identified which developed recrudescent infection and died 7 or more years after initial infection. Such animals can act as reservoirs to spread the infection into naive populations.

Primary SIV induced lesions include: non-suppurative, histiocytic meningoencephalitis with syncytial giant cell formation; giant cell interstitial pneumonia; and disseminated giant cell disease involving the lamina propria of the gastrointestinal track, lymph nodes, and other viscera. Immune stimulation by persistent SIV antigens results in lymphoproliferative disease with lymphadenopathy, splenomegaly, and formation of lymphoid nodules in many organs and tissues. Extranodal B cell lymphomas, or occasionally T cell lymphomas may ensue (possibly associated with Epstein-Barr-like herpesviruses or STLV respectively).

The zoonotic potential of SIV is low, but two laboratory workers have developed antibodies to SIV. One received a needle stick while bleeding an SIV infected monkey; the other had severe dermatitis, was taking corticosteroids and was not wearing gloves while handling infected blood in the laboratory. Neither has shown any evidence of clinical disease. Appropriate precautions for handling nonhuman primates and their blood and tissues should be taken to prevent inadvertent infection.

Human immunodeficiency viruses (HIV's)

The two strains of human immunodeficiency viruses, HIV-1 and HIV-2, have been shown to be infectious for nonhuman primates. Both chimpanzees and gibbons can be persistently infected with HIV-1, the cause of the worldwide pandemic of AIDS. After many years there is little evidence of disease in these animals. HIV-2, the "West African" AIDS virus which is more closely related to the SIV's, can infect several species of macaques and can cause clinical disease. It is unlikely that a nonhuman primate in a zoo setting would be infected with these viruses, but precautions should be taken.
The SIVcmz, isolated from a chimpanzee from Gabon, is more closely related to HIV-1, but infection is rare in chimpanzees.

Conclusion

Retroviral infections have been documented in zoo-housed nonhuman primates. Associated lesions, laboratory findings and clinical signs are given in Table 5. When these conditions are present in group-housed primates, a retroviral etiology should be investigated. Because many primates are potentially infected with retroviruses and other zoonotic agents, precautions given in national guidelines should be followed when handling these species.

SELECTED REFERENCES

TABLE 1. Pathogenic retroviruses of nonhuman primates

<table>
<thead>
<tr>
<th>Agent</th>
<th>Natural host</th>
<th>In wild?</th>
<th>In zoos?</th>
<th>Clinical disease?</th>
</tr>
</thead>
<tbody>
<tr>
<td>GALV</td>
<td>gibbons</td>
<td>Asia?</td>
<td>yes</td>
<td>yes neoplasia</td>
</tr>
<tr>
<td>STLV</td>
<td>many OW monkeys and apes</td>
<td>Asia</td>
<td>yes</td>
<td>yes lymphoma</td>
</tr>
<tr>
<td>SRV</td>
<td>macaques</td>
<td>Asia</td>
<td>yes</td>
<td>uncertain</td>
</tr>
<tr>
<td>SIV</td>
<td>quenons mangabeys baboons chimpanzees</td>
<td>Africa</td>
<td>yes</td>
<td>mild yes/severe in macaques</td>
</tr>
<tr>
<td>HIV</td>
<td>humans</td>
<td>Africa</td>
<td>no</td>
<td>yes minimal in chimps and gibbons</td>
</tr>
</tbody>
</table>

1993 PROCEEDINGS AMERICAN ASSOCIATION OF ZOO VETERINARIANS
TABLE 2. Species of nonhuman primates in which STLV has been detected.

<table>
<thead>
<tr>
<th>Species</th>
<th>STLV-positive Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaques</td>
<td></td>
</tr>
<tr>
<td><em>Macaca arctoides</em></td>
<td><em>M. nigra</em></td>
</tr>
<tr>
<td><em>M. brunnescens</em></td>
<td><em>M. nigrescens</em></td>
</tr>
<tr>
<td><em>M. cyclopis</em></td>
<td><em>M. ochreata</em></td>
</tr>
<tr>
<td><em>M. fascicularis</em></td>
<td><em>M. radiata</em></td>
</tr>
<tr>
<td><em>M. fuscata</em></td>
<td><em>M. sinica</em></td>
</tr>
<tr>
<td><em>M. heckii</em></td>
<td><em>M. silensus</em></td>
</tr>
<tr>
<td><em>M. maura</em></td>
<td><em>M. tonkeana</em></td>
</tr>
<tr>
<td><em>M. mulatta</em></td>
<td></td>
</tr>
<tr>
<td>Baboons</td>
<td></td>
</tr>
<tr>
<td><em>Papio anubis</em></td>
<td><em>P. (Mandrillus) sphinx</em></td>
</tr>
<tr>
<td><em>P. hamadryas</em></td>
<td></td>
</tr>
<tr>
<td>Guenons and their relatives</td>
<td></td>
</tr>
<tr>
<td><em>Cercopithecus aethiops</em></td>
<td><em>C. albogularis kolbii</em></td>
</tr>
<tr>
<td><em>C. cephys</em></td>
<td><em>C. mitis albogularis</em></td>
</tr>
<tr>
<td><em>C. mitis stuhlmanni</em></td>
<td><em>C. sabeus</em></td>
</tr>
<tr>
<td><em>Erythrocebus patas</em></td>
<td><em>Miopithecus talapoin</em></td>
</tr>
<tr>
<td>Mangabeys</td>
<td></td>
</tr>
<tr>
<td><em>Cercocebus (torquatus) atys</em></td>
<td></td>
</tr>
<tr>
<td>Apes</td>
<td></td>
</tr>
<tr>
<td><em>Gorilla gorilla gorilla</em></td>
<td><em>Pan paniscus</em></td>
</tr>
<tr>
<td><em>Pan troglodytes</em></td>
<td><em>Sympalangus syndactylus</em></td>
</tr>
</tbody>
</table>

TABLE 3. Species of macaques in which exogenous type D retroviruses (SRV’s) have been detected

<table>
<thead>
<tr>
<th>Species</th>
<th>SRV-positive Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus (<em>Macaca mulatta</em>)*</td>
<td></td>
</tr>
<tr>
<td>Cynomolgs or long-tailed macaques (<em>M. fascicularis</em>)*</td>
<td></td>
</tr>
<tr>
<td>Sulawesi black crested macaques (<em>M. nigra</em>)*</td>
<td></td>
</tr>
<tr>
<td>Tonkeana macaques (<em>M. tonkeana</em>)</td>
<td></td>
</tr>
<tr>
<td>Pig-tailed macaques (<em>M. nemestrina</em>)*</td>
<td></td>
</tr>
<tr>
<td>Taiwanese rock macaques (<em>M. cyclopis</em>)</td>
<td></td>
</tr>
<tr>
<td>Bonnet monkeys (<em>M. radiata</em>)*</td>
<td></td>
</tr>
<tr>
<td>Japanese macaques (<em>M. fuscata</em>)</td>
<td></td>
</tr>
<tr>
<td>Assamese macaque (<em>M. assamensis</em>)</td>
<td></td>
</tr>
<tr>
<td>Stump-tailed macaque (<em>M. arctoides</em>)</td>
<td></td>
</tr>
</tbody>
</table>

* Indicates that infection is common
<table>
<thead>
<tr>
<th>TABLE 4. Species in which SIV infection has been detected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Guenons and their allies</strong></td>
</tr>
<tr>
<td>African green monkey (Cercopithecus aethiops aethiops)*</td>
</tr>
<tr>
<td>Vervet (C. aethiops pygerythrus)*</td>
</tr>
<tr>
<td>Grivet (C. a. sabaeus)*</td>
</tr>
<tr>
<td>Tantalus monkey (C. a. tantalus)</td>
</tr>
<tr>
<td>Syke’s monkey (aka Kolb’s guenons) (C. mitis albogularis)*</td>
</tr>
<tr>
<td>Lowland Syke’s monkey (C. m. albotorquatus)*</td>
</tr>
<tr>
<td>Blue monkey (C. m. stuhlmani)</td>
</tr>
<tr>
<td>DeBrazza’s monkey (C. neglectus)</td>
</tr>
<tr>
<td>L’hoest’s guenon (C. l’hoestii)</td>
</tr>
<tr>
<td>Hamlyn’s guenon (C. hamlyni)</td>
</tr>
<tr>
<td>Talapoin (Miopithecus talapoin)</td>
</tr>
<tr>
<td><strong>Mangabeys</strong></td>
</tr>
<tr>
<td>Sooty mangabeys (Cercocebus atys)*</td>
</tr>
<tr>
<td><strong>Colobus monkeys</strong></td>
</tr>
<tr>
<td>Black and white colobus (C. guerza kukuyensis)</td>
</tr>
<tr>
<td><strong>Baboons</strong></td>
</tr>
<tr>
<td>Olive baboon (Papio anubis)</td>
</tr>
<tr>
<td>Mandrill (P. sphinx)*</td>
</tr>
<tr>
<td><strong>Apes</strong></td>
</tr>
<tr>
<td>Chimpanzee (Pan troglodytes)*</td>
</tr>
<tr>
<td><strong>Macaques</strong></td>
</tr>
<tr>
<td>Rhesus (M. mulatta)*</td>
</tr>
<tr>
<td>Stump-tailed (M. arctoides)*</td>
</tr>
<tr>
<td>Pig-tailed (M. nemestrina)*</td>
</tr>
<tr>
<td>Cynomolgus (M. fascicularis)*</td>
</tr>
</tbody>
</table>

* Published isolations: (SIVagm, SIVsyk, SIVsmm, SIVmnd, SIVcmz, SIVmac, SIVstm, SIVmne, SIVcyn).
** Infection acquired in captivity from African nonhuman primates - most likely sooty mangabeys
TABLE 5. Clinical conditions reported to be strongly associated with retrovirus infections

<table>
<thead>
<tr>
<th>Lesions, lab findings, signs</th>
<th>Agent(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>GALVSIV, STLV, SRV</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>SRV</td>
</tr>
<tr>
<td>Leukocytosis</td>
<td>GALV, STLV</td>
</tr>
<tr>
<td>Abnormal mononuclear cells</td>
<td>GALV, STLV, SRV</td>
</tr>
<tr>
<td>Lymphoproliferative disease</td>
<td>SRV, SIV, STLV?</td>
</tr>
<tr>
<td>Lymphosarcoma/ leukemia</td>
<td>GALV, STLV, SIV</td>
</tr>
<tr>
<td>Myelogenous leukemia</td>
<td>GALV</td>
</tr>
<tr>
<td>Subcutaneous fibrosarcoma</td>
<td>SSV*, SRV</td>
</tr>
<tr>
<td>Retroperitoneal fibromatosis</td>
<td>SRV</td>
</tr>
<tr>
<td>Chronic diarrhea and wasting</td>
<td>SRV, SIV, STLV?</td>
</tr>
<tr>
<td>Syncytial giant cells</td>
<td>SIV</td>
</tr>
<tr>
<td>Arteriopathy, hypertrophic</td>
<td>SIV</td>
</tr>
<tr>
<td>Aseptic mural endocarditis</td>
<td>SIV</td>
</tr>
<tr>
<td>Lymphocytic periportal hepatitis</td>
<td>SIV, STLV</td>
</tr>
<tr>
<td>Gingivitis (ANUG)</td>
<td>SRV, SIV</td>
</tr>
<tr>
<td>Noma</td>
<td>SRV, SIV?</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>SRV</td>
</tr>
<tr>
<td>Opportunistic infections</td>
<td>SRV, SIV</td>
</tr>
<tr>
<td>Atypical mycobacteriosis</td>
<td>SIV</td>
</tr>
<tr>
<td><em>Rhodococcus enteritis</em></td>
<td>SRV</td>
</tr>
<tr>
<td>Cytomegalovirus infection</td>
<td>SRV, SIV</td>
</tr>
<tr>
<td>SV40/ PML</td>
<td>SIV</td>
</tr>
<tr>
<td><em>Pneumocystis carinii</em></td>
<td>SIV</td>
</tr>
<tr>
<td>Oroesophageal candidiasis</td>
<td>SRV, SIV</td>
</tr>
<tr>
<td>Cryptosporidiosis</td>
<td>SRV, SIV</td>
</tr>
<tr>
<td>Balantidiasis</td>
<td>SRV, SIV, STLV</td>
</tr>
<tr>
<td>Amoebiasis</td>
<td>SIV?</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>SRV, SIV</td>
</tr>
</tbody>
</table>

*SSV is simian sarcoma virus, a one time isolate from a woolly monkey that appears to have been a variant of GALV acquired in captivity.
A ten year retrospective study was performed at the International Wildlife Conservation Park/Bronx Zoo (IWCP) to evaluate the prevalence and assess the implications of shigellosis in captive lowland gorillas (*Gorilla gorilla*). Between 1982 and 1992, the IWCP maintained twenty-six lowland gorillas. During that time period, nine animals had confirmed *Shigella flexneri* isolates from fecal or rectal culture. Two additional animals were exposed to *S. flexneri* infected gorillas developed diarrhea but the organism was not isolated by bacterial culture. The most common clinical signs included mild to severe diarrhea, lethargy and anorexia. Most cases (91%), were in infants between seven and forty months of age. The onset of illness was associated with introduction of the gorilla to other gorillas in the main colony. Treatment protocols varied greatly depending on the severity of disease, response to therapy, antimicrobial sensitivity pattern, age and size of the gorilla. Although there were a number of individuals with severe clinical signs, none of the cases had a fatal outcome.

Beginning in 1990, gorilla shigella isolates became progressively resistant to multiple antibiotics including ampicillin, chloramphenicol, tetracycline and trimethoprim-sulfadiazine. All isolates were sensitive to fluoroquinolones and later generation cephalosporins. Multiple drug resistant *S. flexneri* was also isolated from gorillas at two other zoological institutions which had recently received gorillas from the IWCP.

Using DNA fingerprinting techniques, isolates collected from the other two institutions were compared with previously banked *S. flexneri* isolates from gorillas at the IWCP. Analysis of plasmid and chromosomal DNA indicated that one of the institutions had an identical *S. flexneri* isolate while the second institution had two different and distinct strains. These tests demonstrated that a multidrug resistant *Shigella* strain had been transmitted to another zoological park by an asymptomatic gorilla. The DNA analysis also helped identify three distinct multidrug resistant *S. flexneri* isolates from the gorilla samples tested.

The increased emphasis on relocating gorillas for captive breeding within zoological parks has created new challenges for the management of captive gorilla populations. Conservation institutions, in an attempt to improve gorilla reproductive success and expand genetic diversity, have become increasingly involved with multiple institution breeding programs. The role of infectious disease transmission will play an increasingly important part in the management of these animals.
REFERENCES


LISTERIOSIS CAUSING STILLBIRTHS AND NEONATAL SEPTICEMIA IN OUTDOOR HOUSED MACAQUES (Macaca spp.)

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Listeriosis has been reported to be endemic in outdoor housed macaques and is often manifested as stillbirths and neonatal deaths. In human as well as nonhuman primates, the organism has a predilection for the pregnant uterus causing abortion, stillbirth or neonatal infections with high morbidity and mortality. Sixteen cases of listeriosis occurred at the California Regional Primate Research Center (CRPRC) during the birthing seasons of 1989 and 1990. The cases were associated with stillbirths (n=14) and neonatal septicemia (n=2) without observation of clinical signs in the adult animals. All cases were identified from the outdoor breeding facilities. One early case occurred in a corn crib corral of Macaca radiata, and the remaining cases occurred in seven and the 11 half acre field cages of Macaca mulatta. Each year the cluster of cases occurred during the first nine weeks of the birth season, March through April.

The nature and distribution of gross and histopathologic lesions were characteristic of perinatal listeriosis in human and nonhuman primates. The major histological lesions were fibrinopurulent placentitis, pneumonia and meningitis. Culture swabs taken at necropsy from placenta and multiple fetal tissues yielded Listeria monocytogenes. Following the index case, cold enrichment culture techniques were routinely employed. Serotyping was performed at the National Animal Disease Center in Ames, Iowa, using five isolates from 1989 and two isolates form 1990. All were found to be serotype 1/2 A, factor A. The genomic DNA from these isolates had identical restriction enzyme patterns. Genomic DNA was confirmed as L. monocytogenes using slot blot hybridization with beta hemolysin probe. Southern blot hybridization patterns were also the same for all isolates.

The restriction enzyme analysis employed in this series of cases strongly suggests a point source of contamination. The reservoir could not be identified, although the possibility of carrier animals has not been ruled out. Several rectal swabs were randomly selected following the 1989 cases, and no Listeriase was cultured. In order to screen serum samples, microtiter agglutination was developed at the National Animal Disease Center. Results were confirmed by western blot analysis for the specific strain. Each female rhesus macaque with a known L. monocytogenes related stillbirth or neonatal death was matched for age and conception date with another female in the same field cage. All affected females had positive agglutination titers ≥ 1:80, and unaffected females were < 1:80. To date, western
blot analysis (applied to five affected females and the matched controls) confirms the results obtained by microagglutination. Therefore, it is suspected that only the affected females seroconverted while other females, just as likely to be exposed, did not seroconvert. Uneven distribution of the disease may be due to differences in susceptibility or the infecting agent may not have been uniform in the vehicle. Cold-enrichment culture of fetal tissues was continued through the 1991 birth season. No further cases have been identified to date.
The Toledo Zoo established a formal Animal Enrichment and Training Program in 1992. The Zoo recognized that the use of positive reinforcement techniques with animals in zoological facilities was a valuable management tool. Although animal training in its simplest form had been an integral part of the Zoo's humane captive animal management for over 20 years, its full potential had rarely been attained. The Zoo created the formal program to enhance and maintain the quality, consistency and effectiveness of this effort.

To assist in the implementation of this Program, the Zoo hired a consulting animal trainer (Active Environments), established an Animal Enrichment and Training Committee and created the position of Animal Training and Enrichment Coordinator. The Committee, which meets regularly, is comprised of the Zoo's Assistant Director, the Curator of Mammals, the Veterinarians, the above-mentioned Consultant and Coordinator. The Coordinator is an elephant keeper and elephant manager whose responsibilities include approximately eight hours a week of Program coordination. Keepers meet with the Coordinator prior to each meeting to give him their input. Further, the keepers are encouraged to personally present their views or concerns to the group at meeting times.

The following is a description of the Program functions and components.

The Animal Enrichment and Training Program is designed to integrate the use of positive reinforcement techniques into the implementation of current behavioral, husbandry, health care, investigative, and enrichment programs. Focus is placed on significantly reducing the stressful aspect of routine husbandry and management activities, as well as minor veterinary procedures, by conditioning the animals to voluntarily cooperate in such activities.

To best serve the needs of the staff and animal collection of the Toledo Zoo, the Animal Enrichment and Training Program is designed to meet five basic objectives:

- Enhance the psychological well being of primates and other animals and encourage more natural behaviors;
- On-going instruction of keepers and veterinary staff in the theory, terminology, and application of positive reinforcement operant conditioning techniques;
- Identification, selection, and prioritization of target behavioral projects;
- Assistance, supervision, and support of keepers and veterinary staff engaged in behavioral activities; and
- Assessment and evaluation of progress and results.

Staff Training

Formal and on-going training of keepers in the use of positive reinforcement techniques is critical to the success of the Animal Enrichment and Training Program. Properly trained keepers are more effective in implementing training projects, more efficient in utilizing limited time available for these activities, and attain greater reliability of results in a more timely manner. They also avoid a high degree of frustration and confusion, for themselves and the animals, through skillful application of these techniques. Finally, these enhanced skills allow keepers to conduct training sessions that are more enriching for both themselves and the animals.

A well-developed curriculum for staff training is implemented through recommended reading material, classroom instruction, video tapes and slides, and periodic workshops and skills enrichment activities. Discussion and feedback on behavioral projects provide on-going staff training.

Animal Behavior Activities

Potential behavioral projects are suggested by curators, keepers, veterinarians, or management personnel. The Animal Enrichment and Training Committee selects, prioritizes, and initiates the projects. Primary objectives of behavioral projects include training to:

- Increase reliability of voluntary movement of animals for maximum flexibility;
- Achieve voluntary cooperation in husbandry and veterinary procedures;
- Enhance introductions and integration of animals into social groups;
- Enhance socialization and desired reproduction;
- Combat neurotic and stereotypic behavior;
- Enhance the psychological well-being of primates and other animals;
- Encourage more natural behavior; and
- Support research activities and data collection.

A variety of behaviors have been and currently are being taught to our animals. Many of these were originally taught in the years before our formal Program. It was because of these successes and the advocacy of the keepers, curator and veterinary staff that funds were made available for the formal program.

In the early 1970's, the previous Zoo veterinarian, keepers, and our current mammal curator taught a male chimpanzee (Pan troglodytes) to masturbate into an artificial vagina and a female chimp to present for inseminations. Further, the females were taught to present their perineal regions for reproductive cycle inspection and their arms for hand injections of drugs.

Also in the 1980's, additional training was accomplished with the great apes. Female lowland gorillas (Gorilla gorilla) were taught by keepers to daily present their perineums for labia inspection and to urinate in holding cages for estrous cycle monitoring. Training of a female Bornean orangutan (Pongo pygmaeus) to allow manipulation and milking of her breasts and subsequent placement of her nipple into her infant's mouth resulted in the successful reintroduction of her infant after it had been handraised for three months. Several gorillas, orangutans, and chimps were trained to present for hand or pole syringe injections of immobilizing drugs.

Several species of primates, hoofstock, and carnivores have been trained to transfer through runs, come off exhibit, and enter squeeze cages and/or shipping crates. Colobus monkeys (Colobus guereza), African lions (Panthera leo), leopards (Panthera pardus), and cheetahs (Acinonyx jubatus) have been examined and injected in squeeze or transfer cages. California sea lions (Zalophus californianus) were trained to enter and exit a stock trailer for transfer between exhibits. North American otters (Lutra canadensis) and red pandas (Ailurus fulgens) have been trained to enter sky kennels for weekly weighing (pandas) and shipment (otters). Reticulated giraffes (Giraffa camelopardalis reticulata), nyalas (Tragelaphus angasi), greater kudu (Tragelaphus strepsiceros), impalas (Aepyceros melampus), zebra (Equus burchelli), white rhino (Ceratotherium simum), hippo (Hippopotamus amphibius) move on and off exhibit using visual and verbal cues. Suni antelope (Neotragus moschatus zuluensis) will move into a sky kennel for subsequent transfer to the veterinary hospital. An adult white rhino male was trained to stand for blood sampling from the ear veins.

One outcome of the formal Program has been the increased involvement of the veterinary staff in the training of medically related procedures. This has resulted in the desensitization of the animals to the vets, and has allowed better visual exams of non-sedated animals.

In addition to the previously mentioned behaviors, the following are currently being taught or will be taught by the keepers with assistance from the Coordinator, Consultant and Veterinarians. The keepers spend an average of 30 minutes per day with training. The veterinarians are involved for 1 to 2 hours per week. We are training:
1. Monkeys, bears and cats to enter and remain in squeeze or transfer cages for visual exams, injections and tail venipuncture (felines). Other possibilities include tuberculin testing of primates after presentation of their forearms.

2. Gorillas, chimpanzees and orangutans to:
   (a) Gate as needed on and off exhibit through doors, tunnels, and transfer chutes.
   (b) Separate into individual holding cages.
   (c) Allow individual feeding and training when two or more animals are together.
   (d) Present hands, feet, back and other body parts for exams; open mouth for tongue depressor insertion and teeth inspection; present the ear for tympanic membrane thermometer insertion; and the chest and back for a stethoscope.
   (e) Present and hold arms in position for injections.
   (f) Present fingers and toes for nail trimming.
   (g) Stand on a scale for weight measurements.
   (h) Insert and hold arm in a plastic pipe for venipuncture, injections, tuberculin testing and blood pressure determination.
   (i) Desensitization of the animals to exhibit construction noises and disruptions.

3. Giraffes to enter and stand in a chute/squeeze for physical exam, tuberculin testing, rectal palpation, venipuncture, and hoof trimming.

4. Sea lions to:
   (a) Exit and enter the water on command.
   (b) Come to the trainer on command anywhere in the exhibit area.
   (c) Station on a seat.
   (d) Pick up and present flippers for exam.
   (e) Lay down and roll over for full body examinations.
   (f) Allow blood sampling.
(g) Allow eye and wound treatments.

(h) Work animals in a squeeze.

(i) Condition them to accept an anesthesia mask.

5. Our ostrich (Struthio camelus) to move off and on exhibit on command and allow hooding of the head.

6. Our koalas (Phascolarctos cinereus) for tympanic membrane temperature monitoring.

Another important aspect of the Program has been to aid in the incorporation of behavioral training and enrichment structures in new exhibit designs, for example our Great Ape facility. The committee also has been the facilitator of caging changes to allow for safer keeper/animal interactions during veterinary procedures.

In conclusion, the Animal Enrichment and Training Program at The Toledo Zoo has been a valuable asset to the husbandry, medical care and welfare of our animal collection. Animal training has been a viable, positive and successful alternative to noxious stimuli or immobilizations for movement, introductions, and conditioning of animals to new situations. It has the potential for reducing stress and creating a strong trainer/animal bond while making procedures safer for all involved. It also serves as an excellent means of behavioral and environmental enrichment and can be very rewarding for both animal and trainer.

ACKNOWLEDGEMENTS

I thank all the Toledo Zoo keepers whose dedication to the animals under their care has made this Training Program a reality.
QUARANTINE PROCEDURES FOR FISH AQUARIA

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The term "quarantine" derives from the Italian quarantina, referring to what was the customary 40 day detention of ships or persons immigrating from ports suspected of harboring unwanted infectious disease.³ Forty days (and forty nights) is also the duration of a well-known ancient cruise used by modern zoos and aquaria as a metaphor for their raison d'etre—zoos as latter-day Noah's arks. It is therefore fitting that we address principles and processes of quarantine at this meeting, even though the subjects of this session would have had little use for a literal ark.

Quarantine for the aquarium can be narrowly construed as a set period of isolation and observation of fish prior to their introduction to resident stock or display. It may be more broadly construed as a golden opportunity to practice intensive preventative medicine in all pre-display phases, including collection, shipping, acclimatization, screening for disease, and specific or prophylactic treatment. In either case, quarantine is designed primarily to prevent introduction of disease into a water system from which eradication would be difficult if not impossible, as well as to promote the introduction of vibrant healthy fish for display. When used to its fullest potential quarantine also allows the acclimatization (both physical and behavioral) of newly arrived fish to the captive conditions in a controlled and observable setting, and collection of baseline data for future evaluation in health assessment. Once a fish enters a display, your chances of ever getting your hands on it to affect its health, for better or worse, are inversely proportional to the size and complexity of the display, but are rarely ever as good as during the quarantine period.

The basic principles of quarantine are the same whether you are dealing with aquatic or terrestrial systems. Incoming animals should be isolated from established animals for a given period of time. Thirty days is a recommended minimum for fish,¹ but, depending on the incubation period of the diseases of concern, may need to be longer, especially in cold-water fish, in which disease courses generally take longer to play out than in warm-water fish. Quarantine must be closed: addition of fish of any species to an ongoing fish quarantine requires resetting of the quarantine clock to day zero.¹

Isolation is the essential key to quarantine, and is more difficult to maintain than might be readily apparent. For aquatic systems the surest means of isolation is physical separation (rather than complex filtration and disinfection mechanisms between tanks), with independent tanks, filters, pumps and plumbing to waste.⁹ This is conceptually simple, but costly in capital outlay and space. Fomites and aerosols can easily breach even well-designed isolated quarantine systems. Aerosols caused by air stones and splashing water can conceivably transport water-borne pathogens to any other tank in the same air system.⁹ Aerosol transmissions can be minimized with tank covers and by maintaining quarantine and exhibit tanks in separate rooms.
Fomites capable of spreading water-borne diseases between tanks include nets, air stones, poles, and hands.\textsuperscript{5,9} Quarantine utensils should ideally be used only in quarantine. Utensils used in multiple locations must be chemically disinfected between use. Disinfectants have many drawbacks.\textsuperscript{9} There are no instantly effective broad-spectrum disinfectants. Utensils must be physically clean initially, and then be exposed to the disinfectant for 10 - 60 minutes to be effective. To avoid harming fish, they must be thoroughly rinsed. Effective net dipping thus requires multiple nets to be cycled through the disinfectant bath and rinse—the multiple utensils may as well be dedicated to certain systems.

Utensil disinfection is still a good idea, as added insurance, and to reinforce the idea of the importance of the quarantine process. Chlorine is recommended for saltwater systems, since chlorine can be partially neutralized in the rinse barrel with sodium thiosulfate, and small quantities revert readily to chloride ion.\textsuperscript{9} It is also relatively safe for human exposure, and is easily measured, but has the disadvantage of destroying net material. Potassium permanganate (100 ppm) is a safe and effective disinfectant for freshwater systems.\textsuperscript{5} The purple staining is generally considered a disadvantage of potassium permanganate, but it does allow visual assessment of adequate rinsing. Other disinfectants which may be used include quaternary ammonia compounds, formalin, and povidone-iodines, all of which require complete rinses prior to use.\textsuperscript{5} Air-drying kills many parasites, but not spores and cysts, and it is difficult to completely dry a net in an aquarium situation.\textsuperscript{5} If the same personnel service both display and quarantine areas (the most likely scenario), they should wash their hands thoroughly with a disinfectant soap when going between areas, and should save quarantine work for last if possible.

Quarantine tanks may or may not have established biological filters. If not, in closed systems ammonia and nitrite may build up to lethal levels without frequent water changes.\textsuperscript{5} Water quality parameters are more prone to rapid change in quarantine tanks than in a stable display tank, and should thus be monitored more frequently.

Quarantine tanks should be small for ease of cleaning and fish handling. Although quarantine tanks will usually be more spartan than display tanks, some effort should be made to provide fish with shelter (PVC pipe of appropriate diameter and length works well) to reduce stress and tank-mate trauma.\textsuperscript{2} Fish density should be kept as low as feasible to reduce stress. Temperature should be maintained at the upper end of the species optimum range to speed parasite life-cycles.

If you are lucky, your institution will send you to oversee and arrange specimen acquisition and shipping, so you will know their complete histories first hand. Chances are though, that your first encounter with the new fish will be upon their arrival at your facility. However, knowledge of shipping conditions and procedures is important to you whether you are shipping fish to your own institution, receiving fish into your quarantine facility, or sending them to another institution’s quarantine. Shipping is a stressful procedure. Subclinically diseased pre-shipment fish may well be clinically affected post-transport. If you have control over this aspect of specimen acquisition, these are some guidelines to follow:\textsuperscript{2}
Condition wild-caught fish to the aquarium environment and prepared food before shipping. Screen 2 - 5% of fish for disease by survival techniques (skin scraping, gill and fin biopsy, fecal examination) and treat as indicated. Do not ship fish in sub-optimal health. Minimize stress prior to shipping by avoiding unnecessary handling and overcrowding. Do not feed fish for 12 - 48 h (depending on size) pre-shipment to decrease ammonia production during shipment. Ship smaller fish in 3 - 6 mil plastic bags with a minimum of fresh clear water which matches their previous conditions, and fill with 100% O₂ (at least twice the volume of water)⁷. Ship bags in styrofoam boxes to moderate temperature changes (the darkness will also reduce stress). Large fish may require more elaborate shipping conditions with continuous filtration and aeration.⁸ Minimize transport time (definitely less than 24 h, but the shorter the better) by booking direct flights whenever possible. Do not group incompatible individuals or species in the same shipping container. Treatments of shipping water may or may not be indicated. Acriflavin has been traditionally used and abused in the tropical fish industry for shipping, but may cause more harm as a detriment to water quality and encouraging the selection of resistant bacteria than it does good by its antibacterial and antiprotozoal effects.⁹ The addition of zeolite to absorb ammonia may be helpful when shipping high densities of fish or ammonia-intolerant species. Light sedation (e.g. with 30 - 50 mg/L tricaine methanesulfonate) may likewise allow higher densities to be shipped, by reducing O₂ consumption and waste production, but it is not routinely recommended. Alternatively one can ship lower densities of fish. For shipping freshwater fish, the addition of salt (0.1 - 1% solution) can be beneficial.⁶ Stress-induced cortisol surges increase gill permeability to Na⁺ and Cl⁻, resulting in potentially life-threatening reductions in plasma osmolality. Adding salt reduces the osmotic gradient and allows the fish to maintain electrolyte homeostasis.

Once the fish have arrived begin your quarantine examination. Although hands-on examination for diagnostic procedures should wait for 4 - 7 days (unless fish are showing clinical signs of disease) this prudent delay does not preclude other aspects of examination such as observation, obtaining a complete history, and fecal examination, as well as acclimatizing the fish to the new conditions.¹⁰

A complete history is as essential for an aquatic quarantine examination as it is for terrestrial species. Were the fish wild caught or captive bred? If wild caught how were they collected? Were they shipped direct or via a wholesaler? How were they shipped and how long were they in transit? How many and what species were in the consignment? Were they treated with any therapeutic agents? Is the facility of origin known for any disease problems? What were the water quality parameters (temperature, pH, salinity, hardness, dissolved oxygen, ammonia, etc.) pre-shipment and in the shipping water? What was their diet? How do these conditions compare to their destination conditions? These questions are all interrelated and their answers will affect the quarantine procedures for each lot of incoming fish in a unique manner.

Acclimatization involves both physical and behavioral aspects, although we tend to focus on the physical aspects (temperature, pH, salinity, hardness, dissolved O₂, ammonia, nitrite, alkalinity, etc.).² There are rules of thumb on acclimatization to new water conditions (e.g. Δ pH < 0.5/h); in practice however the initial process involves floating the transport bag in
the quarantine water system (or adding the shipping water and fish to a fresh tank) and siphoning system water into the bag at a slow rate (via airstone tubing for instance) to effect a complete transition to the new water conditions in 1 - 3 hours. It is a good idea to test the transport water and compare to the quarantine system water parameters (which you should already know) to make any necessary adjustments to the procedure. For instance if the pH or temperature differences are great you may want to slow the water change; if the shipment water ammonia levels are very high and dissolved O₂ low and the fish are gilling alarmingly you may want to speed up the process and add an airstone to the bag. During the initial acclimatization is a good time to compare the invoice with what you actually received and obtain accurate counts and species identifications for accession purposes. For the next 24 hours the fish should be kept in a dimly lit area and left undisturbed.

During the 4 - 7 day hands off period the fish should be observed for signs of illness. Check for fraying of the fins, areas of discoloration or depigmentation, and grossly visible external parasites. Abnormal behaviors which could indicate a diseased fish include piping, coughing, labored respiration, lethargy, flashing, color changes, curling, fin-clamping, seclusion, prolonged anorexia, favoring one side, whirling and disequilibrium. A fecal specimen can often be obtained in the course of the observational examination and, depending on the volume of the sample, fecal direct, flotation, and sediment examinations can be performed.

After fish have had a chance to acclimatize to their quarantine surroundings, procedures requiring handling may commence. A guideline for conducting non-lethal diagnostic techniques is to randomly sample 2 - 5% of fish for ectoparasites by skin scrapings and gill biopsies. Large numbers of small fish may dictate a smaller percentage of fish sampled; on the other hand, it may be wise if feasible to sample 100% of small numbers of large valuable fish. This is a good time to permanently mark (e.g., fin clips, transponders) those fish which warrant individual identification. Baseline hematology, clinical chemistries, and microbiology could also be pursued at this time. Species-specific diseases should be screened for on a case by case basis (e.g., Hexamita in cichlid species). Terminal diagnostic techniques (necropsy, histopathology, squash preparations of organs, kidney cultures) may be useful in dealing with large numbers of fish for which individuals are perceived as less valuable than the school or the tank, but in some cases lethal diagnostic tests would be unacceptable. In the event of significant fish mortality, sufficient sick and dying fish should be necropsied and diagnostic tests pursued until a diagnosis is reached. Ideally fish should be examined (according to predetermined protocol for a given species and shipment) upon entry to and exit from quarantine, and should not be allowed out of quarantine unless all diagnostic tests pursued are normal (or unless those problems identified are already endemic in the exhibit tank).

Any problem identified should be treated specifically, and retested to verify efficacy of treatment prior to release from quarantine. In addition, wild caught fish may be assumed to be subclinical carriers of a wide variety of parasites, and prophylactic treatments are in order. Fish from facilities with known infectious disease problems also warrant prophylactic treatment. A series of 1 - 3 alternate day dips in freshwater for saltwater fish, or in 25 g/L saltwater for freshwater fish, lasting 3 - 5 minutes, or until signs of distress are evident, can
reduce external parasite burdens with minimal detrimental effects on fish.\textsuperscript{5,7,9} (Although note that \textit{Corydoras} catfish do not tolerate salt;\textsuperscript{5} if you are unsure how a certain species will react, treat only 1 or 2 individuals initially.)

Prophylactic copper treatments are also recommended for marine tropical fish of wild or unknown origin to reduce or eliminate external parasite burdens.\textsuperscript{9} After fish have had a chance to acclimate, copper levels are raised gradually to 0.15 ppm over the course of 3 days, maintained at that level for 21 days, and removed over the next 3 days or less. Copper treatment is not 100\% efficacious, and has the disadvantage of being immunosuppressive. It is therefore important to allow fish an acclimatization period both before and after copper treatment. Fish showing signs of bacterial or viral diseases should not be subjected to copper treatment, or should have copper treatment suspended as soon as such disease becomes evident.

If the quarantine and display waters are truly completely distinct and separate, the fish should be exposed to display water prior to their release from quarantine, in order to acclimate to any endemic diseases while they are still accessible for possible treatment.\textsuperscript{9}

From the foregoing, you may well imagine that the biggest obstacles to an effective quarantine are economic constraints. The strongest argument in favor of an effective quarantine are likewise economic, although ethical considerations would not be out of place. The one-time cost of treating a large display system coupled with revenue loss associated with display of unhealthy fish and bad publicity could make the initial investment in adequate quarantine facilities look very attractive; unfortunately this cost-benefit analysis is usually only performed retrospectively.

These guidelines should not be interpreted as requirements. An open system aquarium stocking its exhibits exclusively from its source waters may not need as strict a quarantine protocol as a closed system aquarium supplied by many sources. Group fecal examinations may be more appropriate than individual fecals for large schools of fish. Pre-shipment quarantine may be applicable for facilities with no means to quarantine on site. The person or persons responsible for maintaining fish health at an institution should have the leeway to adapt quarantine principles to each shipment on a case by case basis, keeping uppermost in mind their obligation to prevent disease introduction by acclimatizing the incoming fish, assessing their health, and treating any disease prior to releasing the fish from quarantine.

\textbf{LITERATURE CITED}
PREVENTATIVE MEDICINE FOR FRESHWATER AND MARINE AQUARIUM FISHES

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Introduction

When dealing with any captive animal, whether it be aquatic or terrestrial, preventative medicine is always the best medicine. Since the logistics of treating captive fishes can frequently be difficult, the concept of preventative medicine is especially important. Included in this broad category of aquarium fish preventative medicine is the crucial area of good water quality. When water quality parameters are adequate then many of the other factors relating to fish preventative medicine fall readily into place. There are currently several very good general texts that deal broadly with the topic of aquarium fish preventative medicine. The goal of this paper is to highlight and organize the various components of proper preventative aquarium fish medicine.

Species Compatibility

Once proper quarantine procedures have been instituted the next consideration when introducing new fishes into an exhibit or holding area is species compatibility. This is a very familiar concept to zoo veterinarians as it should be to aquarium veterinarians. As a rule the aquarist or curator should be familiar with the species in question and will usually know when to expect a compatibility problem. Unfortunately it is not always as simple as species x not liking species y. Many variables such as stocking density, availability of food, water quality parameters and the availability of security areas (cover) all come into play. Veterinarians that routinely deal with captive fishes are well advised to become familiar with the natural history of fishes in the collection they are associated with. Subscribing to one or more of the hobby publications or purchasing a general atlas of aquarium fish species is advisable, especially if this type of information is not generally available at the aquarium or zoo. Table 1 lists some of the major hobby magazines and book publishers.

Compatibility does not begin and end with nipped fins or total consumption of submissive individuals. In certain circumstances the dominant individuals will simply intimidate the submissive fishes, forcing them to hide and causing them to slowly starve to death since they may not be getting any of the available food. A simple lack of gross lesions does not necessarily mean that there is not a compatibility problem.

Water Quality and Environmental Control

Poor water quality or other environmental management problems are a sure way to precipitate disease problems among aquarium fishes. Understanding basic water quality parameters as well as aquarium system management is to the aquatic clinician’s advantage. Several references on this subject are readily accessible to veterinarians.
A number of important water parameters must be controlled and monitored on a regular basis. These include dissolved oxygen, ammonia, nitrite, nitrate, temperature and pH. Under normal circumstances public aquaria will have a laboratory and technicians that will handle monitoring and any necessary adjustments. Table 2 summarizes the important parameters and includes critical information about each one. Table 3 lists some manufacturers of water quality test kits. Fishes which have been quarantined and placed in holding should be slowly acclimated to the display aquarium water before being introduced into the new tank. A gradual mixing (over a period of hours or even days) is recommended for marine fishes while thirty to sixty minutes is usually adequate for freshwater species. These time periods assume that the holding facility and display facility environments are very similar. Acclimating fishes from cold to warm water and vice versa may take weeks to months.

Nutrition

An adequate nutritional plan is at the heart of any effective preventative medicine program. Spotte has produced a comprehensive chapter on the subject of fish nutrition. Most quantitative work done in this area centers on food fishes such as salmonids, catfish and eels. As the aquarium hobby becomes more sophisticated more scientific information on nutritional requirements of captive tropical fishes will become available.

The first point to consider is that if it is not being eaten it is of no value to the fish. Some species of fishes readily eat just about anything that is put into the aquarium. Other species (especially certain marine reef fishes) have very specialized diets that may be difficult or even impossible to duplicate in captivity. When possible, a varied diet is always recommended. Many of the community or schooling fishes are omnivores in the wild. Many of the commercially prepared flake and pelleted foods are well balanced and very palatable. Such foods should be tightly covered between uses and optimally stored in the refrigerator or freezer to help preserve unstable nutrients like vitamin C. Some piscivorous fishes will not consume prepared foods and must be fed live or thawed fish. Feeding live fishes has the obvious disadvantage of introducing potential pathogens into the aquarium. For this reason I recommend live foods as a last resort in a fishes nutritional program. Some fishes such as certain species of South American plecostomus seem to require driftwood in the aquarium upon which they graze and likely extract some nutrients. Other species enjoy fresh zucchini or even raw potatoes. Experienced aquarists are usually knowledgeable on the feeding habits and requirements of the fishes in their care. The prudent fish clinician will try to assimilate this information and record it for future reference.

A frequently asked question is how often should aquarium fishes be fed? Since thousands of species of fishes are kept in captivity this is a difficult question to answer simply. As a rule smaller fishes like freshwater tetras do better with frequent small volume feedings (three to ten times daily) while larger animals like freshwater catfishes or marine sharks may thrive on a single feeding per day or even one meal every other day. The natural history and husbandry requirements of any aquarium fish should be thoroughly studied before introducing the animal into the display aquarium. Planning before hand can save many headaches in the future.
Physical Examination and Disease Control

Once proper quarantine and introduction procedures have been followed and everything appears to be running smoothly, how can the clinician monitor the health of the fishes in the aquarium? Unlike many terrestrial animal species, fishes tend to have a very short period of morbidity before succumbing to a disease problem. Time is a critical component in maintaining a healthy aquarium. Any fish showing gross lesions or behaving abnormally should be immediately removed from the aquarium and isolated from the rest of the animals. Once this has been accomplished, the clinician can decide whether a biopsy or necropsy is in order. The goal in this situation is to head off problems in the aquarium even if it means sacrificing the sick individual to gain more knowledge about the problem at hand. Skin, fin, and gill biopsies can reveal a lot of valuable information in a short period of time for next to no expense. A thorough gross necropsy including tissue squash preparations and fecal and blood examination will commonly lead the clinician to an accurate diagnosis without having to wait for microbiological or histopathological results. Fish should be examined as soon after death as possible due to the fast rate of autolysis in these animals. Moribund fishes can be euthanatized with an overdose of tricaine methanesulfonate (MS-222) or a sharp blade may be used to sever the cervical spine.

Assuming that there are no disease or water quality problems in an aquarium, fish health can still be monitored in the form of fecal examinations, blood tests, and skin and gill biopsies. Such diagnostics are not practical on each fish in the exhibit but occasionally sampling fish from an aquarium is a good idea, especially if new animals have been added to the exhibit recently. If a parasitic problem is suspected, then certain parasiticides can be added to the diet and this medicated food fed to the fishes which are or are thought to be infected. Stoskopf provides a thorough formulary of compounds effective in treating fish diseases. A recipe for a gelatinized diet has been included in this paper since medications can easily be incorporated into this form of easy to produce food (Table 4).

Vaccination

This form of preventative medicine has very little application in today's aquarium industry. There are currently only three commercially available fish vaccines and these are used almost exclusively in the food fish industry. Vibriosis, furunculosis, and enteric-redmouth disease are the diseases vaccinated for, and all three vaccines are formalin-inactivated whole cell preparations. Methods of vaccine application include immersion, intraperitoneal injection, and oral administration. There are currently no properly controlled studies in the literature on the efficacy of vaccines for diseases of marine tropical fishes. Developing vaccines for use in aquarium fishes is currently an area of focus in several research laboratories, and we may see this field develop into an important part of fish preventative medicine in the near future.

Sanitation, Disinfection and Sterilization

Three very important aspects of good aquarium fish preventative medicine are proper sanitation, disinfection, and sterilization techniques when working around aquariums.
Sanitation- The use of measures designed to promote health and prevent disease; the development and establishment of conditions in the environment favorable to health.  
Disinfection- The destruction of pathogenic microorganisms or their toxins or vectors.  
Sterilization- The destruction of all microorganisms in or about an object.

Sanitary protocols are largely based on common sense. Aquariums should be maintained and kept as free from unnecessary debris as possible. Proper filter and filter media maintenance also falls into this category. Conscientious aquarists will normally take care of this vital component of preventative medicine.

The terms disinfection and sterilization are frequently used interchangeably despite subtle differences in their definitions. Instruments which may be transferred from tank to tank such as a net or feeding stick should be disinfected between uses. Most net dips or baths are designed to actually sterilize the net which is the safest way to insure that pathogens are not transferred from aquarium to aquarium. Quaternary ammonium compounds such as Nolvasan® or Roccal-D® are effective net dips when used at a concentration of between 10 and 20 ppm for at least 5 minutes. A 20 ppm chlorine solution works well as a disinfectant but it, like all dips, should be kept in a separate container safely away from the aquarium. Air drying of nets and other instruments will kill many but not all infectious pathogens. The use of formalin as a net dip should be discouraged because of its toxicity to humans. Solutions containing antibiotics are not recommended as net disinfectants since they may select for certain pathogens and leave others viable.

There are currently two methods commonly employed to help remove circulating pathogens in an aquarium system. These are ozonation and ultraviolet light sterilization. Both techniques act as contact sterilizers and by definition only affect the stream of water in direct contact with the device. Efficiency depends upon flow rates and proper maintenance of the equipment. Ozone generators inject ozone gas into a water contact chamber which should always be separate from the actual aquarium since ozone is toxic to fish and other vertebrate and invertebrate animals. Ultraviolet sterilizers usually consist of a polyvinyl chloride (pvc) tube which contains an ultraviolet light bulb. Numerous bulbs may be placed in series to increase the potency of the filter. According to Blasiola ultraviolet units should provide at least 35,000 microwatt seconds per square centimeter (u W/cm²) to effectively kill circulating microbes.

Some public and private aquariums circulate copper compounds in their water on a continual basis as a form of prophylaxis against ectoparasitic diseases. Spotte recommends against this practice since therapeutic levels are not well documented and the fate of copper in aquarium systems is unpredictable. Copper is toxic to all animals and levels that will kill protozoal and metazoan parasites will also adversely affect marine invertebrates. Copper can be especially dangerous in freshwater systems and its use should be discouraged. Proper quarantine protocols combined with a solid preventative medicine plan should preclude the need for the use of circulating toxins like copper.
LITERATURE CITED


Table 1: Aquarium Hobbyist Periodicals and Book Publishers

Aquarium Fish Magazine, Fancy Publications, Inc., 3 Burroughs, Irvine, California 92178
Freshwater and Marine Aquarium, R/C Modeler Corporation, 144 West Sierra Madre Boulevard, Sierra Madre, California 91024.
Tropical Fish Hobbyist, T.F.H. Publications, One TFH Plaza, Neptune City, New Jersey 07753.
T.F.H. Publications, One TFH Plaza, Neptune City, New Jersey 07753.
Tetra Press, 201 Tabor Road, Morris Plains, New Jersey 07950.

Table 2: Important Water Quality Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>Essential for life support. Most aquarium fishes require between 6 and 10 ppm. Temperature, pressure and salinity influence dissolved oxygen.</td>
</tr>
<tr>
<td>Temperature</td>
<td>Must be monitored accurately. Most tropical fishes require aquarium temperatures between 24° and 27° C (76° to 80° F). Temperate and cold water species have much different requirements.</td>
</tr>
<tr>
<td>pH</td>
<td>This represents the number of dissolved hydrogen ions in the water. Most marine aquaria should be between 8.0 and 8.3. Freshwater systems vary widely but a neutral pH of 7.0 is a good initial target when information on specific requirements is lacking. Closely related to toxicity of ammonia.</td>
</tr>
<tr>
<td>Ammonia</td>
<td>Single most important toxin to control. Detectable levels indicate a filtration or biological load imbalance. Most test kits measure the total ammonia concentration which includes both the toxic unionized form (NH₃) and the relatively non-toxic ionized form (NH₄⁺). As pH levels increase so do levels of toxic unionized ammonia.</td>
</tr>
<tr>
<td>Nitrite</td>
<td>Intermediate compound in the nitrogen cycle. Nitrifying bacteria in a healthy biological filter convert ammonia to nitrite and nitrite to nitrate. Elevated levels are more dangerous for freshwater species than marine forms. Can cause methemoglobinemia (brown blood disease). Presence of this compound indicates inadequate filtration or early stages of biological filter establishment.</td>
</tr>
</tbody>
</table>
Nitrate

Final compound in nitrogen cycle. Generally non-toxic but levels over 20 ppm indicate the need for water changes. Utilized by aquatic plants in aquarium systems.

Chlorine/Chloramine

Most municipal water supplies contain one of these compounds to help provide sanitary drinking water to the public. Both are toxic to fishes. Many commercially available water conditioners contain sodium thiosulfate which neutralizes chlorine. For bigger jobs 3 grams of sodium thiosulfate will remove 2.0 ppm chlorine from 250 gallons of water. When the chloramine bond is broken a small amount of ammonia will be released which should be taken care of by good biological filtration.

Salinity

Must be monitored in marine systems. A simple hydrometer is used to help maintain consistent levels. Most reef fishes require a specific gravity of between 1.021 and 1.023. Some marine species prefer lower salinities and brackish fishes even lower still. Salt can be used prophylactically in freshwater to help reduce stress, replace electrolytes and kill ectoparasites (some protozoans). Hydrometer levels of 1.0015 to 1.003 are safe for most freshwater aquarium fishes. Sea salt mixtures are recommended for this purpose.

Table 3: Water Test Kit Manufacturers

| Hach Company, P.O. Box 389, Loveland, Colorado | 80539 800-227-4224. |
| La Motte Chemicals, P.O. Box 329, Chestertown, Maryland | 21260 301-778-3100. |
| Orion Research, Inc., 840 Memorial Drive, Cambridge, Massachusetts | 02139 617-864-5400. |

Table 4: Gelatinized Food Recipe (Makes 750 to 1000 grams of food).*

| Ingredients: |
| 1. 250 grams of a well balanced flake or pelleted food. |
| 2. 1 can of sardines in oil. |
| 3. 1000 milliliters of clean fresh water. |
| 4. 30 grams of gelatin powder. |
| 5. 25 milliliters cod liver oil** |
| 6. 25 milliliters vegetable oil** |
| 7. 100 grams of fresh zucchini, spinach or vegetable baby food |
| 8. Multivitamin or any medication desired** |

| Directions: |
| Place fish food, sardines, oils, vegetable and medication(s) in blender with 500 milliliters of room temperature water. Blend until you have a smooth slurry (should be the consistency of a thick milk shake, add more water if necessary). In a separate sauce pan heat 500 milliliters of water to a boil and add the gelatin powder. Stir until gelatin is dissolved and allow this mixture to cool for about 10-15 minutes before adding the food slurry to the gelatin. Stir well and place in refrigerator to solidify. Mixture may be fed in pieces or frozen and sliced into fine pieces with a potato peeler or cheese grater. |

* Modified from a recipe by John B. Gratzek DVM, Ph.D., University of Georgia.
** Optional ingredients.
DIAGNOSTIC PROCEDURES IN PISCINE BACTERIOLOGY AT THE JOHN G. SHEDD AQUARIUM

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Introduction

A perusal of the literature of fish bacteriology from the past two decades reveals several features of interest to veterinary clinicians. Revisions in the systematics and nomenclature are constantly being made involving both reclassifications of previously described organisms and descriptions of newly discovered bacterial taxa. Members of the clinically important family Vibrionaceae and Aeromonadaceae, in particular, seem to demonstrate a high degree of phenotypic heterogeneity, i.e., variable reactions in the differential biochemical media. Indeed, some authors advocate a departure from the use of the differential media, dichotomous keys and diagnostic tables traditionally used to classify bacteria.1 Certainly, the use of chemotaxonomic methods such as determination of electrophoretic protein and fatty acid profiles, as well as the use of DNA hybridization probes, would greatly enhance both the speed and specificity of bacterial identification. Budgetary constraints, however, prohibit most institutions from investing in these kinds of technologies.

It cannot be overemphasized that many differences exist between mammalian and piscine diagnostic bacteriology. This is true regarding both the types of bacteria involved and the standard isolation and identification techniques. Subsequently, rapid I.D. systems such as the API 20E™ (BioMerieux Vitek, Inc., Hazelwood, MO 63042, USA), developed primarily for identification of the Enterobacteriaceae of medical importance to man, have somewhat limited applications in fish health. With this system, the recommended incubation temperature (37 C) and duration (24 and 48 hours) are inappropriate for many of the fish pathogens which tend to be mesophilic (25 - 40 C) to psychrophilic (15 - 20 C) and often require 48 to 72 hours (or more) to be isolated. The presence of plasmid DNA may result in positive sugar fermentation reactions on the API strip in organisms not normally associated with such ability.1 Nevertheless, certain gram-negative fermentative rods will key out very well on API. See Table 1 for fish pathogens currently listed in the API 20E database.1 In our identification scheme, API 20E is primarily utilized for comparative purposes.

Another feature of the fish bacteriology literature is an emphasis on the pathogens of cultured food fish. As a result, cold and temperate water species and their diseases are overrepresented. Moreover, successful isolation techniques often assume the economic practicality of sacrificing multiple specimens to find a causative agent. This latter approach is often both ethically and economically undesirable in zoo and public aquarium specimens.
The fish bacteriological literature tends to focus on a species-by-species approach to isolation and identification. This can become expensive in terms of media and technician time. Clinical situations, on the other hand, often warrant rapid and economical isolation and identification procedures as well as the generation of useful antibiotic sensitivity spectra.

Institutions with substantial fish collections typically utilize large-scale, flow-through filtration systems. To avert bacterial epizootics, these institutions have a real need to track incidence of infection, antibiotic susceptibility patterns and emerging antibiotic resistance. Therefore, identifying bacterial pathogens to species level and keeping accurate records of sensitivity trends becomes more important. In the interest of economy, institutions with smaller fish collections and more physically isolated tanks may prefer to identify pathogens to genus level only, but retain accurate antibiotic sensitivity records.

The goal of the fish health management program at the John G. Shedd Aquarium was to develop a scheme for rapid isolation and identification of bacterial pathogens that would not compromise accuracy. The scheme that has evolved is derived from the method developed by Shotts and Bullock.2 It entails initial inoculation on both selective and nonselective media. The number and kinds of differential media and reagents utilized to speciate primary isolates can then be dictated by an institution's diagnostic needs and economic constraints. The beauty of this system is that common pathogens can be identified quickly in-house. Identification of less frequently encountered pathogens can then be pursued with either the in-house lab or an outside reference lab.

Clinical signs of bacterial infections may include any one or a number of the following: lethargy; anorexia; emaciation; dyspnea; 'whirling'; hyperesthesia; pigment changes; exophthalmia; hyphema; keratopathies; oral mucosal hyperemia to hemorrhaging; erosions of the jaws, mouth, gill, fins, or tail; discoloration of gill tissue; gill nodules; ascites; hyperemic fins; hyperemia to hemorrhaging of the body surface, fin base, or vent; dermal ulcers; 'boils,' furuncles and hematomas. A list of bacterial fish pathogens and their associated diseases has been provided (Table 1). It is readily apparent from this list that the relative number of 'obligate' fish pathogens is low compared to the secondary or 'facultative' fish pathogens. In fact, many of the pseudomonads, aeromonads, and Vibrio/Listonella spp. are usually present in the aquatic environment and both on and in clinically normal fish. Therefore, fish often succumb to bacterial infections under stressful situations involving cortisol-mediated immunosuppression, e.g., parasitic infestations, poor nutrition, shipping, water quality deterioration and thermal extremes. Disruption of the protective body mucus layer and/or underlying epidermis from trauma (netting, handling, or tankmate aggression) or external parasitism can create a portal of entry for opportunistic bacteria as well.

**Sampling Techniques**

Due to rapid postmortem invasion of tissues by normal flora, ideally only clinically affected fish that have been euthanatized should be sampled for bacterial culture. For bacteremic conditions, the kidney, spleen, liver and heart blood are good tissues for obtaining cultures. If time or economy prohibit multiple organ sampling, a kidney swab often permits the most satisfactory isolation of pathogens. To obtain cultures from small fish (less than 30 gms),
the dorsal fin is clipped and the dorsal surface is disinfected by either searing with a heated spatula or wiping with a disinfectant such as 70% alcohol. A sterile scalpel blade is used to cut into the middle of the disinfected area until the spinal column is severed. By bending the head and tail together ventrally, the kidney is exposed, allowing a sample to be obtained with a sterile swab or inoculating loop.

For larger specimens, the body surface is sterilized by wiping with a disinfectant. The organs of the coelomic cavity then are exposed as in a necropsy. The kidneys are located retroperitoneally along the spinal column dorsal to the swim bladder. They are exposed by displacing the associated coelomic organs ventrally. The serosal surface and fascia covering the kidney then may be disinfected again. With a sterile scalpel blade, the tissue overlying the kidney is incised so that a culture may be obtained.

When obtaining cultures from skin or fins, the sample should be taken from the leading edge of the lesion if it is apparent. If an edge is not apparent, a swab of grossly normal skin or fin tissue should be inoculated on media for comparison. Normal fauna of the body surfaces can obscure the presence of a significant pathogen if a comparative culture is not performed.

It is best to confirm bacterial morphology and gram reaction from blood smears or tissue impression smears before culture is attempted. Often, however, limitations of specimen numbers, sample size and/or technician time makes multiple gram staining impractical. To ensure timely isolation, the authors often forgo gram staining and initiate bacterial isolation with the fortuitously available "fresh" specimen. This is especially appropriate if there is a reasonable index of suspicion of a bacterial etiology.

Isolation and Identification Procedures

For primary isolation of aerobes from freshwater fish, the following media plates are used: two tri-plates with tryptic soy agar (TSA), brain-heart infusion agar (BHIA) supplemented with 5% defibrinated sheep's blood (S.B.) (v/v) and Pseudocel™ agar (containing cetrimide for selective inhibition of nonpseudomonads); one Rimler-Shotts (R-S) agar plate (selective for aeromonads and some enterics). One tri-plate and the R-S agar plate are incubated at 35 C. The other tri-plate is incubated at 22 C. For marine fish, primary isolation employs the same media and incubation temperatures as above; however, 2% NaCl (w/v) is added to the TSA because of the halophilic nature of the common marine fish pathogens.

It is not uncommon for isolates to require 48 to 72 hours to grow. No plates should be recorded as negative until they have been incubated for seven days. As soon as growth is apparent, colony morphology and hemolysis pattern (if any) can be noted. Then a gram stain should be performed to determine size, shape, grouping and gram reaction. Gram-negative rods should be swabbed onto Mueller-Hinton (M-H) plates for antibiotic sensitivity testing according to the standard Kirby-Bauer method.
Gram-positive bacteria

The cocci can be separated on the basis of the catalase test into *Staphylococcus* spp. (+) and *Streptococcus* spp. (-). *Streptococcus* spp. are best isolated on BHIA + 5% S.B. Likewise, streptococcal antibiotic susceptibilities must be determined from a blood agar plate since these organisms will not grow on M-H. Many of the aerobic or facultative gram-positive rods are considered to be contaminants. One exception is *Lactobacillus piscicola*, the causative agent of 'pseudokidney' disease in salmonids in the U.K. and North America. This organism is a gram-positive, aerobic coccobacillus that readily grows on TSA or BHIA at 22 - 24 C. *Renibacterium salmoninarum*, the agent of bacterial kidney disease (BKD) in cultured and wild salmonids, is another gram-positive coccobacillus; however, it is a fastidious organism requiring cysteine-supplemented media, low incubation temperatures (15 - 18 C) and up to six weeks of incubation.1 Granulomatous renal lesions from salmonids in which groups of gram-positive coccobacilli can be demonstrated should be submitted to a reference lab for attempted isolation of the BKD agent. Serological assays for the BKD agent are also available.

*Mycobacterium* spp. will stain weakly gram-positive; but the presence of these organisms is usually implicated by the typical chronic granulomatous host-tissue response seen on wet mounts of organ squash preparations. The kidney, spleen and liver will often be affected; the granulomata may be grossly apparent. Confirmation is easily accomplished with an acid-fast stain. If acid-fast bacilli are found, a Lowenstein-Jensen (L-J) tube may be inoculated and incubated at room temperature. Growth may take from 2 to 28 days at 15 - 22 C. Colonial morphology is yellow and moist. Both *Cytophaga* species show gliding motility on wet mount preparations (Figure 1).

Short (1-3μm) gram-negative rods should initially be tested for motility ('hanging drop' or tubed media) and cytochrome oxidase (C.O.) reaction. Following the Shotts-Bullock method,2 rapid identification of the commonly encountered pseudomonads and aeromonads is possible (Figure 1). The genus *Pseudomonas* is selected by the Pseudocel™ agar, with yellow-green pigmentation produced by the fish pathogen *P. fluorescens*. The presence of brown pigment production on TSA is characteristic of 'typical' strains of *Aeromonas*.
*A. salmonicida* is nonmotile, C.O.-positive, and does not grow at 35°C. Confirmation with serological tests is possible. Two nonpigment-producing, nonmotile, C.O.-positive bacteria are *A. salmonicida* ('atypical' strains) and *Pasteurella piscicida*. This triad may be separated based on cell morphology, acid/gas production on O/F media, gelatinase production and growth on salt-supplemented TSA (Figure 1). Yellow colonies on R-S agar at 35°C after 24 hours of incubation that are C.O.-positive can presumptively be identified as members of the motile aeromonad complex (*A. hydrophila* et al). Use colonies from the TSA for the C.O. test since it can be inhibited by acid produced on R-S. The API 20E™ provides good confirmation of the motile aeromonad complex. It is noteworthy that the more virulent strains of motile aeromonads typically change from yellow to green colonies on R-S after 48 hours of incubation.

The motile C.O.-positive colonies from TSA, BHIA + 5% S.B., or TSA + 2% NaCl are most likely members of either *Vibrio* spp., *Listonella* spp., *Aeromonas* spp., or *Pseudomonas* spp. *Listonella anguillara* is nonhalophilic and will grow on unsupplemented TSA. The halophilic *Vibrio* spp. and *Listonella* spp. will demonstrate enhanced growth on TSA + 2% NaCl. These four genera can be separated based on novobiocin (10 μg) and/or O/129 (150 μg) sensitivity, O/F glucose reactions and gas production in O/F glucose. A good confirmatory test for *Vibrio* spp./*Listonella* spp. is growth in tryptic soy broth (TSB) supplemented with 6% NaCl (w/v). For further speciation of *Vibrio* spp./*Listonella* spp., refer to Figure 2. As previously discussed, motile aeromonads produce yellow colonies. *Proteus vulgaris* and some *Citrobacter* spp. are also yellow on R-S; however, these colonies usually have black centers indicative of H₂S production. *Aeromonas salmonicida* also produces yellow colonies, but growth is inhibited at 37°C. *Yersinia ruckeri* produces yellow colonies on R-S as well; this organism and the *Citrobacter* spp. can be differentiated from the motile aeromonad complex based upon lack of C.O. production (Figure 1). These latter two organisms are separated based on lysine decarboxylation, H₂S production and indole production.

In addition to being a selective medium for isolation of motile aeromonads, R-S agar will also support the growth of pseudomonads and some clinically significant members of the family Enterobacteriaceae. As previously discussed, motile aeromonads produce yellow colonies. *Proteus vulgaris* and some *Citrobacter* spp. are also yellow on R-S; however, these colonies usually have black centers indicative of H₂S production. *Aeromonas salmonicida* also produces yellow colonies, but growth is inhibited at 37°C. *Yersinia ruckeri* produces yellow colonies on R-S as well; this organism and the *Citrobacter* spp. can be differentiated from the motile aeromonad complex based upon lack of C.O. production (Figure 1). These latter two organisms are separated based on lysine decarboxylation, H₂S production and indole production.
*Pseudomonas*, *Escherichia* and *Enterobacter* produce green colonies on R-S; those of *Edwardsiella* are green with black centers (H₂S). The nonyellow colonies on R-S should be inoculated onto TSI slants and tested for C.O. production (use colonies from TSA or BHIA). They then can be grouped according to the flowchart and further delineated on the basis of motility, indole production, lysine decarboxylation, urease production and the Voges-Proskauer test (V-P)(Figure 1).

Although this key will enable identification of most of the common fish pathogens, there are a number of atypical variants and facultative pathogens that will not be identified. At this point, either submission to a reference lab, reliance on API 20E™ or use of additional differential media are possible options. At the Shedd Aquarium, additional tests performed include the following: catalase production; ornithine decarboxylase production; arginine dihydrolase production; gelatinase production (if not already tested); nitrate reduction; citrate utilization (Simmon's citrate); growth at 42°C, growth and lactose fermentation on MacConkey agar and motility at 37°C. The results from these tests are combined with results from TSI, glucose O/F, C.O. and urea agar. Differentiation of glucose-fermenters and glucose-nonfermenters (glucose-inert and oxidative) can be made from TSI and glucose O/F (open). The nonfermenters can then usually be identified. The fermenters are further tested as follows: esculin hydrolysis, phenylalanine deamination, methyl red/Voges-Proskauer, malonate utilization, and lysine decarboxylation (if not already done). Fermenters are simultaneously inoculated onto API 20E™ for comparison. The same identification scheme utilized for nonfermenters is then followed for fermenters.

**ACKNOWLEDGEMENTS**

The authors thank Jill Arnold, Dr. Jeff Teska, Dr. Larisa Ford and Dr. Rocco Cipriano for their assistance and advice. We also thank Blair Duff for assisting with the manuscript preparation.

**LITERATURE CITED**

3. Austin, B., and D.A. Austin. 1987. Bacterial Fish Pathogens: Disease in Farmed and Wild Fish. Ellis Horwood Ltd., Chichester, U.K.
8. Teska, J.D. Personal communication.
<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GRAM-NEGATIVE PATHOGENS</strong></td>
<td></td>
</tr>
<tr>
<td>Listonella anguillara$^{1,2,9}$</td>
<td>Listonellosis</td>
</tr>
<tr>
<td>Listonella ordalii$^{1,2,9}$</td>
<td>Listonellosis</td>
</tr>
<tr>
<td>Vibrio alginolyticus$^4$</td>
<td>Vibriosis</td>
</tr>
<tr>
<td>Listonella damsela$^{4,9}$</td>
<td>Listonellosis</td>
</tr>
<tr>
<td>Vibrio cholerae (non-01)</td>
<td>Vibriosis</td>
</tr>
<tr>
<td>Vibrio vulnificus (Biogroup 2)$^4$</td>
<td>Vibriosis</td>
</tr>
<tr>
<td>Aeromonas salmonicida$^{1,2,4}$</td>
<td>Furunculosis</td>
</tr>
<tr>
<td>Motile Aeromonad Complex$^{1,3,4,6}$</td>
<td>Motile Aeromonad Septicemia</td>
</tr>
<tr>
<td>Proteus shigelloides$^{4,10}$</td>
<td>Proteus Septicemia</td>
</tr>
<tr>
<td>Pasteurella piscicida$^{1,5}$</td>
<td>Pasturelllosis</td>
</tr>
<tr>
<td>Providencia rettgeri$^{3,4}$</td>
<td>Hemorrhagic Septicemia</td>
</tr>
<tr>
<td>Edwardsiella tarda$^{3,4}$</td>
<td>Edwardsielloasis</td>
</tr>
<tr>
<td>Edwardsiella ictaluri$^1$</td>
<td>Enteric Septicemia</td>
</tr>
<tr>
<td>Yersinia ruckeri$^{1,4}$</td>
<td>Enteric Redmouth Disease</td>
</tr>
<tr>
<td>Acinetobacter sp.$^4$</td>
<td>Acinetobacteriosis</td>
</tr>
<tr>
<td>Pseudomonas anguilliseptica</td>
<td>Pseudomonas Septicemia</td>
</tr>
<tr>
<td>Pseudomonas chlororaphis</td>
<td>Pseudomonas Septicemia</td>
</tr>
<tr>
<td>Pseudomonas fluorescens$^{3,4}$</td>
<td>Pseudomonas Septicemia</td>
</tr>
<tr>
<td>Cytophaga psychrophila$^1$</td>
<td>Bacterial Coldwater Disease</td>
</tr>
<tr>
<td>Cytophaga spp.$^1$</td>
<td>Fin Rot, Bacterial Gill Disease</td>
</tr>
<tr>
<td>Cytophaga columnaris$^{1,7}$</td>
<td>Columnaris Disease</td>
</tr>
<tr>
<td>Cytophaga marina$^8$</td>
<td>'Flexibacteriosis'</td>
</tr>
<tr>
<td>Sporocytophaga sp.</td>
<td>Salt Water Columnaris</td>
</tr>
<tr>
<td>Flavobacterium sp.$^4$</td>
<td>Bacterial Gill Disease, Mollie Granuloma</td>
</tr>
<tr>
<td><strong>GRAM-POSITIVE PATHOGENS</strong></td>
<td></td>
</tr>
<tr>
<td>Renibacterium salmoninarum$^{1,2}$</td>
<td>Bacterial Kidney Disease</td>
</tr>
<tr>
<td>Eubacterium tarantellus</td>
<td>Eubacterial Meningitis</td>
</tr>
<tr>
<td>Lactobacillus piscicola$^5$</td>
<td>Pseudokidney Disease</td>
</tr>
<tr>
<td>Staphylococcus epidermidis$^3$</td>
<td>Staphylococcosis</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>Strep. Septicemia</td>
</tr>
<tr>
<td>Clostridium botulinum</td>
<td>Botulism</td>
</tr>
<tr>
<td>Myxococcus piscicola</td>
<td>White Mouth</td>
</tr>
<tr>
<td><strong>ACID-FAST PATHOGENS</strong></td>
<td></td>
</tr>
<tr>
<td>Mycobacterium marinum$^3$</td>
<td>Mycobacteriosis</td>
</tr>
<tr>
<td>Mycobacterium fortuitum$^5$</td>
<td>Mycobacteriosis</td>
</tr>
<tr>
<td>Mycobacterium chelonel$^3$</td>
<td>Mycobacteriosis</td>
</tr>
<tr>
<td>Nocardia asteroides$^3$</td>
<td>Nocardiosis</td>
</tr>
<tr>
<td>Nocardia kampachi$^{1,2,5}$</td>
<td>Nocardiosis</td>
</tr>
</tbody>
</table>

$^1$Major fin fish pathogen  
$^2$Obligate fin fish pathogen  
$^3$Zoonotic potential  
$^4$Listed in API 20E database  
$^5$Currently not recognized in Bergey’s Manual$^{13}$  
$^6$Formerly Aeromonas hydrophila (et al)  
$^7$Formerly Flexibacter columnaris  
$^8$Formerly Flexibacter maritimus  
$^9$Formerly in the genus Vibrio  
$^{10}$Formerly in the genus Plesiomonas
FIGURE 1

Gram-Negative Bacteria

Short Rods 1-3 microns by 0.5-0.8 microns

Growth in 48 hours

22 C

PS

TSA

BHA +

5% S B

Try-plate

22 C +/- 35 C

Escherichia coli, e.g., Vibrio damsel, Streptococcus spp.

Pseudomonas fluorescens

Misc. Psychrophilic Pseudomonads

Acid, gas on O2; glucose; Pseudomonas salmonicida 'typical' (brown pigment on TSA)

Acid only on glucose

Pantoea piscicida (safety pin morphology)

Aeromonas salmonicida 'regicid' (erases C to C; enhances growth)

Nonmotile; O2 sensitive; CH4 gas

Flbro spp.

Aeromonas spp.

Pseudomonas spp.

Growth on O2 NO3, Other O- rods (refer to text)

Motile Aeromonad Complex

Cytoschrome oxidase

Citrobacter freundii Papers

18-24 hour

PS = Pseudoseq TM

BHA + 5% S B = Brain-Heart Infusion Agar with 5% Sheep's blood

TSA = Tryptic Soy Agar

F = Fermentative; O = Oxidative; NO = Nonreactive

1 slant / butt K = alkaline (red), A = acid (yellow), G = gas (bubbles)

*Motility Indole Lysine Decarboxylase

- - - - (validity)

Modified from Shotts and Bullock 1975
FIGURE 2

Vibrio Flowchart

Genus Vibrio

- lysine
  - + sucrose
    - + bile-esculin
      - V. vulnificus
    - + malonate
      - + ornithine
        - V. parahemolyticus
        - V. mimicus
    - + malonate
      - Tween 80
        - V. cholerae
        - V. metschnikovii
      - + ornithine
        - V. alginolyticus
        - V. logei
        - V. carchariae (sharks)
  - + mannose
    - + mannitol
      - V. ordalii
    - + ornithine
      - V. natriegens
      - V. diazotrophicus
      - + sucrose
        - V. alginolyticus
        - V. salmonicida

- Citrate
  - + ornithine
    - V. alginolyticus
    - Tween 80
      - V. anguillarum
    - + sucrose
      - V. anguillarum
      - V. damsela

- + malonate
  - + Tween 80
    - V. fluviialis
  - + ornithine
    - V. tubiashii
    - V. pelagius

V. cholerae  V. aestuarianus

* Growth at 42 C / Positive V-P
** No growth at 42 C / Negative V-P
TABLE 2. Selective Media Formulas & Sources

**Pseudocel™**: medium for the selective isolation of pseudomonads.

**Tryptone-Yeast-Gelatin (TYG)**: medium for the selective isolation of *C. columnaris*.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Gelatin</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>q.s. 1,000 mL water</td>
<td></td>
</tr>
<tr>
<td>Autoclave at 15 psi for 15 min</td>
<td></td>
</tr>
<tr>
<td>Cool to 45 C</td>
<td></td>
</tr>
<tr>
<td>Add filter decontaminated neomycin sulfate^2^ (4.0 μg/ml) then pour</td>
<td></td>
</tr>
</tbody>
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**Rimler-Shotts (R-S)**: medium for selective isolation of *A. hydrophila* et al.

<table>
<thead>
<tr>
<th>Ingredient</th>
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<tbody>
<tr>
<td>L-lysine-HCl^3^</td>
<td>5.0 g</td>
</tr>
<tr>
<td>L-ornithine-HCl^3^</td>
<td>6.5 g</td>
</tr>
<tr>
<td>Maltose</td>
<td>3.5 g</td>
</tr>
<tr>
<td>Sodium thiosulfate^3^</td>
<td>6.8 g</td>
</tr>
<tr>
<td>L-cysteine-HCl^3^</td>
<td>0.3 g</td>
</tr>
<tr>
<td>Ferric ammonium citrate^1^</td>
<td>0.8 g</td>
</tr>
<tr>
<td>Bromothymol blue^1^</td>
<td>0.03 g</td>
</tr>
<tr>
<td>Sodium deoxycholate^1^</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Novobiocin^3^</td>
<td>0.005 g</td>
</tr>
<tr>
<td>Yeast extract^1^</td>
<td>3.0 g</td>
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<tr>
<td>Sodium chloride^3^</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Agar^1^</td>
<td>13.5 g</td>
</tr>
<tr>
<td>q.s. 1,000 mL water</td>
<td></td>
</tr>
<tr>
<td>Adjust pH to 7.0</td>
<td></td>
</tr>
<tr>
<td>Heat to boiling for 1 min</td>
<td></td>
</tr>
<tr>
<td>Cool to 45 C then pour</td>
<td></td>
</tr>
</tbody>
</table>

^1 Baxter Diagnostics, McGraw Park, IL, 60085  
^2 Chemaqua, Oxnard, CA, 93033  
^3 Sigma Chemical Co., St. Louis, MO, 63178
TABLE 3. Media, Reagents, and Sources

| 1. Arginine decarboxylase broth (Remel) |
| 2. Bile esculin agar slants (Remel) |
| 3. Brain-heart infusion agar (powdered) (Baxter) |
| 4. Simmon's citrate agar slants (Remel) |
| 5. Oxidase Bactidrop DM (Cytochrome Oxidase Test) (Remel) |
| 6. Gelatin medium (12%) tubes (Remel) |
| 7. Indole broth tubes (Remel) |
| 8. Lowenstein-Jensen agar slants (Remel) |
| 9. Lysine decarboxylase broth (Remel) |
| 10. MacConkey's agar plates (Remel) |
| 11. Malonate broth (Ewing modification) (Remel) |
| 12. Phenol red broth with 1% mannitol (Remel) |
| 13. Phenol red broth with 1% mannose (Remel) |
| 14. Methyl red/Voges-Proskauer (MR/VP) broth-5 ml (Remel) |
| 15. Motility test medium with TTC (Remel) |
| 16. Mueller-Hinton agar plates (Remel) |
| 17. Nitrate broth with Durham tubes (Remel) |
| 18. Novobiocin sensitivity discs (10 ug) (Remel) |
| 19. O/129 sensitivity discs (10 ug & 150 ug) (Remel) |
| 20. O/F medium with 1% dextrose (Remel) |
| 21. Ornithine decarboxylase broth (Remel) |
| 22. Phenylalanine agar (Remel) |
| 23. Phenol red broth with 1% sucrose (Remel) |
| 24. Tryptic soy agar (Baxter) |
| 25. Tryptic soy broth (Baxter) |
| 26. Tween 80 hydrolysis substrate (Remel) |
| 27. Urea broth (Stuart's) (Remel) |

Baxter Diagnostics, McGraw Park, IL, 60085
Remel Microbiology Products, Lenexa, KS, 66215
For information regarding preparation, use, and interpretation of results from the media and reagents listed above, refer to references 14 and 15 in the 'literature cited' section.
THE DIAGNOSIS AND TREATMENT OF CORNEAL ULCERS IN FISH

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National Aquarium in Baltimore, 501 E. Pratt St., Baltimore, Maryland 21202, USA

Corneal disease is commonly observed in captive fishes. The majority of cases are the result of trauma due to aggression, parasitism, capture, or transportation. If left untreated, vision may be permanently impaired or lost as a frequent manifestation is corneal ulceration and subsequent rupture of the eye. While fish in general will survive this loss, the animal is often considered "exhibit dead" and thus removed from display.

The general anatomy of the eye is similar to that of other animals. The retina of salmonids for instance, consists of eight layers including: the capillary layer of the choroid, the pigment epithelium, a layer of rods and cones, an outer nuclear layer of rods and cones, an outer plexiform layer, an inner nuclear layer, an inner plexiform layer, a ganglion cell layer, and a nerve fiber layer. The lens of fish is spherical and unlike that of mammals lacks elasticity. Accommodation therefore, does not occur by altering the shape of the lens, but by drawing it towards the retina using the powerful retractor lensis muscle. The cornea consists of several distinct layers including an epithelium, a condensed superficial stroma resembling Bowman's membrane of primates, a dermally derived deep stroma, Descemet's membrane, and Descemet's endothelium (plate 1). In general, marine fish have a much thinner corneal epithelium than do freshwater fish.

The initial evaluation is made by simply observing the animal in its habitat. Fish with ocular disease are often dark in coloration and isolated from the main group. A strong, focal light beam is helpful in making the examination, although ambient light is often sufficient. Frequently, the affected eye will be turned away from the observer providing a challenge and requiring patience. Assessment of vision may be accomplished by determining the animals ability to avoid an object placed in front of the affected eye(s). The loss of corneal tissue in teleosts is usually easy to appreciate as compromise of the corneal epithelium often leads to stromal edema and an opaque eye.

Once the initial observations have been made anesthetics, such as Tricaine methanesulfonate (MS-222, TMS, Finquel), must be employed for a more complete examination and subsequent treatment. A dosage of 50-200 mg/L of water is appropriate for most teleost. Latex gloves should be worn to minimize disruption of the skin's protective layer of mucous and scales. Assessment of both eyes should occur as in other species with minor variations. Equipment used may include a penlight, ophthalmoscope, and slit-lamp, and ultrasonography. Special stains, such as fluorescein when used with a cobalt blue light, may help detect subtle corneal ulcers. Impression smears and corneal scrapings aid in the detection of fungi, bacteria, and parasites. Microbial cultures often reveal organisms commonly found in the environment, such as Aeromonas spp. in freshwater, and Vibrio spp. in saltwater. Pupillary light reflexes in most fish are very slow and difficult to discern.

Corneal ulcers in fish must be diagnosed and treated immediately to improve the likelihood of success. An apparently minor ulceration may progress to full thickness resulting in
rupture of the eye within 24-48 hours. Two methods of treatment utilizing n-butyl cyanocrylate (Nexaband Ophthalmic) and a higher homolog (Nexaband S/C) have been used at the National Aquarium in Baltimore dependant upon the severity and depth of the ulcer. These products serve to create a barrier to water, the latter for 5-10 days, protecting the damaged cornea from further irritation and infection during the healing process. In addition, a small amount of formalin is released from the glue as it cures, which provides a bacteriocidal effect.

Minor ulcerations are considered to be lesions involving less than 50% of the cornea. We have had tremendous success when treating these ulcers with the following procedure: The fish is anesthetized and held out of the water while the affected area is dried using sterile Q-tips. The eye is flooded with chloramphenicol ophthalmic solution and Q-tips are used to remove loose necrotic tissue (plate 1). Additional chloramphenicol is applied and allowed to stand for 30 seconds to 1 minute. The area is then dried using the Q-tips and cyanocrylate is applied using a 25 gauge needle (plate 2). It is important to place only a thin layer of glue over the lesion to maintain elasticity of the patch. In addition, large "tags" of cyanocrylate trailing from the eye creates drag as the fish swims, and may disrupt the repair. Once the glue has fully cured, the animal is returned to its tank for recovery. Antimicrobial treatment may be indicated if the fish is extremely stressed predisposing it to secondary disease.

Treatment of major ulcerations, those involving 50% or more of the cornea, have been less rewarding. The addition of a collagen corneal shield to the above procedure, however, has recently provided encouragement. This is accomplished by trimming a shield so that the periphery of the lens can be glued to healthy corneal tissue. Antimicrobial drugs may be necessary, especially if a uveitis is suspected.

Rapid treatment of corneal ulcers using cyanocrylate greatly increases the probability of success. This is most likely due to the control and prevention of secondary infection. Healing is usually complete within 2-4 weeks, leaving a slight opacity where corneal scarring has occurred. The collagen corneal shield has shown great promise in the treatment of serious corneal ulcers, although further work is necessary to fully explore its potential benefit.

ACKNOWLEDGEMENTS

Special thanks to Katherine Lattie, a second year Medical Illustration student at The Johns Hopkins University School of Medicine, who spent numerous hours observing our procedures and preparing the illustrations.

LITERATURE CITED

Examination of eye to determine extent of ulcer

Roll swab soaked with chloramphenicol ophthalmic solution to debride ulcer

A. Corneal epithelium
B. Superficial stroma
C. Deep stroma
D. Descemet's membrane
E. Descemet's endothelium

PLATE 1
Apply a thin layer of sterile cyanoacrylate to defect with a tuberculin syringe.

Place a few drops of chloramphenicol ophthalmic solution on eye.

After 30 seconds, use a clean swab to wipe dry.

2-3 weeks post-operative, eye may retain slight opacity.
USE OF DIAGNOSTIC CYTOLOGY IN MARINE MAMMAL MEDICINE

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Cytology can be a useful antemortem diagnostic tool in the evaluation of marine mammal patients. Cytologic examination of the blow, oral cavity, skin, rectocolon, vagina, stomach, and urine [which is more easily obtained from females] is easily performed in cetaceans. Cytologic techniques described for domestic mammals can be applied to pinnipeds, manatees, and otters.

Blow samples for cytologic evaluation can be obtained by direct application of a sterile swab into the blow or collecting material from the blow by voluntary exhalation onto a sterile petri dish. The material collected in the petri dish can be transferred to a microscope slide either by the use of a sterile cotton swab or by suspending the cellular material in sterile saline and using a cell concentration technique. In a study performed at Sea World of Florida, the cellularity of blow samples [N=25] collected into petri dishes and transferred to microscope slides either by cotton swab or suspension into saline were compared. The results of the study suggested that if particulate material [which provided the best sample for cytologic examination] was not obtained from a blow sample, then the cellularity was improved by suspending the sample in saline [0.5-1.0ml] and concentrating the cells as one would do with a wash sample. A simple cell concentration device using gravity and filter paper was used to concentrate the cells on a microscope slide. All cytology slides were stained with Wright's stain.

In a study at Sea World of Florida, blow samples [N=46] from healthy killer whales [Orcinus orca], false killer whales [Pseudorca crassidens], beluga whales [Delphinapterus leucas], and Atlantic bottlenose dolphins [Tursiops truncatus] were examined. The normal blow sample from cetaceans is poorly cellular and contains primarily squamous epithelial cells and a small amount of noncellular debris in the background. Extracellular bacteria occur in low numbers and are often associated with squamous epithelial cells and represented by a variety of morphologic types. Blow samples from cetaceans occasionally contain large filamentous bacteria that are usually associated with squamous epithelial cells. These bacteria appear to be part of the normal blow flora and are not associated with inflammation. These large bacteria could be members of the Simonsiellaceae family. Blow samples also revealed the presence of holotrichus ciliate protozoa [Kyaroikeus odontocetis, proposed name] from healthy killer whales [four of seven], false killer whales [three of three], and beluga whales [four of four]. These protozoa have also been found in blow samples from healthy Atlantic bottlenose dolphins. These rigid, cylindroid ciliates exhibit little movement on wet mounts. They average 25 um in width and 120 um in length. They stain deeply basophilic with Wright's stain; have linear rows of cilia; and have an eosinophilic triangular posterior podite. These protozoa appear to be nonpathogenic.

Abnormal blow cytologic findings include the presence of numerous inflammatory cells, large numbers of yeast, parasite ova, and bacterial phagocytosis by leukocytes. Triangular, operculated ova of Nasitrema, a trematode commonly found in the pterygoid sinus of cetaceans, may be seen in infected animals.
Rectocolon samples from cetaceans are collected using a tube [i.e. Levin tube, Davol Inc, Cranston, RI, which is an 18 Fr., 1.27m long tube marked at intervals to determine distance] inserted into the anus and passed into the rectocolon. The liquid fecal material will usually flow into the tube without aspiration. Sterile saline can be used as a wash sample if an adequate sample is not obtained in the initial passage of the tube, which may occur if the animal had defecated just prior to sampling. Excessive pressure should be avoided if aspiration is required to prevent injury to the mucosa. Rectocolon samples [N=20] stained with Wright's stain from healthy killer whales, false killer whales, beluga whales, and Atlantic bottlenose dolphins were examined. Normal samples are poorly cellular and contain primarily squamous epithelial cells. Rare leukocytes [usually nondegenerate neutrophils] are present [0-2 per high power field in undiluted samples. The background contains a variable number of bacteria represented by a variety of morphologic types and noncellular debris.

Abnormal rectocolon cytology included increased numbers of inflammatory cells; increased numbers of erythrocytes; bacterial phagocytosis by leukocytes; the presence of yeast, especially those showing hyphae formation; high numbers of bacteria predominated by one morphologic type; and the presence of numerous bacterial rods containing swollen ends suggestive of terminal spores.

Scrapings of the oral cavity from normal marine mammals reveal samples of variable cellularity [usually poor to moderate cellularity] that contain primarily squamous epithelial cells. Normal cytologic samples from the oral cavity will contain a variable amount of noncellular background debris and usually low numbers of bacteria represented by a variety of morphologic types. Occasionally, cetaceans will possess a white or yellow film in the oral cavity or along the margins of the mouth. A scraping of this material usually reveals dense sheets of cornified squamous epithelial cells and most likely represents retention of cells that normally slough. This condition appears benign and is typically self-limiting. Abnormal cytologic findings of the oral cavity include high numbers of leukocytes indicative of inflammation and numerous yeast as seen with candidiasis.

The normal cytology of skin scrapings is typically poorly cellular [depending upon the force of the scraping] and represented by squamous epithelial cells. Common abnormal cytologic findings include increased number of leukocytes indicative of inflammation. Numerous narrowly-based yeast are indicative of cutaneous candidiasis. Inflammatory lesions may reveal bacterial phagocytosis by leukocytes in cases of septic inflammation.

Collection of samples from the vagina can be accomplished by passing a sterile tube [i.e. Levine tube] into the cranial vault of the vagina. Normally, fluid for cytologic examination flows passively into the tube. If only a small quantity of fluid is obtained, it may be necessary to irrigate the vagina with small quantities of sterile saline [5ml] to allow aspiration of cellular material for examination. In a study conducted at Sea World of Florida, the course of the vaginal cytology of two adult killer whales was followed through their estrous cycle. The results suggested that the vaginal cellularity is not as distinctive as the estrous cycle of some domestic species of mammals, such as the dog. Female cetaceans in estrus produce a copious flow of thick mucus that contains relatively few cells. Following estrus, the volume and viscosity of the mucus decreases and the cytology shows a variable amount of cornified
squamous epithelial cells. Cornified squamous epithelial cells also appear in variable numbers in cytologic samples just prior to the stage of heavy mucus production [estrus]. A small number of leukocytes, primarily nondegenerate neutrophils, appear in samples taken several days [i.e. five to seven days] following the heavy mucus flow. This stage most likely represents a post-estrus stage [metestrus]. Epithelial cells at this stage are not as cornified and appear as oval to round nucleated epithelial cells. The vaginal cytology of juvenile cetaceans and those in anestrus shoe low cellularity that consists primarily of round to oval nucleated epithelial cells. The vaginal cytology changes in cetaceans is not as distinct as seen in the dog and may not be as helpful in determining the stage of estrus for breeding purposes. The stage of heavy mucus production usually corresponds with the behavior and hormonal signs of estrus. Abnormal vaginal cytology includes a marked increase in leukocytes [especially those showing degenerative changes] and erythrocytes. Such a cellular response would be indicative of vaginitis or metritis.

The cytology of the stomach can be performed on fluid samples collected from a tube passed into the stomach. Fluid volume is usually not a problem, however a small amount of saline can be used as a wash if needed. The cytology of the normal stomach [only the first compartment of the cetacean stomach can be sample directly] usually reveals a variable number of epithelial cells and a marked amount of noncellular background debris. The epithelial cells appear as cornified and noncornified squamous epithelial cells. Abnormal cytologic findings include large numbers of leukocytes and erythrocytes, suggestive of gastric ulcers.

Urine cytology is part of the routine urinalysis. The cytology of urine collected from marine mammals is interpreted in the same manner as that of domestic mammals. Urine can be collected by catheterization or as a free flow sample. Cetaceans can be trained to slide out of the water on their side and urinate on command so that a free flow urine sample can be collected. A microscopic examination of urine sediment [from five milliliters of urine] is a routine part of the urinalysis. Normal urine contains few leukocytes [0-3/hpf] and erythrocytes [0-3/hpf]. Epithelial cells appear in small numbers in normal urine. Casts are rare in normal urine and appear as hyaline or finely granular casts when present. Bacteria are rare in normal urine and when present, most likely represent contamination of the sample during catheterization or midstream collection. Normal urine contains a variable amount of crystals, usually represented by amorphous phosphate crystals.

In general, the cytologic interpretation of samples obtained from marine mammals is identical to that of domestic mammals. Inflammatory lesions are classified based upon the types of leukocytes present. Neutrophilic inflammation, where greater than 80% of the leukocytes are neutrophils is usually associated with severe irritation and although the etiology is usually nonspecific, certain etiologies, such as trauma and bacterial infections are common. Septic inflammation is identified by the presence of bacterial phagocytosis by leukocytes. Degenerate neutrophils are usually associated with a microenvironment that is toxic to the cells, such as the presence of bacterial toxins. If degenerate neutrophils are present or bacteria are seen within phagocytes, the lesion should be cultured. Mixed cell inflammation, where fifty percent or more of the inflammatory cells are neutrophils and the rest are mononuclear leukocytes, such as macrophages, lymphocytes, and plasma cell, implies
an established less severe inflammation where the etiology is of low pathogenicity. Mixed cell inflammation may also be associated with resolving neutrophilic inflammatory lesions. The neutrophils of mixed cell inflammation is characterized by a predominance of macrophages [greater than fifty percent of the inflammatory cells]. Macrophagic inflammation is usually associated with certain etiologies, such as mycotic infections and foreign bodies. Other etiologies, although rarely reported in marine mammals would include mycobacterial and actinobacterial infections. Hematomas are often associated with mixed cell or macrophagic inflammation where macrophages demonstrate erythrophagocytosis.

Noninflammatory lesions include tissue hyperplasia/benign neoplasia and malignant neoplasia. Benign neoplasia cannot be differentiated from normal cells or cellular hyperplasia based upon cytology. Criteria of malignant neoplasia fall into the following categories: general criteria, nuclear features, cytoplasmic features, and structural criteria.

The use of cytology as part of the routine physical examination of marine mammals [i.e. blow evaluations] or the diagnostic protocol of medical cases can provide important information in the assessment of the patient. Cytology is a quick, inexpensive diagnostic tool that can easily be performed in the medical laboratory of the zoological park.
IN VITRO FERTILIZATION AND EMBRYO TRANSFER IN A WESTERN LOWLAND GORILLA (Gorilla gorilla gorilla)

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Introduction

Advances in assisted reproductive technologies (ART) such as in vitro fertilization (IVF) and gamete intrafallopian transfer (GIFT) have successfully provided a method of treatment for infertility in the human. Recently, a small number of ART procedures have been applied to the lowland gorilla. They include zygote intrafallopian transfer (ZIFT), GIFT, artificial insemination, and IVF. Because this species is preciously rare, such attempts have usually been limited to animals that have failed to reproduce naturally. This case describes an attempt at genetic expression in a 31 yr old, infertile female gorilla with bilateral occluded oviducts, using in vitro fertilization and cryopreservation.

Objectives

The procedures on this gorilla included superovulation, collection and fertilization of ova in vitro, and cryopreservation of the resulting embryos for transfer at a later time. The original intent was to transfer embryos back to the donor female, but a uterine adenocarcinoma was discovered which necessitated a hysterectomy. A 30 yr old, multiparous female was then selected as a recipient. Four oocyte retrievals were attempted over a 5-year period. The fourth retrieval was performed at the time of the hysterectomy.

Methods

Hyperstimulation of the ovaries was performed using either clomiphene citrate (trial #1 and 2) (Serono Laboratories, Inc., Norwell, MA 02061) or urofollitropin (trial #3 and 4) (Serono Laboratories, Inc., Norwell, MA 02061). Following follicular development, HCG was given to induce oocyte maturation. HCG was administered 2 and 5 days after the last treatment of urofollitropin and clomiphene, respectively. The drugs used to promote follicular growth in these four attempts are summarized in table 1. Oocyte retrieval (follicle aspiration) was timed to occur just prior to the ovulation event (36 hr after HCG). The ovaries were visualized, and follicles were aspirated via laparotomy. Laparoscopy was not possible in these

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attempts because of preexisting pelvic adhesions. There were 1, 0, 5, and 6 oocytes retrieved respectively. In trial 2, where clomiphene was used for the second time, there was no visual evidence of follicular stimulation (table 1).

Table 1
Overview of Four Attempts at IVF in the Gorilla

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Follicle Stim. (A)</td>
<td>Clomiphene 100 mg x 6d</td>
<td>Clomiphene 150 mg x 5d</td>
<td>Urofollitropin 450 IU x 10d</td>
<td>Urofollitropin 600 IU x 9d</td>
</tr>
<tr>
<td>HCG Injection (B)</td>
<td>5,000 IU</td>
<td>10,000 IU</td>
<td>10,000 IU</td>
<td>20,000 IU</td>
</tr>
<tr>
<td>Interval A-B</td>
<td>5 days</td>
<td>5 days</td>
<td>2 days</td>
<td>2 days</td>
</tr>
<tr>
<td>Coll. Interval After HCG</td>
<td>36 hr</td>
<td>36 hr</td>
<td>36 hr</td>
<td>36 hr</td>
</tr>
<tr>
<td># Ova Collected</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Source of Thawed Semen Used</td>
<td>None</td>
<td>-</td>
<td>Epididymal semen</td>
<td>Electroejaculated semen</td>
</tr>
<tr>
<td>Quality of Post-Thaw Embryos</td>
<td>-</td>
<td>4-Good</td>
<td>1-Atretic</td>
<td>1-Good 5-Atretic</td>
</tr>
<tr>
<td>2-Cell Stage Post-Thaw</td>
<td></td>
<td>3</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

Culture Conditions: All media utilized were a modified Nutrient Mixture Ham’s F-10 (MHF-10) (Gibco Laboratories, Grand Island, NY 14072). Three basic types of working media were used for retrieval and incubation:

1. Insemination Medium (IM); MHF-10 supplemented with 10% maternal serum. Used for sperm preparation, cleaning the oocytes and final incubation medium for insemination.

2. Growth Medium (GM); MHF-10 supplemented with 20% maternal serum. Used for post-fertilization events of embryo growth and culture.

3. Flush medium (FM); a 20mM HEPES buffered MHF-10 medium with pH adjusted to 7.4. This is medium that is not dependent on 5% CO₂ atmosphere to maintain pH. It is used for oocyte pick-up and initial holding medium.

Prior to use all media were pre-equilibrated overnight in a humidified atmosphere of 5% CO₂ in air at 37°C. Autologous, heat-inactivated gorilla serum was used for IM and GM protein supplementation. Serum preparation included heat-inactivation at 56°C for one hr and filter sterilization. Follicular fluid containing the oocyte was aspirated into culture tubes containing either Heps-buffered Ham’s F-10 (Gibco Laboratories, Grand Island, NY 14072) or Dulbecco’s phosphate-buffered saline solution (Gibco Laboratories, Grand Island, NY 14072).
The aspirated fluid was poured into a flat culture dish and examined with a dissecting microscope. The oocytes were then located and transferred to an organ culture dish containing Ham's F-10 supplemented with 10% maternal serum. Prior to oocyte collection, the medium had been placed in an incubator at 37°C with 5% CO₂ in air for approximately 12 hr. The oocytes were cultured in this environment for 4 hr and then evaluated for maturity.

As described in table 1, a total of five oocytes were collected from retrieval #3. Four of these oocytes appeared to be mature based upon the radiant coronal cells and the equal dispersion of associated cumulus cells. The fifth oocyte appeared to be atretic. Four of the six oocytes from retrieval #4 were classified as mature, one was immature and the other appeared to be degenerate. On each occasion, the oocytes from the retrieval were consolidated into a single organ culture dish for incubation and insemination. The frozen semen used in trial 3 was epididymal semen collected postmortem. In trial 4, the frozen semen used had been obtained by electroejaculation.

Approximately 13 hr following insemination, the oocytes were examined for fertilization. All four of the mature oocytes from trial 3 appeared to be fertilized. The atretic oocyte appeared to be an empty zona at this time. Of the six oocytes collected in trial 4, several had one to as many as four pronuclei present. Vacuoles were also present in several oocytes. At approximately 16 hr post-insemination, the oocytes were again evaluated prior to cryopreservation. While the three oocytes from trial 3 were still in good condition, only one of the oocytes from trial 4 was considered to be of good quality; however, all six were cryopreserved. Cryopreservation was performed in 2.5 ml straws utilizing propanediol as the cryoprotectant, and a slow-freeze procedure.

In preparation for transfer, the recipient female was primed with an intramuscular injection of 3.75 mg leuprolide acetate (Lupron Depot, TAP Pharmaceuticals Inc., Deerfield, IL 60015) at the time of menses. This was followed by oral supplementation with estradiol and micronized progesterone (Pharma-Tek, Huntington, NY 11743) (table 2). Transfer took place 18 days later, on November 7, 1992.

On November 6, 1992, at 4:00 pm, the fertilized eggs from treatment cycles 3 and 4 were thawed. The frozen straws were removed from cryostorage and warmed in a 37°C water bath. The thawed embryos were transferred through a dilution series of propanediol to remove the cryoprotectant, and were then placed in GM. The embryos were cultured overnight and reexamined on November 7. It was found that three out of the four embryos from trial 3 had survived the thawing and culturing process. One oocyte had divided into a 2-cell embryo with blastomeres of equal size. Each blastomere had a darkened central area. Another fertilized oocyte had divided into a 2-cell embryo similar to the one described, except that there appeared to be a nuclear structure in each of the blastomeres. The nuclear structures seemed light when compared to their surrounding, darkened, granular-like ooplasm. The third fertilized oocyte appeared to have two pronuclei still within the single cell. It was thought that perhaps it was unchanged or possibly had arrested. Later

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in the day and prior to transfer, however, this fertilized oocyte was again examined. It too had divided into a 2-cell embryo with blastomeres of equal size. The fourth fertilized oocyte was dark and granular. These are indications of a nonviable embryo or atretic oocyte.

The six oocytes and embryos from retrieval #4 were also thawed on the same day. Five embryos appeared to be degenerate, which would be consistent with freezing nonfertilized oocytes. The remaining oocyte appeared to be a 2-pronuclear stage embryo. It had two pronuclear-like areas in the center, surrounded by darkened granular ooplasm. On the following day, this particular oocyte had not divided, but was transferred along with the 2-cell embryos from trial 3.

Prior to the transfer, the vulva was cleansed with saline solution. The cervix was cleansed with FM culture medium. The uterus was then sounded with a practice embryo transfer catheter. For embryo transfer, the embryos were taken out of the incubator and transferred to HEPES medium. They were placed in a culture tube for transport to the zoo facility. Once at the zoo, the embryos were transferred from the transport tube to GM organ culture dishes and reevaluated for development. Three 2-cell embryos were evident. One arrested pronuclear intact embryo was also evident. All four embryos were placed in an organ culture dish containing 100% pre-equilibrated serum. They were then loaded into a side-opened Monash embryo transfer catheter. The transfer catheter was smoothly inserted through the cervical canal and into the uterus. The total depth of insertion was 6.5 cm. The amount of fluid transferred was approximately 40-50 microliters.
Table 2
Protocol for Recipient Gorilla

<table>
<thead>
<tr>
<th>Cycle Day</th>
<th>Estradiol</th>
<th>Cycle Day</th>
<th>Estradiol</th>
<th>Progesterone</th>
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<tbody>
<tr>
<td>*1</td>
<td>1mg BID</td>
<td>15</td>
<td>2mg BID</td>
<td>100mg TID</td>
</tr>
<tr>
<td>2</td>
<td>1mg BID</td>
<td>16</td>
<td>2mg BID</td>
<td>200mg TID</td>
</tr>
<tr>
<td>3</td>
<td>1mg BID</td>
<td>17</td>
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</tr>
<tr>
<td>4</td>
<td>1mg BID</td>
<td>T 18</td>
<td>2mg BID</td>
<td>200mg TID</td>
</tr>
<tr>
<td>5</td>
<td>1mg BID</td>
<td>19</td>
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<td>1mg BID</td>
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<tr>
<td>7</td>
<td>2mg BID</td>
<td>21</td>
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<tr>
<td>9</td>
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<td>10</td>
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<td>11</td>
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<tr>
<td>14</td>
<td>2mg TID</td>
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*3.75 mg leuprilide was given IM on day 1 of the cycle.

Discussion

All available embryos were used in a single transfer in order to maximize the chances for establishing pregnancy. In humans there is a 10-15% pregnancy rate when IVF and embryo transfer techniques are used. It is noteworthy that post-thaw embryos divided to the two-cell stage. This suggests that gorilla embryos, collected and fertilized as described, can be viably preserved using propanediol methods.

The age of the recipient (30 yr) may have lessened the chances for implantation, though specific reproductive problems were not identified. Fecundity in gorillas in natural breeding situations is known to decrease in their late twenties.
Summary

Although this trial did not produce a pregnancy, it did demonstrate that in vitro fertilization techniques, including freezing and thawing embryos, is a viable alternative breeding technique in gorillas with bilaterally occluded oviducts.

Conclusions

The use of artificial breeding techniques similar to those used in human infertility cases, and those already applied to gorillas, should be pursued in certain cases of gorilla reproductive disorders.

LITERATURE CITED

DILATED CARDIOMYOPATHY IN AN ANTILOPINE KANGAROO (Macropus antilopinus)

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An 8 yr old, male antilopine kangaroo (Macropus antilopinus) presented with clinical signs of lethargy, depression, anorexia and weakness. The onset of clinical signs was acute occurring several days after heavy equipment had been working in the area surrounding the exhibit. This animal was housed alone.

Physical findings on initial examination were bradycardia (44 bpm), diminished lung sounds bilaterally, and depression. Therapy included an injection of vitamin B complex, ceftiofur sodium and subcutaneous fluid support. Blood was submitted for CBC and serum chemistry evaluation, as well as a toxoplasma titer. The CBC and chemistries were within normal limits. Nucleated red blood cells were present and the toxoplasma titer was negative.

The kangaroo initially responded to therapy, becoming more alert and aggressive. He also began to show some interest in food. However, on the second day, he was severely depressed and laterally recumbent. Peripheral edema and cooling of the extremities was noted. The heart rate continued to be slow and irregular. Thoracic radiographs demonstrated cardiomegaly with biventricular enlargement. On electrocardiogram, no P waves were observed and the rate was slow and irregular ranging from 40 to 80 bpm. The ventricular beats appeared to be either supraventricular in origin with aberration or idioventricular. Echocardiogram confirmed the tentative diagnosis of dilated cardiomyopathy with biventricular enlargement and decreased myocardial contractility.

Because of the poor prognosis associated with cardiomyopathy and the difficulty monitoring response to therapy in this type of patient, euthanasia was indicated. The major finding at necropsy was biventricular cardiac enlargement. The right ventricular wall thickness was 0.5-1.0 cm and the left ventricular wall thickness was 1.5 - 2.1 cm. Histologically, there was marked, diffuse myofiber disorganization and disorientation with moderate, nonsuppurative, lymphocytic myocarditis. Disseminated myofiber vacuolar degeneration, interstitial fibrosis, sarcotlemmal proliferation and myofiber cytomegaly were also noted. This was consistent with the diagnosis of dilated cardiomyopathy. Post mortem vitamin E and selenium levels were within normal reference ranges for most mammals.

It is unusual for dilated cardiomyopathy to cause bradycardia. The absence of P waves would be consistent with SA node arrest, ventricular rhythm with no sinus node activity, or atrial fibrillation with a very slow ventricular response rate. Hyperkalemia can cause atrial standstill; however, the patient's serum potassium level was normal. It was, therefore, theorized that the SA node had been damaged as a result of the degenerative process resulting in atrial standstill.
In domestic cats, dilated cardiomyopathy has been associated with taurine deficiency. In dogs, a deficiency of myocardial L-carnitine has been associated with dilated cardiomyopathy. A relatively small percentage of dogs with dilated cardiomyopathy respond to carnitine supplementation. Taurine and carnitine levels were not evaluated in this kangaroo. The effects of stress associated with the heavy equipment working in the area of this animal exhibit remain speculative. No reports of cardiomyopathy in macropods were found in a review of the literature.
SEVERE DIFFUSE PULMONARY BULLOUS EMPHYSEMA SECONDARY TO PULMONARY PAPILLARY ADENOCARCINOMA IN AN AFRICAN LION (Panthera leo)

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A 15 yr old male African lion (Panthera leo) presented with an acute onset of weakness, lethargy, anorexia and labored breathing. From a distance "fluid sounds" could be heard during respiration. It was possible to elevate the lip with a pole to evaluate his mucous membrane color without resistance. The membranes were pale but did not appear cyanotic. The animal was treated with flunixin meglumine at 1 mg/kg IM using a blow dart and immobilization for workup was scheduled for the following day.

The next day his attitude had improved dramatically. He charged and growled during attempts to dart him for immobilization. Because of concerns for the stability of his cardiopulmonary system, 20 min following the preanesthetic dose of xylazine, ketamine was administered in gradual increments until approach was possible. The animal weighed 250 kg and received a total dose of 75 mg xylazine and 1 g ketamine. As soon as the lion was approachable, supplemental oxygen was provided. Mucous membranes were pale and cyanotic.

During endotracheal intubation, it was noted that a brownish, blood tinged fluid was draining from the trachea through the larynx. As the venous catheter was placed, it was noted that the blood was very cyanotic. The xylazine was reversed with 0.12 mg/kg yohimbine IV. Based on the movement of air through the rebreathing bag, it appeared subjectively that the lions tidal volume was very low (approximately 2 l). Blood gas determination and pulse oximetry were not available.

Thoracic radiographs were made and demonstrated severe, diffuse pulmonary bullous emphysema. In spite of therapy including aminophylline, prednisolone sodium succinate, and dopamine the animal became apneic and did not respond to resuscitation attempts.

At necropsy, the radiographic diagnosis of diffuse pulmonary emphysema was confirmed. Numerous large bullae were present, as well as smaller bullae throughout the lung parenchyma. Approximately 90% of the lungs were affected. Histologically, it was determined that the emphysema was secondary to pulmonary papillary adenocarcinoma with necrotizing cavitation. The malignancy had infiltrated the bronchi and bronchioles, weakening their architectural support. Subsequent loss of passive recoil in the lung parenchyma caused forced expiratory efforts which resulted in alveolar rupture and bulla formation.

The development of primary lung tumors in animals is uncommon. The incidence in dogs and cats has been estimated to be 1.24% and 0.38%, respectively. Approximately 50% of pulmonary adenocarcinomas have metastasized at the time of diagnosis. Other pulmonary locations are the most common site of metastasis. Adenocarcinoma is the most common...
histologic type found in dogs and cats. Squamous cell carcinoma and anaplastic carcinoma are more rare. Many lung tumors are advanced in their course at the time of diagnosis, making determination of the cell of origin difficult. Although risk factors for the development of pulmonary neoplasia have been defined in humans, the role of environment and pollutants in the development of lung cancer in animals is unsettled. Most lung tumors cause no clinical signs in their early stages and are often incidental findings on routine thoracic radiography. Cavitation may occur as a result of tumor necrosis. No reports of primary pulmonary neoplasia in exotic felids were found in the literature.
CEREBRAL INFARCTION ASSOCIATED WITH COARCTATION OF THE AORTA IN A LOWLAND GORILLA (Gorilla gorilla)

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Coarctation of the aorta is a well-recognized, but uncommon, congenital defect in man. It occurs as a discrete narrowing or segmental occlusion of the aortic arch. In the proximal form, the defect lies proximal to the ductus arteriosus, and is usually associated with severe left heart failure in infancy. The distal form most commonly involves the aortic arch, distal to the origin of the left subclavian artery, and is often associated with adult-onset hypertension. Coarctation of the aorta has rarely been reported in non-human primates. It has never been described in the gorilla (Gorilla gorilla). We report an unusual case of proximal aortic coarctation in a lowland gorilla which produced clinically significant cerebrovascular disease.

In 1987, a 7 yr old male lowland gorilla developed slowly progressive left focal seizures and left hemiparesis. Weak left sided peripheral pulses with profound pulse deficits were noted on repeated physical examinations. At 10 yr of age, magnetic resonance imaging and computed tomography revealed diffuse atrophy of the right frontal lobe of the cerebrum, with calcified gyri. Serum and cerebrospinal fluid antibody titers to herpesvirus 1 and Epstein-Barr virus were consistently elevated, and the animal was treated with acyclovir (Zovirax, Burroughs-Wellcome, Research Triangle Park, North Carolina). Seizures were initially controlled with phenytoin (Dilantin, Parke-Davis, Morris Plains, New Jersey). Anticonvulsant therapy was changed to phenobarbital due to the development of severe gingival hyperplasia requiring gingivectomy, and then to valproic acid (Depakene, Abbot Laboratories, North Chicago, Illinois) because of drowsiness caused by phenobarbital. The hemiparesis gradually worsened, and seizures recurred in the face of increasing anticonvulsant drug dosages. On 11 February, 1992, the animal was found in status epilepticus, which was controlled with phenobarbital and midazolam (Versed, Roche, Nutley, New Jersey). On awakening, there was right-sided hemiplegia, and decreased alertness. Due to extensive debility and poor prognosis for recovery, the animal was euthanized.

Post-mortem examination revealed marked left-sided subcutaneous edema. There was diffuse left ventricular hypertrophy, and a complete coarctation of the aorta immediately proximal to the left subclavian artery. Collateral circulation around the coarctation was provided by branches of the left subclavian artery. There was marked atrophy of the right cerebral hemisphere, and severe arteriosclerosis of the middle cerebral artery and circle of Willis. Histologic examination of the brain revealed chronic and acute ischemic changes in the right cerebral cortex, and in watershed areas supplied by the middle cerebral artery. Atrophy and gliosis were present within the thalamus and basal ganglia.
In this case, although the location of the coarctation is of the more proximal type, it is believed to have resulted in chronic cerebrovascular hypertension and arteriosclerosis of the middle cerebral artery. This resulted in repeated episodes of ischemia and infarction of the right cerebral hemisphere and thalamus. The cortical scarring or thalamic injuries may have served as the seizure focus. Similar cerebrovascular hypertension with aneurysmal dilatation of the circle of Willis is associated with coarctation of the aorta in man. Chronic hypertension can be found in numerous disorders and has been associated with stroke, lacunar infarction, and arteriosclerosis of the middle cerebral artery in humans.
THE USE OF HEAVY MOVING EQUIPMENT TO FACILITATE IMMOBILIZATION AND LOADING PROCEDURES FOR ELEPHANTS

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A variety of techniques have been described for the manual and chemical restraint of captive Asian elephants (Elephas maximus); however, there is little published information available relative to the use of these techniques in captive African elephants (Loxodonta africana). Unless trained or conditioned to a particular restraint device, such as a hydraulic crush, African elephants represent a significant risk to handlers and clinicians whether these animals need to be moved to another enclosure or transported to a different facility. To a large extent, this may be a function of facility design and construction, but other factors often enter into the decision-making process, the least of which includes the size of the elephants involved. As an alternative to traditionally used techniques, heavy moving equipment and specially constructed steel cages were used to facilitate the immobilization, movement, and eventual transport of 32 untrained African elephants during the fall of 1991. Weights ranged from 4500 to 8000 pounds (2045-3636 kg). Carfentanil and xylazine were used for immobilization and sedation respectively.

The success of the procedures involving these untrained African elephants, suggests that alternative techniques should be considered for loading and transport in addition to those currently recommended or published in the zoo husbandry and medicine literature.
INTEGRATING BASELINE HEALTH STATUS INFORMATION FOR FREE-RANGING AND CAPTIVE-HELD WILDLIFE

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Monitoring the health status of wild populations is necessary to ensure the success of conservation, reintroduction, repatriation, and translocation programs (CRRT). Biomedical data that could be useful for monitoring wildlife population health is obtained by many sectors. These usually include veterinarians, rehabilitators, and biologists. In most cases, however, data is recorded and stored at the receiving facility, and becomes inaccessible to other parties. In an effort to pool findings, a project is underway to provide the means of compiling and disseminating information about the health status of free-ranging wildlife.

The following project was initiated as a result of the needs defined during the Disease Conference*, held in Oakland, California, in November 1992. Participants of the conference generally agreed that infectious diseases are a component of wildlife management strategies. However, developing guidelines that incorporate disease considerations into management strategies is difficult. This difficulty arises from the lack of baseline information on the health status of many free-ranging populations. To enhance programs for monitoring, investigation, and surveillance of wildlife disease, the working group recommended that a communication network be established to facilitate the exchange of information between agencies, institutions, and individuals working with wildlife.

The ultimate goals of the current project are: a) to establish a centralized accessible repository of health status information for free-ranging wildlife, and b) to enhance communication among people involved in wildlife work. Regional offices where data can be submitted and screened are being established. Methods for collecting, disseminating, and accessing the data are being defined. The repository will be a computerized system that interfaces as well as possible with databases commonly used for managing captive-held wildlife. The Veterinary Group of the International Union for the Conservation of Nature / Species Survival Commission (IUCN/SSC) is the agency recommended for coordinating the project. A mechanism for sharing information is being delineated that allows the contributing party to maintain ownership of the data.

To date there is a paucity of data on the geographic distribution, host susceptibility, and pathogenic capability of pathogens among many wildlife populations. One of the most important aspects of infectious diseases is not the disease itself but what it can imply of processes occurring in the environment. To enable epidemiologic analyses of animals in defined geographic regions, the database uses an inventory system based on species...
identification and location. The location information includes universal transmeridian (UTM) coordinates. The project focuses on North America, but maintains an international perspective through association with the Office International des Epizooties (OIE) ad hoc Working Group on Wildlife Diseases and with the IUCN/SSC Veterinary Group.

Collaboration between agencies is a priority. The effectiveness of the project requires cultivating relationships between groups working with captive-held and free-ranging wildlife. Groups involved in CRRT programs must make the needs of the programs known. Rehabilitators, veterinary practitioners, and wildlife biologists are encouraged to participate in the project.

Due to limitations of personnel and finances, extensive monitoring programs cannot be established in all areas. Nevertheless, rehabilitators and veterinarians regularly encounter injured and ill wildlife from areas of interest. The information compiled from these cases can contribute greatly to health monitoring programs. By screening the wild animals that come through their facilities, veterinarians and rehabilitators can help establish baseline health data of species otherwise neglected and/or from areas not yet studied. Any historical parameters that are available can be very useful for exposing what is happening in the region.

Compiling basic information for the database requires making careful observations, maintaining complete written records, and sharing information. Noting that an animal is in good health is also important. Minimum information collected should include: species identification, date obtained, age, location of origin, health when obtained, all medical problems, treatment, weight, measurements, and final disposition. When possible, a complete diagnosis should be made and recorded. Treatments should be recorded and monitored for success. The utility of pooled data is dictated by the quality of the data that is included. Certain standards for data collection must be met to ensure quality results. Standards for many procedures are outlined in the manual of the OIE.3

This project provides direction for coordinating the collection, storage, and sharing of data from a wide variety of sources. Individuals and groups alike are encouraged to participate and offer ideas to this project so the databank may be useful to all sectors.

LITERATURE CITED

ONE APPROACH TO THE REMOVAL OF AN AURAL Rhabdomyoma IN A 7 YEAR OLD AFRICAN ELEPHANT (Loxodonta africana)

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Case report

Successful removal of a large benign tumor from an elephant weighing more than 1500 kg presents many husbandry, surgical and anesthetic challenges. A firm aural mass had been noted on the anterior aspect of the pinna of a 7 yr old female African elephant (Loxodonta africana). It had been measured quarterly since the animal’s arrival in the U.S. four years earlier. The mass was located medial to the rest of the pinna. It extended ventrally to a point close to the zygomaticoauricularis (ZyAur) muscle, which controls anterior ear movement. There was concern that if the tumor extended into the ZyAur muscle, ear movement might be impeded. Repeated needle aspirations had yielded no bacterial or fungal growth. Doppler examination of the ear noted no major arterial branches within the mass. After 24 months, when significant anterior mass growth and the appearance of two new posterior masses were noted, needle biopsies of the anterior mass were performed under local anesthesia. Biopsied tissue revealed a rhabdomyoma. No dermal erosion or irritation was noted around the anterior or posterior masses at any time prior to surgery. Non-cardiac rhabdomyomas are very rare in the veterinary literature, but a few cases of human pediatric auricular rhabdomyomas have been documented.1,2,8 Since the human cases had been successfully managed by surgery, it was decided to attempt removal before the patient or the tumor grew much larger.

There were multiple crucial steps in the preplanning of the surgery. The elephant was trained to lay in right lateral recumbency on mattresses while wearing restraint belts. A frame capable of lifting and holding the animal upright was moved into the exhibit the day of surgery. It was to be used in case of difficulty with post-operative ambulation. Approximately 10 min after sedation with 150 mg of xylazine, the elephant followed commands to lay on the mattresses and was physically restrained. Twenty minutes later, 300 mg of ketamine IV followed by 100 mg of butorphanol IV were used to induce general anesthesia. Two 10 sec episodes of generalized myoclonus approximately 2 min apart were noted during induction. Endotracheal intubation was facilitated by a combination of a fiberoptic colonoscope placed inside the 26 mm endotracheal tube and digital manipulation of the bulbous tongue and epiglottis. Anesthesia was maintained with 1% isoflurane supplemented with 30 mg butorphanol 90 min after intubation. Anesthetic monitoring was performed by using an automated cuff sphygmomanometer at the base of the tail, limb E.C.G., pulse oximetry on the tongue, endotracheal capnography and measurement of expiratory isoflurane concentration. Values were recorded every 5 min for the duration of
anesthesia (Tables 1, 2 & 3). End tidal CO₂ was kept below 40 mm/Hg by manually compressing a 30 L non-rebreathing bag. Ventilation was assisted as needed. Cardiac arrhythmias were not noted during the period of monitoring.

A 30 cm vertical incision was made over the anterior aspect of the tumor. The tumor was easily dissected free of skin flaps and cartilage with an ultrasonic scalpel. Tissue planes marking the margins of the tumor appeared to be clearly definable and no invasion into the adjacent structures was noted. A 600 gm mass measuring 18x10x8 cm was removed. The ultrasonic scalpel was able to provide adequate hemostasis throughout the dissection and no major vessels were encountered. Dissection was also attempted with electrocautery and standard #22 surgical scalpels. The ultrasonic scalpel provided the best combination of control, sensitivity and hemostasis, although it was somewhat slow. Subjectively, electrocautery was noted to be faster. Standard stainless steel scalpel blades became unacceptably dull very quickly. The tumor did not penetrate the auricular cartilage as had been originally suspected. The masses on the posterior aspect of the ear appeared to be sites where the cartilage had buckled posteriorly in response to pressure from the tumor mass. Their removal was not deemed necessary. The dermal incision was closed with 2 layers of #1 polydioxanone (PDS) in an interrupted subcuticular pattern. Stainless steel wire retention sutures were considered but not utilized. It was felt that the interrupted PDS closure would be able to resist anything but deliberate and determined manipulation. Such manipulation was thought to be likely to disrupt wire sutures as well. The wire could potentially cause even greater tissue damage.

Prophylactic antibiotic therapy consisted of 30 gm of cefotetan IV intraoperatively. Two gm of ceftiofur in 200 ml saline were flushed into the wound prior to its closure. An 8 french IV catheter was placed in the right saphenous vein and 20 L of lactated ringers solution was administered during surgery.

Following completion of the surgical procedure, the patient was recovered. Ventilation was assisted for a period of 10 min. The end tidal isoflurane concentration had decreased to less than 0.2% by the end of assisted respiration. One hundred mg of naloxone IV produced an increase in skeletal muscle tone and respiratory rate. Simultaneous second doses of naloxone (100 mg IV and 100 mg IM) were given. After one circulation time (100 seconds), the elephant regained her swallowing reflex and was extubated. A drape was kept over her face for two minutes until she removed it with her trunk. The elephant then stood on her first attempt. She was fed 15 min after anesthetic recovery because she demonstrated great interest in the hay bales around her.

Postoperatively, a mild lameness of the left forelimb was noted for three days, but no myoglobin was detected in urine. After seven days of 24 hr monitoring, the elephant was allowed into the outdoor exhibit area unsupervised. She rubbed the incision until the suture line broke down. The open wound was then managed conservatively by fresh water irrigation twice daily followed by mechanical debridement. After cleansing, the granulating surface was covered with silver sulfadiazine cream 1% (Silvadene) or zinc oxide cream to prevent contact and adhesion of debris. The wound healed by second intention in 3 months. The vertical nature of the wound, as well as the shape of the ear, prevented the adherence
of occlusive or protective dressings. Second intention healing was not complicated by an excessive proliferation of granulation tissue. Histologic examination of multiple portions of the tumor by two pathologists confirmed the diagnosis of rhabdomyoma.

Discussion

Elephant surgery is never a simple process, but it no longer has to be as dangerous to the patient and staff as has been in the past. Training the animal to lie in lateral recumbency and accept restraints can minimize the danger of a fall during induction. Endotracheal intubation is a challenge, but long arms and endoscopic guidance make it possible. It was probably due to intubation and assisted respiration that hypoxia and hypercarbia were not evidenced during 3 hr of anesthesia. It is likely that respiratory management to avoid hypoxia also decreased the possibility of cardiac arrhythmias. Measurements of heart rate derived from pulse oximetry, ECG and sphygmomanometry concurred throughout the session, allowing greater confidence in measured data. The initial concern over the length of time that would be required to exhale isoflurane was relieved by observing the rapid decrease in the expired isoflurane level when respiration was assisted. Supplemental respiration occurred at a rate of six additional ventilations per minute for a period of 10 min. An end expiratory concentration of less than 0.2% isoflurane has consistently been observed in other species at the same approximate time when the swallowing reflex is regained and the animal is extubated.

Postoperative wound care may be a problem for an elephant, since the animal may pick at sutures with its trunk or rub against objects. We had previously utilized subcuticular closures in many wild species without difficulty or manipulation by the patient; however, the patient in this case opened her incision the first time that she was left alone in the outdoor exhibit, seven days postoperatively. While antiseptic creams may impede wound healing in some situations, dirt and hay on a wound surface was judged to be a greater problem in this case. Application of a barrier after vigorous debridement protected the granulating surface from delayed healing due to clinically significant microbial contamination.

Careful patient training, aggressive anesthetic monitoring to prevent hypoxia and hypercarbia, high quality surgical instruments, and meticulous post operative wound care can be combined to successfully remove a tumor from a difficult patient.

ACKNOWLEDGEMENTS

We wish to thank: Kim Bokelhaide, M.D., Ph.D. and Don Nichols, D.V.M., for the histopathologic diagnosis. Julie Pelto, D.V.M. and Sue Lynch, D.V.M., for preliminary clinical evaluations. Dr. M. Schmidt for anesthesia advice. The elephant keepers Jim Pugh and David Martini for the hours spent on training and wound care.

INSTRUMENTATION

NELLCOR 2500, pulse oximeter/capnograph/anesthetic gas analyser.
Critikon, Dinamap 8300 Vet. Automated Sphygmomanometer. Life pak 8, ECG monitor.

DONATIONS

Anaquest for isoflurane, Ethicon for suture and Stuart Pharmaceuticals for cefotetan.

1993 PROCEEDINGS AMERICAN ASSOCIATION OF ZOO VETERINARIANS
LITERATURE CITED

Table 1. Data collected during the anesthetic session

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<th>Blood Pressure (mm/Hg)</th>
<th>SaO₂ (%)</th>
<th>ET CO₂ (mm/Hg)</th>
<th>Resp. rate (rpm)</th>
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Table 2. Real time and pharmaceutical administration during anesthetic session

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<td>Xylazine (150 mg) I.M.</td>
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<tr>
<td>Ketamine (300mg) and Butorphanol (100mg) I.V.</td>
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<td>Initiate Gas Anesthesia</td>
<td>9:34</td>
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<tr>
<td>Butorphanol (30mg) I.V.</td>
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Table 3. Allometric data calculated during anesthetic session

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<td>Tidal volume</td>
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<td>Circulation time</td>
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HERPES ASSOCIATED LYMPHOMA IN A SLOW LORIS (Nycticebus coucang)

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Case report

A 960 g, 7 yr old intact male slow loris (Nycticebus coucang) with a history of chronic intermittent upper respiratory infections and severe dental disease was presented to The Wildlife Health Center of the NYZS/The Wildlife Conservation Society. Physical examination revealed bilateral mucopurulent ocular discharge, oligodontia, gingivitis, periodontitis, bilateral submandibular lymphadenopathy and increased lung sounds in all fields. Further medical evaluation included a complete blood count (CBC), serum biochemical analysis, whole body radiography and aerobic culture and sensitivity of the ocular discharge. Radiographs revealed a generalized patchy pulmonary infiltrate with a soft tissue mass effect just cranial to the heart. Klebsiella pneumoniae and Pasteurella multocida were isolated from the conjunctiva. CBC test results are shown in table 1.

A diagnosis of lymphoid leukemia was made based upon lymphocyte morphology and total white cell count. Due to the severity of the disease and the poor long term prognosis, the loris was humanely euthanized. Blood was collected for viral isolation, viral serology and serum banking. A bone marrow aspirate was obtained from the humerus at that time.

Gross necropsy findings included marked hepatosplenomegaly, mediastinal and submandibular lymphadenopathy, and pale, firm lungs. Microscopically, lymphosarcoma was observed in the spleen, submandibular and mediastinal lymph nodes, lung, bone marrow, and conjunctiva. The neoplastic cells were primarily medium size lymphocytes with round nuclei and scant eosinophilic cytoplasm. The nuclei had a coarse chromatin pattern with one to three prominent nucleoli. One to three mitotic figures per high power field were observed. In the lung, lymphosarcoma effaced bronchial and bronchiolar walls. The alveoli contained a mixture of neoplastic lymphocytes, foamy macrophages, neutrophils, eosinophils and microorganisms resembling Pneumocystis carinii. Severe, diffuse, chronic membranoproliferative glomerulonephritis was also noted.

Whole blood was submitted for viral isolation to the Center for Reproduction of Endangered Species, Zoological Society of San Diego. Mononuclear cells were isolated from heparinized blood samples on Ficoll Paque gradients (Pharmacia LKB, Piscataway, NJ USA). Cells were cultured in an RPMI-160 medium (Grand Island Biological Co., Grand Island, NY USA) which contained 15 % heat-inactivated fetal bovine serum, 10 ug/ml phytohemagglutinin (Sigma Chemical Co., St. Louis, MO USA), and 200 U/ml recombinant human interleukin-2.
(Cellular Products, Buffalo, NY USA). Complete medium was replaced at 3-4 day intervals. Thirteen days after initiation of the culture, cells in suspension were pelleted, washed, and fixed in 2.5% glutaraldehyde in preparation for electron microscopy. Examination of cultured mononuclear cells revealed a nearly homogenous population of lymphoid cells. Scattered among this population was an occasional cell containing viral nucleocapsid particles in the nucleus and near complete virus particles in the cytoplasm. Both types of particles were indistinguishable in size and morphology from a herpesvirus previously described in cultured lymphocytes of the slow loris. 10

Discussion

The primary medical problems in this slow loris included lymphoid leukemia and a herpes viremia. A direct cause and effect relationship between the viral infection and development of neoplastic disease cannot be proven. The two diseases may have occurred independently or a leukemia associated immunosuppression may have allowed expression of a latent herpes infection. Herpesvirus induced neoplasia has been documented in a number of other non-human primates. *Herpes ateles* and *Herpes saimiri* are DNA oncoviruses which cause malignant transformation of lymphocytes. Reports in the human medical literature have demonstrated a correlation between malignant lymphoma and Epstein-Barr virus infected individuals. Herpesvirus infections in slow lorises have been associated with an increased incidence of upper respiratory disease, dental disease and neoplasia. The loris in this report had these same clinical features.

Table 1. Complete blood count (CBC) in a slow loris (*Nycticebus coucang*) with herpes associated lymphoma

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC x10³</td>
<td>280.6</td>
</tr>
<tr>
<td>HGB gm</td>
<td>12.7</td>
</tr>
<tr>
<td>HCT %</td>
<td>45</td>
</tr>
<tr>
<td>MCHC %</td>
<td>28.22</td>
</tr>
<tr>
<td>TOTAL SOLIDS</td>
<td>6.5</td>
</tr>
<tr>
<td>DIFFERENTIAL</td>
<td></td>
</tr>
<tr>
<td>MONO</td>
<td>1</td>
</tr>
<tr>
<td>LYMPH</td>
<td>93</td>
</tr>
<tr>
<td>SEG</td>
<td>6</td>
</tr>
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</table>

REFERENCES


USE OF DENTAL X-RAY FILM FOR NON-DENTAL RADIOGRAPHY IN ZOOLOGICAL MEDICINE

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The unique characteristics of human dental X-ray film can be utilized to provide non-dental radiographs of exceptional quality. Dental film was developed to yield an image with high contrast and resolution of a small focal area. The film itself has a number of particular characteristics which make it useful in zoological medicine. Dental film is an ultra high speed, nonscreen radiographic film which is available in a variety of shapes and sizes. The film is encased within a thin disposable paper lining which protects it from light exposure and mechanical damage during use. The packaged film is flexible and can be manipulated into areas which are otherwise difficult to access. It is particularly useful for whole body radiographs of very small species or for focal areas of interest in larger animals.

The same basic principles of radiography apply when using dental film. There are some features which help ensure quality films. A closer focal distance is necessary for dental film; twenty centimeters is commonly used. Most radiographs can be taken with a mAs (milliamperage X seconds) of 5 and a peak kilovoltage (kVp) of 55-65. Several combinations of milliamperage and time can be used to arrive at a mAs of 5 (50 mA X 1/10 second or 100 mA x 1/20 second). The settings must be adjusted for the size and density of the object being radiographed. A technique chart should be created for each X-ray machine to ensure high quality radiographs.

Standard X-ray machines can be used for dental film radiography. Dental X-ray units can also be used but are not indispensable. These units are much smaller and have a wider range of potential positions.

Dental film can be developed either by manual processing or with an automatic processor. The paper packaging which covers each piece of film must be removed in a darkroom before processing. A dental film hanger should be purchased when using dip tanks. If developing manually with standard dip tanks, an extended time in the developer is required in comparison to routine radiographs. The exact processing time is dependent on the type of processing chemicals and the temperature of the processing fluids. Time and temperature guidelines for manual film development are given in Table 1. A "Chairside Darkroom" is available for table-top processing (Henry Schein Inc., Port Washington, New York 11050, USA). This small box unit has built in gloves and miniature fluid baths for manual developing without a darkroom. Rapid process chemicals are available with the chairside darkroom for radiographic developing in less than a minute. Dental film automatic processors are available, but have not been used by the author. Standard radiographic automatic processors do not compensate for the smaller size and longer developing times of dental film and thus provide radiographs of inferior quality.
Dental film should be placed immediately next to the anatomical site being radiographed. The stippled, white outer surface must face the X-ray beam. The x-ray tube is adjusted to an appropriate height; usually 20 cm. Dental film is thin and can be placed in the mouth for mandibular, maxillary or dental radiographs. If there are special size or shape requirements, the film can be cut and shaped accordingly. A portion of the film is cut away in the dark room and the open edge is closed with opaque tape.

Dental film is available in two different size groups. Periapical film is narrower and longer than the occlusal film. The periapical film is available in three different sizes while the occlusal is only available in one size (Table 2). There are two types of high speed dental film; the Ultraspeed (Eastman Kodak Company, Rochester, New York 14650, USA) and the Ektaspeed (Eastman Kodak Company). The latter requires 50% less radiation exposure. The Ektaspeed film lacks some of the high detail imaging which the Ultraspeed film provides. Ultraspeed occlusal film (DF-50) is the most versatile of the various dental films and is the type preferred by the author.

REFERENCES

Table 1. Guidelines for manual processing of dental radiograph film indicated for Kodak Ultra-speed and Kodak Ektaspeed processed with Kodak GBX developer and fixer.

<table>
<thead>
<tr>
<th>Temp</th>
<th>Developer Rinse*</th>
<th>Fix+</th>
<th>Wash</th>
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</thead>
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<tr>
<td>68°F</td>
<td>5 min 30 sec</td>
<td>4 min</td>
<td>10 min</td>
</tr>
<tr>
<td>72°F</td>
<td>4 min 30 sec</td>
<td>4 min</td>
<td>10 min</td>
</tr>
<tr>
<td>76°F</td>
<td>3 min 30 sec</td>
<td>4 min</td>
<td>10 min</td>
</tr>
</tbody>
</table>

* Continuous agitation.
+ Intermittent agitation.

Table 2. Types of ultraspeed dental radiograph film.

<table>
<thead>
<tr>
<th>Type</th>
<th>Dental use</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF-54</td>
<td>Periapical</td>
<td>2.2 x 3.5 cm</td>
</tr>
<tr>
<td>DF-56</td>
<td>Periapical</td>
<td>2.4 x 4.0 cm</td>
</tr>
<tr>
<td>DF-58</td>
<td>Periapical</td>
<td>3.1 x 4.1 cm</td>
</tr>
<tr>
<td>DF-50</td>
<td>Occlusal</td>
<td>5.7 x 7.6 cm</td>
</tr>
</tbody>
</table>
NUTRITIONAL SECONDARY HYPERPARATHYROIDISM IN AN INFANT GORILLA (Gorilla gorilla)

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An 8 month old male gorilla being mother-reared presented for lethargy and depression. Serum chemistry results revealed marked hypocalcemia, hypokalemia and hyponatremia. Serum alkaline phosphatase and lactate dehydrogenase levels were elevated. Whole body radiographs showed no evidence of rickets. The intact parathyroid hormone level was 996 pg/ml (Normal value in humans 15-55 pg/ml). A diagnosis of nutritional secondary hyperparathyroidism due to vitamin D deficiency was made.

Treatment was instituted with injectable Vitamin D$_2^*$ and oral vitamin supplementation. Intravenous fluid therapy was instituted to supplement calcium and potassium. Nasogastric tube feeding was instituted to provide nutritional support. Serum calcium and potassium levels remained low in spite of intensive IV supplementation and phosphorus levels decreased such that oral supplementation was required. Intravenous electrolyte supplementation was maintained for 12 days and nasogastric tube feeding for 30 days before returning to normal feeding.

The reason for the delayed response to electrolyte therapy was thought to be renal Fanconi Syndrome. This syndrome is characterized by a generalized defect in renal proximal tubule transport including impaired reabsorption of glucose, phosphate amino acids and the excess excretion of calcium, magnesium, sodium, potassium and water.$^1$ This syndrome has been associated with Vitamin D deficiency.$^2$

Secondary nutritional hyperparathyroidism results from vitamin D deficiency, diets low in calcium or diets with excessive phosphorus and low to normal calcium. Breast milk has been shown to be low in Vitamin D and breast fed human infants are at risk of Vitamin D deficiency when not given vitamin D supplementation.$^3$ Great ape infants being mother reared indoors should be given supplemental vitamin D.

LITERATURE CITED


* Ergocalciferol in oil

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ACHY BREAKY ARACHNIDS

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Introduction

Tarantulas have been heavily utilized in both the pet trade and zoological gardens. These spiders are in demand as pets, and offer a unique opportunity to interpret the most charismatic members of the class Arachnida. Due to their popularity in "hands on" programming they are occasionally dropped. The nature of invertebrate anatomy makes these falls potentially life threatening. This paper will describe some of the therapeutic techniques that may be employed to save split spiders.

Within the past six years there has been an increasing amount of literature devoted to the medical aspects of Arachnids. The cost of an adult tarantula runs from $75.00 - $250.00, females of many species are long lived (20 + years) and one of the most popular pet species and thus most exploited, the Mexican red kneed (Brachypelma smithi), is now listed in CITES II. Thus, it behooves zoo veterinarians and private practitioners to become familiar with arachnid medicine.

Biology

The spiders that most of us recognize as tarantulas are members of the class Arachnida, order Araneae, and sub-order Orthognatha or Mygalomorph. They belong to four of the 15 families of Mygalomorphs: Theraphosidae, Dipluridae, Ctenizidae and Atypidae. The majority of tarantulas commonly encountered are members of the family Theraphosidae or the "hairy Mygalomorphs."

Anatomy

As with any species, in order to become an effective clinician it is important to become conversant with the anatomy and physiology of that species. The arachnid body is divided into two major parts: 1) the cephalothorax or prosoma which includes the appendages, made up of four pairs of walking legs, one pair of pedipalps, and the chelicerae; and 2) the abdomen or opisthosoma. (Figure 1)

Cephalothorax

The prosoma is composed of the dorsal carapace and ventral sternum. The eyes are located at the anterior portion of the carapace, most species having eight. All of the appendages arise from the cephalothorax. The chelicerae are composed of the fangs, teeth and poison glands. The pedipalps are utilized for the manipulation of prey and in males, copulation. The four pairs of walking legs are attached to the prosoma at the coxae. The prosoma also contains: part of the digestive system, the mouth, esophagus, and sucking stomach; a large part of the central nervous system; and the anterior aorta. (Figure 2)
Abdomen

The opisthosoma contains the majority of the internal organs of the spider. The heart is located along the dorsal midline. Beneath the heart is the midgut, from which lateral branches or diverticula extend surrounding the viscera laterally. The midgut empties into the stercoral pocket which is connected by a short hindgut to the anus at the caudal end of the spider. The malpighian tubules or excretory organs are located dorsally and also empty into the stercoral pocket. Ventral to the midgut, starting proximally are the two pairs of book lungs, ovaries and genital opening(epigastric furrow), plus spinning glands and spinnerets. These structures all have openings through the exoskeleton and thus are important to recognize.6 (Figure 3)

Pedicle

The cephalothorax is connected to the abdomen by the pedicle. Through this narrow portion of the spider runs the aorta, midgut and abdominal nerve.(Figure 3)

Exoskeleton

The exoskeleton is made up of four layers of cuticle, the epi-, exo-, meso- and endocuticle. The exocuticle appears to be the layer with the greatest strength and rigidity. This layer is absent in the cuticle of the abdomen and leg joint membranes. These cuticular layers are supported by the underlying epithelial cell or hypodermis. Pore canals within the cuticular layers allow for epithelial materials to be transported for repair of the outer layers of the exoskeleton.6 Details on repair of the cuticular layers post trauma are lacking.

Hemolymph

Hemolymph is composed of a large number of blood cells termed hemocytes. Four types, including granulocytes have been identified. These cells are believed to be involved in clotting functions, phagocytosis and storage. The hemocytes are derived from the myocardium cells. The respiratory pigment in spiders is hemocyanin. Hemocyanin gives the hemolymph a very slight bluish color. The ionic composition of tarantula hemolymph has been studied.

220 mM/L - NA+ or 220 meq/L
5 mM/L - K+ or 5 meq/L
4 mM/L - Ca++ or 8 meq/L
1.1 mM/L - Mg++ or 2.2 meq/L
3 mM/L - HCO3- or 3 meq/L

The pH is approximately 7.3 and osmolarity between 400 and 600 mOsm/L.

Soluble proteins, primarily hemocyanin make up 7% of the soluble substances. There is also a soluble fatty portion. The major carbohydrate of hemolymph is glucose. The hemolymph accounts for approximately 20% of a spiders body weight.6
Anesthesia

Arachnid anesthesia has been described by a number of sources. Short periods of hypothermia (30 min. at 4°C) have been described and advocated for brief sedation. General anesthesia utilizing anesthetic gasses (ether, methoxyflurane, Halothane, Isoflurane) delivered by chamber induction have also been described. The use of carbon dioxide as a canister gas or from dry ice has also produced effective although slightly risky anesthesia. Maintenance of anesthesia has not been described, and anesthetic time appears limited to recovery time or between 5 - 20 minutes.

Traumatic injuries

Loss of walking legs due to trauma or interference during the moult, and their subsequent treatment have been reported. Lost limbs will regenerate during the next moult, so the only worry of the practitioner is to stop any excessive leakage of hemolymph. This has been accomplished using cyanoacrylate, Cyanomethacrolate glue, paraffin wax or plasticine.

There is little reported information concerning the treatment for fractures of the abdomen secondary to trauma. At the Minnesota Zoo there have been five instances of abdominal fracture in four individual tarantulas. Three of these falls resulted in the death of the animal, two were successfully treated.

Although anesthesia techniques have been described, none of the cases have shown excessive movement (a reaction to avoid noxious stimuli) during the repair process. Abdominal rents also tend to leak large amounts of hemolymph, due the extra time needed for induction of anesthesia it seems justified to proceed directly with the repair.

A basic understanding of the spiders anatomy will greatly aid the clinician in determining the prognosis for the case. Dorsal splits appear to carry the best prognosis. Both of the successful repairs were splits involving the dorsal or lateral aspects of the abdomen. Splits involving the ventrum have not been successfully repaired. The book lung’s slits, genital opening, anal opening and spinerettes occupy the majority of the exoskeletal space on the ventrum of the tarantula. It appears impossible to suffer a split in this area without involving a major organ. One split involved the pedicle. During manipulation of this area the spider responded as if in extreme discomfort. This would make sense as the abdominal nerve travels through this area. All deaths that occurred from splits, occurred shortly after or within three to four days of the injury.

Repair materials included, 5-0 suture (Coated Vicryl, Gastro-intestinal J-314H, Ethicon Inc., Somerville, New Jersey 08876-0151, USA), cyanoacrylate (Nexaband, TRI-POINT MEDICAL L.P., Raleigh, North Carolina 27604, USA), and transparent dressing (Tegaderm, Medical products division/3M, St. Paul, Minnesota 55144, USA). All repair materials were left in place and were shed during the next moult. Repair materials did not result in an abnormal or difficult moult.
Spiders drink water for hydration have been reported to drink saline.² As in any species that has suffered acute hemorrhage, replacement of body fluids would appear critical. This author has not attempted to use "spider ringers".

LITERATURE CITED

Figure 1. The two major parts of the spider, prosoma or cephalothorax and opisthosoma or abdomen. a. Lateral view. b. Ventral view. Adapted from, Rainer, F.F. 1982. The Biology of Spiders. Harvard University Press. Cambridge, Massachusetts.
Figure 2. Longitudinal section of the cephalothorax. Note the Aorta, Intestine and Abdominal nerve as they course into the pedicle at the caudal end of the cephalothorax. Adapted from, Rainer, F.F.1982. The Biology of Spiders. Harvard University Press. Cambridge, Massachusetts.

Figure 3. Longitudinal section of the abdomen. Note multiple organ openings in the ventral aspect of the exoskeleton. Adapted from, Rainer, F.F.1982. The Biology of Spiders. Harvard University Press. Cambridge, Massachusetts.
Table 1. Tarantula Oral Replacement Solution

<table>
<thead>
<tr>
<th>Composition</th>
<th>SI Units (mMol/L)</th>
<th>Spider hemolymph Common Units (mEq/L)</th>
<th>Ringers (mEq/L)</th>
<th>* Spider Ringers (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>220</td>
<td>220</td>
<td>147</td>
<td>220</td>
</tr>
<tr>
<td>K</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Ca</td>
<td>4</td>
<td>2.2</td>
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<td>4</td>
</tr>
<tr>
<td>Mg</td>
<td>1.1</td>
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<td>0</td>
<td>2.7</td>
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<tr>
<td>HCO₃</td>
<td>3</td>
<td>3</td>
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<td>3</td>
</tr>
<tr>
<td>Cl to balance</td>
<td>224</td>
<td>155</td>
<td>226</td>
<td></td>
</tr>
<tr>
<td>SO₄ to balance</td>
<td>2.7</td>
<td>0</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>mOsm/L</td>
<td>460</td>
<td>310</td>
<td>461</td>
<td></td>
</tr>
</tbody>
</table>

* Spider Ringers Solution: To one liter of Ringers Solution add the following:

- 333 mg MgSO₄
- 252 mg NaHCO₃
- 4090 mg NaCl
- 75 mg KCl

* Spider Ringers Solution from scratch: As specified in common units column.

- 160 mg CaCl dihydrate
- 373 mg KCl
- 333 mg MgSO₄ heptahydrate
- 252 mg NaHCO₃
- 12680 mg NaCl
- q.s. to one liter of H₂O

Can add up to 1.3 gm of dextrose for palatability and still keep mOsm/L less than maximum for hemolymph of 600.
CRYPTOCOCCOSIS IN A COMMON ANACONDA (Eunectes murinus)

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Libero Ajello, PhD and Arvind A. Padhye, PhD
Division of Mycotic Diseases, Centers for Infectious Diseases, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA, USA

Systemic cryptococcosis was diagnosed in a common anaconda (Eunectes murinus) that died after a 2.5 month history of progressive neurologic disease. Pathological examination revealed granulomatous pneumonia and meningoencephalitis associated with yeast organisms morphologically consistent with Cryptococcus sp. Cryptococcus neoformans was confirmed as the etiologic agent using a specific fluorescent antibody (FA) test for this pathogenic yeast. To our knowledge, this is the first reported case of C. neoformans infection in an ectothermic host.
REMOTE DELIVERED AND REVERSIBLE CONTRACEPTION OF BLACK-TAILED DEER (Odocoileus hemionus columbianus) BY NORGESTOMET BALLISTIC IMPLANTS

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California Department of Fish and Game, 1701 Nimbus Rd. *D*Rancho Cordova, California 95670 and Wildlife Health Program, School of Veterinary Medicine, University of California, Davis, California 95616, USA

Nadine K. Jacobsen, PhD
Department of Wildlife and Fisheries, University of California, Davis, California 95616, USA

Darrel J. Kesler, PhD
Department of Animal Sciences, University of Illinois, Urbana, Illinois 61801, USA

Management of wild ungulate populations is often complicated by political, economic and practical concerns. When population control is desirable, contraception provides wildlife managers with another option to sport hunting, culling, or capture and removal. Progestin steroids in the form of silastic surgical implants containing melengesterol acetate (MGA) or ethinylestradiol (EE) have been used to contracept wild ungulates and other species for many years.\(^1\) The need for capture, anesthesia and sterile surgical placement of these implants has limited their applicability in free-ranging wildlife. We investigated the efficacy of a remotely delivered, biocompatible ballistic implant in deer. Implants consisted of a hydroxypropyl cellulose biobullet containing a central silastic rod impregnated with 42 mg of the synthetic progestin norgestomet (17α-acetoxy-11β-methyl-5,20-dione).

Seven adult (2 to 9 yr old) female captive reared black-tailed deer (Odocoileus hemionus columbianus) were remotely implanted with a BallistiVet rifle in October of 1991, approximately 30 days before their normal breeding season. Three females housed with these does acted as a comparison group. Subsequently two proven males (3 and 5 yr old) were allowed access to the ten does within a 1.5-ha enclosure for 5.5 months. None of the treated does showed estrus behavior, nor did males tend these does, and none produced fawns in the spring of 1992. The untreated does were courted by the bucks, bred normally on their first cycle, and produced an average of 2.33 fawns per doe in May 1992. Daily observations of behavior and activity, food intake, and health of animals were made throughout this study and abnormalities were not noted.

An in vitro assay system utilizing bovine serum incubated at 37°C was used to profile norgestomet release from the biocompatible implants. Results suggest that the release rate of norgestomet decreases linearly, that the release rate at the end of the fourth month is 100μg per day, and that by 252 days norgestomet release ceases. In the fall of 1992 all ten does bred normally and all were determined to be pregnant by 90 days post-breeding using serum progesterone and pregnancy specific protein B assays.

The norgestomet-biobullet and BallistiVet rifle offer a simple, convenient remote delivery system and reversible fertility control without loss of genetic potential or disruption of social behavior. No microencapsulation, adjuvant or booster dose was necessary. The only problem with this system appears to be associated with acute pain and localized trauma at the

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implant site. Three of 7 animals had noticeable swelling at the implant site and carried the hind limb for several hours. In one animal this lasted 21 days. No permanent debility, local necrosis or abscessation occurred. Reducing implant size as well as muzzle velocity could decrease local trauma. This is the first documented use of a single dose, remotely implanted and reversible contraceptive in a wild ungulate. Details of this work are reported elsewhere.²

ACKNOWLEDGEMENTS

We thank Dr. A. Kjemprup of the U.C. Davis, Wildlife Health Program, Terry McDermott of BallistiVet, Inc, our respective agencies and institutions for support and Drs. Bill Lasley and Jay Kirkpatrick for review and comment.

LITERATURE CITED

THERAPEUTIC USE OF METHOCARBAMOL IN A DEMOISELLE CRANE (Anthropoides virgo) WITH SEVERE ATAXIA AND LATEROFLEXION OF THE NECK

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Present address: Philadelphia Zoo, 3400 West Girard Avenue, Philadelphia, PA 19104, USA (Dr. Ialeggio)

An adult, male Demoiselle crane (Anthropoides virgo), estimated age 20 yr, was presented to the hospital after running at high speed into a fence. Clinical signs included ataxia, inability to stand, and severe lateroflexion of the base of the neck with inability to support its head. Initial therapy for severe shock included intravenous prednisolone sodium succinate, and lactated ringers solution. Intramuscular sodium ascorbate, vitamin E/selenium and vitamin B complex were also administered. In addition, diazepam was given IM for its anxiolytic and muscle relaxant properties. Initial cervical radiography showed increased soft tissue density ventrally at C13-14; no skeletal abnormalities were appreciated.

Improvement occurred over the next few days with continued treatment with lactated ringers solution and corticosteroids.

On both the eighth and ninth days of hospitalization, the crane ran forcefully into the wall of his stall during release to the exercise yard. It stumbled, fell into sternal recumbency, then stood with marked ataxia. On day 13 of hospitalization it was not making any attempts to stand. It was placed in a mesh sling and intravenous treatment with prednisolone sodium succinate and lactated ringers solution was reinstituted, as was intramuscular administration of diazepam. Over the next 11 days its condition worsened, despite sling support, and ongoing corticosteroid therapy. The caudal cervical area was very stiff, with markedly reduced lateral and dorsoventral range of motion. Radiography showed a distinct curvature at C13-14 and C9-10. Euthanasia was considered.

It was decided to initiate treatment with methocarbamol (Robaxin-VR, A.H. Robins), a centrally acting muscle relaxant which has used to treat muscle spasticity in equines, and intervertebral disc syndrome and muscle trauma in dogs and cats. No published use of this drug in birds was found. Methocarbamol (50mg/kg IV) was administered every 12 hr on day 25 of hospitalization. Prednisolone sodium succinate, lactated ringers solution, and diazepam were also administered at the same time. This was repeated over the next four days.

The crane began to show signs of improvement within several hours of the first methocarbamol treatment. The caudal cervical area became palpably more flexible. Over the next few days the degree of lateroflexion became gradually less severe, and its head, neck, and leg posture became normal. On the fifth day of methocarbamol treatment, the dosage was decreased to 22mg/kg IV every 12 hrs., and the diazepam dose was reduced. On the sixth treatment day methocarbamol was administered once daily and the diazepam was discontinued. On day seven of the methocarbamol treatment the crane was removed from the sling for several hours. The crane appeared more agitated when removed from the sling, so diazepam therapy was reinstituted on the eighth day of methocarbamol, at the
original dosage rate (45mg/kg IM). There was no further improvement in the degree of lateroflexion of the neck. On the ninth day of the methocarbamol treatment, the crane was placed in a pool of water to allow for a 30-minute assisted passive therapy swimming session. Afterwards the crane was not repositioned in the sling. Because there had been no improvement of the lateroflexion of the neck during the preceding three days, the methocarbamol dose was increased to the original dose of 50mg/kg IV every 12 hours. The IV prednisolone sodium succinate was tapered off along with the IV lactated ringers solution. Diazepam was administered orally.

On the 11th day of methocarbamol treatment the crane appeared much improved. It was able to stand unassisted for short periods of time with greatly improved coordination, steadiness and strength. Lateroflexion of the neck was much less pronounced. The methocarbamol was changed to 32.5 mg/kg PO every 12 hours.

Over the next few weeks the crane became stronger, more coordinated, and exhibited less muscle rigidity. Methocarbamol (32.5mg/kg) was continued orally in food for 18 days. Oral diazepam therapy was continued for two months.

This case report suggests that methocarbamol may be beneficial in the treatment of muscle spasm in birds. Methocarbamol has been used in human rehabilitation medicine for muscle spasm, as well as in equines for muscle spasticity. It is thought to produce muscle relaxation by decreasing transmission in spinal and supraspinal polysynthetic pathways. Since the pharmacokinetics of methocarbamol in birds is not known, it was necessary to extrapolate a dosage from those already established. The dose for dogs and cats is highly variable (40-220mg/kg IV). The equine dose is less variable (5-50 mg/kg).

It appears that the 50mg/kg dose given intravenously every 12 hours is effective, administered over several days with subsequent oral doses at 32.5mg/kg. Results of hematology and serum chemical analyses performed after methocarbamol treatment had been discontinued were consistent with those from normal Demoiselle cranes, suggesting that, at least in the short term, methocarbamol did not appear to adversely effect renal and hepatic function.

During preparation of this case report we learned that methocarbamol has been used successfully in a trumpeter swan at the Raptor Center at the University of Minnesota. The swan, which had suffered blunt muscle trauma, presented with a Creatinine Phosphokinase (CPK) level of over 70,000. All other parameters were within normal limits. It was treated daily for three days with methocarbamol (44mg/kg IV), following initial standard treatment for shock utilizing lactated ringers solution and 4mg of dexamethasone given intravenously. The swan was clinically normal within one week's time. The CPK decreased dramatically over the first week of treatment and was normal after nine days (Roberto Aguilar, DVM, personal communication).
USE OF NEUROLEPTIC AGENTS IN THE CONTROL OF INTRASPECIFIC AGGRESSION IN GREAT APES

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Neuroleptic drugs have been used frequently in human medicine to control schizophrenia, alter mood or attitude, or to reduce behavioral abnormalities. Their use improves social adaptation and reduces belligerent, dominant or aggressive behavior. The use of these drugs in zoo animals has generally been limited to ungulates, since they show a higher incidence of capture-related stress and trauma.

Two intermediate acting neuroleptic agents (Haloperidol and Thioridazine) were used in combination with an antiparkinsonian drug (Biperiden) to control aggression and maladaptive behavior in gorillas and chimpanzees. The following are summaries of two cases.

Case 1:

A 12 yr old 65 kg male chimpanzee (Pan troglodytes) who had been living in isolation for several years was donated to the Jerez Zoo. Introduction to an adult female who had recently lost her mate was attempted. Initial association through a barred door allowed visual, olfactory and auditory contact. After a period of several weeks, the animals were allowed to interact without barriers. The male displayed constant aggression toward the female. The following treatment protocol was instilled in order to control the male's aggression (Table 1).

Case 2:

An adult 11 yr old 65 kg female lowland gorilla (Gorilla gorillagorilla) was introduced to an established pair of adult handraised animals. The pair (one 14 yr old 180 kg male and a 13 yr old 70 kg female) had been showing abnormal behavior (aberrant social behavior, coprophagia, and regurgitation followed by ingestion) for some time. The male displayed constant aggression towards the new female. In two cases, interaction resulted in severe injury and the female had to be separated for some time. This group was considered important for breeding purposes. The following protocol was used in an attempt to control aggression as well as abnormal behavior (Table 2).

The protocols were helpful in controlling aggression and decreasing physical aggressive interaction in both cases. Improvement in acceptance and cohabitation was noted during the introductory periods. In the animals showing aberrant behavior, however, the patterns were unchanged. As in human medicine, neuroleptics were found to be helpful tools for short periods of time. They must not be used as a permanent form of therapy.
Table 1 Treatment protocol followed to decrease aggression in a chimpanzee (Pan troglodytes)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Frequency</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haloperidol</td>
<td>20 mg</td>
<td>sid</td>
<td>10 days</td>
</tr>
<tr>
<td>Biperiden</td>
<td>2 mg</td>
<td>sid</td>
<td>10 days</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>15 mg</td>
<td>sid</td>
<td>3 months</td>
</tr>
<tr>
<td>Biperiden</td>
<td>1 mg</td>
<td>sid</td>
<td>3 months</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>30 mg</td>
<td>sid</td>
<td>3 months</td>
</tr>
</tbody>
</table>

Table 2 Treatment protocol followed to decrease intraspecific aggression in gorillas (Gorilla gorilla)

![Graphs showing dose changes over time for male and female gorillas.]
CUTANEOUS LUPUS IN A GREY SEAL (Halichoerus grypus)

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An autoimmune disease was suspected in a 24 yr old grey seal (Halichoerus grypus) which had continuous ulcerative nasal dermatitis and intermittent ulcerative dermatitis of the nail beds and dorsum of the body for 9 years. Lesions were non-responsive to topical and systemic antibacterial and antifungal treatment.

Histopathological findings were typical of cutaneous lupus erythematosus. The lesions were characterized by a lymphoplasmacytic interface dermatitis, hydropic degeneration of basal epithelial cells, acanthosis, and hyperkeratosis. Intralesional immunoglobulins were identified by immunofluorescence. Secondary ulceration with associated suppurrative and granulomatous dermatitis also were present.

Mycobacterium avium-intracellular-scrofulaceum (MAIS) complex and Staphylococcus aureus were isolated from affected skin, but no fungi were identified. Hematology, serum chemistries, and thyroid hormone levels were within normal limits except for low total leukocyte and red blood cell levels.

Treatment with systemic prednisone, trimethoprim-sulfadiazine, enrofloxacin, ketoconazole, tetracycline or niacinamide did not resolve the lesions. Furthermore, the lesions did not resolve with topical clobetasol propionate, regional debridement, or protection from ultraviolet radiation (indoor housing and topical sunblock).

The seal died during its second week of treatment with hydroxychloroquine sulfate. In addition to skin lesions, gross necropsy results included congested lungs and calcified and caseous mediastinal lymph nodes. Final necropsy results are pending.
IMMOLIZATION OF NORTH AMERICAN RIVER OTTERS (Lutra canadensis) WITH MEDETOMIDINE-KETAMINE AND REVERSAL BY ATIPAMEZOLE

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Five doses of medetomidine (10, 25, 50, 75 and 100 ug/kg, i.m.) combined with ketamine (2.5 mg/kg, i.m.) were evaluated in North American river otters (Lutra canadensis). Otters were immobilized in cooperation with the North Carolina Wildlife Resources Commission Otter Restoration Project as previously described.¹⁰ The initial sedative effect of medetomidine-ketamine occurred rapidly and consistently in otters receiving medetomidine at 25 ug/kg and 50 ug/kg combined with ketamine 2.5 mg/kg. Median induction times were also similar for both groups. Most otters were sedated 1-2 minutes post-injection, and were recumbent within 3-5 min. All otters receiving the higher doses (50, 75, or 100 ug/kg) and most of those receiving 25 ug/kg of medetomidine remained completely immobilized throughout the 30 min period.

Dose-dependent effects of medetomidine were apparent for relative oxyhemoglobin saturation and respiratory rate. Apnea and hypoxemia developed during induction in one-half of the otters immobilized with medetomidine 50 ug/kg-ketamine 2.5 mg/kg and in all those immobilized with medetomidine 75 or 100 ug/kg-ketamine 2.5 mg/kg. Respiratory rates and relative percent oxyhemoglobin saturation were initially lower with increasing doses of medetomidine. In response to earlier hypoventilation, respiratory rates increased later in the immobilization period.

Atipamezole effectively reversed medetomidine-ketamine in river otters. When atipamezole was administered as a standard dose (100 ug/kg), recovery was faster in otters immobilized with lower doses of medetomidine. Total reversal time decreased with increasing ratios of atipamezole:medetomidine (w/w) and was shortest at a ratio of 4:1. Otters immobilized with medetomidine-ketamine recovered gradually and quietly after atipamezole reversal. The absence of residual ketamine effects was attributed to the low dose of ketamine (2.5 mg/kg) used in the medetomidine combination.

We recommend medetomidine 25 ug/kg combined with 2.5 mg/kg ketamine in river otters as an induction dose prior to inhalation anesthesia or as an immobilizing dose adequate for short procedures, including minor surgery. A higher dose of medetomidine (50 ug/kg) in combination with ketamine (2.5 mg/kg) is required for procedures of 30 min or longer but respiratory depression may occur. The doses reported here are lower than those...
recommended for dogs and cats (medetomidine 40-80 ug/kg-ketamine 5 mg/kg)\textsuperscript{0,11-13} and for other small, nondomestic carnivores (medetomidine 100 ug/kg-ketamine 5 mg/kg)\textsuperscript{1-8}. For anesthetic reversal in river otters, atipamezole administered at a ratio of 4:1 relative to the dose of medetomidine worked best in these trials.

**ACKNOWLEDGEMENTS**

This manuscript is dedicated in memory of Harry H. Jalanka, D.V.M., Ph.D., whose advice and generosity made this study possible.

**LITERATURE CITED**


MEDETOMIDINE-KETAMINE-ISOFLURANE ANESTHESIA IN CAPTIVE CHEETAH (Acinonyx jubatus) AND ANTAGONISM WITH ATIPAMEZOL

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Fourteen captive cheetah (Acinonyx jubatus), 9 males, 5 females, aged 4.8 ± 3.5 (0.7-14) yr (x ± S.D. (range)) and weighing 36.1 ± 7.8 (23-49) kg, were immobilized and anesthetized with medetomidine, ketamine (MK) and isoflurane (Aerrane: Anaquest, Madison, Wisconsin 53713) -O2 for annual physical exam, flea bath, dental prophylaxis and other minor procedures. Three MK dose rates were used based on estimated body weight: 70 µg/kg M + 2.5 mg/kg K (n=3), 50 µg/kg M + 2.5 mg/kg K (n=7) and 40 µg/kg M + 2.5 (n=1) or 3.0 mg/kg K (n=3). All drugs were delivered by projectile syringe to animals maintained in outdoor pens or paddocks. After initial immobilization, the cheetahs were transported to the animal clinic. Blood gases were determined on 15 femoral arterial blood samples drawn from 10 of the cheetahs while they were breathing ambient air spontaneously, at 2-7 and 9-13 minutes after immobilization. ISO was given by mask, if necessary, to provide airway relaxation, and after endotracheal intubation, anesthesia was continued with ISO. HR and RR were monitored during the immobilization and anesthetic period. When the clinical procedures were completed, ISO was discontinued, the cheetahs were placed in wooden transport crates to recover from anesthesia, and atipamezol (ATI) 300 µg/kg (n=10) or 150 µg/kg (n=4) total dose was given (one quarter IV and three-quarters SQ).

Two cheetahs receiving 40 µg/kg M were not adequately immobilized. In cheetahs receiving 50 or 70 µg/kg M, complete immobilization occurred in 8.8 ± 3.0 (6-16) min. Induction was very quiet. No excitement stage or seizure activity occurred. Muscle relaxation was profound and palpebral reflexes were slight to absent.

In cheetahs receiving 50 or 70 µg/kg M, heart rates recorded during MK immobilization before ISO were 66.5 ± 9.0 (44-80) BPM and during ISO were 53 ± 13.7 (36-80) BPM. Respiratory sinus arrhythmia was common. Spontaneous respiratory rates were 20.8 ± 8.1 (12-13) before ISO and 15.0 ± 4.5 (6-32) during ISO. A transient period of clinically apparent respiratory depression (very shallow breathing) occurred in some cheetahs at 10-12 min after MK administration. Two cheetahs were intubated and ventilated manually at this time. One was given atropine to treat concurrent bradycardia. PaO2, PaCO2 were 90.0 ± 13.2 and 35.0 ± 6.4 mmHg, respectively 2-7 min after immobilization and were 82.7 ± 11.5 and 34.7 ± 6.1 mmHg, respectively 9-13 min after immobilization. The lowest PaO2 was 65 mmHg, the highest PaCO2 was 48 mmHg. ISO was begun 11-23 min after immobilization and ISO anesthesia time was 78.5 ± 16.2 (60-105) min. Average delivered ISO concentration was 0.40 ± 0.27, carried in an O2 flow of 1 LPM or 0.70 ± 0.56 during closed circuit anesthesia.
ATI was given 97.0 ± 13.8 min (68-112) after MK administration. After ATI 300 μg/kg, animals were sternal or sitting in 6.1 ± 1.7 (4-9) min and standing in 8.8 ± 3.0 (6-16) min. Two cheetahs receiving 150 μg/kg ATI had a slow, awkward recovery and stood at 23 and 27 min. Recovery was generally quiet. Generalized mild muscle tremors occurred in one cheetah after ATI administration. One major anesthetic complication occurred. A 14 year old female developed a period of slow, wide QRS idioventricular rhythm during ISO at 76 min after MK administration. Initial resuscitation and recovery were slow but appeared to be successful. After doing well for approximately 2 weeks, she developed renal insufficiency which did not respond to treatment, and she was euthanized 3 weeks after immobilization. Post mortem examination revealed chronic renal disease.

ACKNOWLEDGEMENT

The authors wish to thank Wildlife Pharmaceuticals, Fort Collins, Colorado 80524, for providing the medetomidine, ketamine, and atipamezol for these immobilizations.
LOW-DOSE CARFENTANIL TOGETHER WITH XYLAZINE IN WAPITI

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Traditionally, doses of carfentanil for ungulates have been in the range of 2.5 - 15 μg/kg. When xylazine has been mixed with the opioid, the carfentanil doses have been at the low end of this range. For wapiti doses of 2.5 - 5.0 μg/kg of carfentanil with doses in the range of 0.1 mg/kg xylazine have been reported as effective. The objective of this study was to test the effect of reversing the ratio of carfentanil:xyazine in wapiti. Twenty-three farmed wapiti stags of known weight (between 235 and 450 kg) were immobilized with doses of 0.5 μg/kg of carfentanil and 0.5 mg/kg of xylazine. Drugs were administered by blowgun or by hand-held syringe into heavy muscles of the hind limb. All of the animals became recumbent within 12 minutes (mean 7.9 minutes). The animals were immobilized for 10-15 minutes prior to administration of reversal agents. After routine procedures, including velvet antler removal and anthelmintic treatment, and been carried out, opioid and α-2 adrenergic antagonists were administered. Naltrexone was given at the rate of 100:1 naltrexone: carfentanil, and yohimbine was given at 0.12 mg/kg. No cases of recycling were seen during the next 72 hours when animals were intermittently monitored. At the doses used, the combination of carfentanil and xylazine proved to be both safe and reliable. A principle benefit was cost saving, both for carfentanil and naltrexone.
Zoo veterinarians frequently use potent immobilization agents which are potentially hazardous to humans. The risk of using these drugs and the need to develop a protocol to deal with accidental human exposure to them have been previously identified.\(^2\)\(^5\)\(^6\)\(^10\) The intent of this paper is to present one such protocol that has been developed at the Minnesota Zoological Garden, to describe the process involved in developing this protocol, to explore some of the ways in which accidental exposures have occurred, and to review guidelines for using immobilization agents.

The need to develop these protocols cannot be overemphasized. Although there have been only a handful of serious accidents involving immobilization agents, the use of these drugs is becoming more common, and the agents are becoming increasingly more potent and fast-acting. Of particular concern are the narcotic drugs used to immobilize hoofstock. Although the human lethal dose of carfentanil citrate (Wildnil, Wildlife Laboratories, 1401 Duff Drive, Ft. Collins, Colorado 80524, USA) is unknown, the minimum lethal dosage of 3-methylfentanyl, an illegal fentanyl derivative with a chemical structure amazingly similar to carfentanil, is only a few micrograms.\(^4\) In addition to their potency, the fentanyl compounds are very lipophilic and reach maximal brain concentration within 1 minute after IV injection.\(^4\) Victims of fentanyl analogue abuse have been found dead with the needle still in their arm.\(^9\) The treatment recommended for fentanyl and fentanyl derivative drug overdose is mechanical ventilation, preparation for total cardiopulmonary resuscitation, and high doses of naloxone.\(^4\)

In March 1993 a brief survey regarding the use of immobilization agents was sent to zoo veterinarians at 152 institutions which were members of the American Association of Zoological Parks and Aquariums. The intent of this survey was to attain information about the incidence of drug accidents that occur in zoos and the methods currently being suggested to handle these emergencies. Of the 108 institutions that responded, 77% currently use carfentanil or etorphine. Many other zoos indicated they would be using carfentanil in the near future. Approximately three-fourths of zoos currently using opioids conduct staff training and have written protocols regarding accidental human exposure to these agents. However, the content of the 51 protocols that were returned with the survey varied considerably.

The Minnesota Zoo recently enlisted the aide of the local medical community to help develop protocols for human exposure to immobilizing agents. An example of one of these protocols, the emergency protocol for accidental narcotic exposure, can be found in
Appendix A. Protocols were also developed regarding human exposure to the alpha agonists, xylazine and detomidine.

To develop these protocols we first contacted a toxicologist, a pharmacologist, and the physician in charge of emergency medicine from the Hennepin County Medical Center. We sent information to them about the agents we were using and how they were being used. The veterinarians and first aide staff from our zoo then met with these individuals to draft the protocols. After several revisions we came up with a final product.

The protocols were then presented to the hospital near the zoo where an accident victim would likely be transported. Our consulting physician contacted his emergency room colleagues at that hospital to discuss our rather unique situation and the content of the protocols.

The protocols were also presented to our local ambulance service. They developed a detailed policy of their own for emergency response and patient care. Their policy incorporated the one we had developed, described additional treatment and monitoring, and outlined how and where victims were to be transported. It also detailed the notification process, e.g. zoo personnel calls 911, who in turn dispatches the ambulance and air transport rescue service. The policy describes specifically where and how rescue units should respond.

Once the protocols had been accepted by the medical community, copies were laminated and placed in the immobilization and emergency kits. Further copies were distributed to staff and posted at all work areas where immobilization agents are used.

Finally, we conducted in house staff training. All staff that potentially would be present during immobilizations and the first aide staff were included. People were cautioned about the potency of the drugs being used and the importance of adhering to set guidelines for their use. We reviewed these guidelines and explained the treatment procedures that had been developed. Staff were shown where the emergency kit and antidotes are kept. They practiced opening needles and syringes, drawing up the antidote, and operating the oxygen tank and mask. In addition, formal first aide and cardiopulmonary resuscitation training is mandatory for all staff present during immobilizations. We also discussed the risks, clinical signs, and action to take in the event of human exposure to other drugs for which specific antidotes are not available, such as ketamine and tiletamine.

Although it is beyond the scope of this paper to review all aspects of emergency medicine that may pertain to treating a drug overdose, several aspects are worthy of review. The narcotic overdose treatment suggested in the Minnesota Zoo protocol (Appendix A) differs in several ways from earlier recommendations, and from most of the protocols returned with the survey.

First, most protocols reviewed recommended treating a narcotic overdose with 0.4 to 2 mg naloxone IV, with repeat injections given to effect every 2-3 minutes as needed. However, concern has been expressed that in some cases these doses may not be adequate. Our consulting physicians recommended that a patient who is losing consciousness due to a
narcotic as potent as carfentanil receive an initial dose of 30 mg naloxone hydrochloride (Narcan, Dupont Pharmaceuticals, P.O. Box 363, Manati, Puerto Rico 00701) intravenously. (See Appendix A for details.) Although smaller amounts of naloxone may be adequate in certain exposure cases when the amount of drug absorbed is quite small, one should be prepared for the worst case situation. The maximum effective human dose of naloxone has not been established, but it is not unusual to administer 8 mg of naloxone to unresponsive narcotic overdose patients, and doses up to 30 mg have been used in the Hennepin County Medical Center emergency room to treat cases of opioid overdose. (Keyler, Ling, Borys, per. comm.) In one study, doses of up to 4 mg/kg were administered intravenously to healthy volunteers with no significant toxic effects.3

Secondly, almost all protocols recommended using a tourniquet proximal to the injection site to help delay drug absorption. We could not find any evidence to support the effectiveness of a tourniquet for this purpose and felt that its application in this situation would not only take up valuable time, but may be dangerous.

Lastly, most protocols recommended injecting naloxone into the base of the tongue if intravenous access cannot be established. Although there are reports of successful narcotic reversal in humans using this route of administration, the use of naloxone sublingually or intralingually is controversial. Only a small volume can be injected by this route and there is also risk of intraoral bleeding and obstruction of the airway. We have therefore deleted this from our protocol.

As always, prevention is the best cure. Accidents involving immobilizing drugs happen in a variety of ways. Eighteen institutions surveyed (17% of all respondents) reported accidents. Thirty-nine percent of these (7 episodes) were due to sprays from blowdart syringes. Four of these accidents occurred during dart loading. One spray occurred when a dart impacted an animal being immobilized in a trailer. In another case, the contents of a blowdart syringe sprayed into the face of the operator when fired using an air pump. Another exposure resulted when a cooperative orangutan kindly returned a loaded dart to a keeper and in the process dislodged the needle sleeve causing the syringe to discharge in the keeper's face.

Three accidents occurred while administering drugs by hand. In one of these the hub of the syringe broke off during injection. In another, the syringe blew off the needle. In both cases the drugs sprayed into the face of the operator. In a third case, the animal jumped and the needle scratched the hand of the operator.

Four accidents reported were due to punctures from contaminated needles; several of these occurred during recapping. Two accidents happened during clean-up, one from being sprayed in the face while cleaning a half emptied dart and one while cleaning up powder from a broken bottle. A contaminated needle cap placed in the mouth was responsible for another exposure. And in one accident, a fired dart stopped at the end of the rifle barrel, the dart discharged and sprayed the operator in the face.
Fourteen of the eighteen cases reported involved etorphine. In three of these cases, naloxone was administered. One person required repeated injections at 15 to 30 minute intervals. Another etorphine victim was given approximately 0.5 ml of diprenorphine IM and exhibited adverse effects for three days. No treatment beyond immediate flushing of the exposed area was required in the remaining cases of etorphine exposure. Two exposures to telazol were reported. In one, mild symptoms developed but no treatment was required. One accident involved xylazine. Again, mild symptoms were experienced but no treatment was required. In the one ketamine exposure reported, no symptoms were experienced.

Guidelines for handling potent narcotics which may help reduce accidents are offered in Appendix B. It is important to develop a well thought out method of handling these agents, then follow these guidelines no matter how tense the clinical situation becomes. No immobilization is worth your life!
APPENDIX 1

EMERGENCY PROTOCOL FOR ACCIDENTAL NARCOTIC (CARFENTANIL/ETORPHINE)
EXPOSURE-MINNESOTA ZOOLOGICAL GARDEN

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Revised 20 March, 1993

Introduction

Carfentanil (Wildnil™) and etorphine (M99™) are synthetic opiates with a clinical potency 10,000 times that of morphine. They have a morphine like analgesic mode of action and produce a rapid immobilization following intramuscular injection. Carfentanil or etorphine are to be used only by a licensed veterinarian for the immobilization of exotic animals.

Assuming that a naloxone dose of 0.53-2.0mg/kg will be needed to reverse the effects of carfentanil in humans, the dose of naloxone for a 70 kg person will be 37.1-140.0mg. Naloxone is supplied for human use in 10 ml, 1 mg/ml, multiple dose vials. Thus, up to 14 vials may be required to reverse narcotic toxicity. Naloxone has a rapid onset (IV—one minute, IM—15 minutes) and short duration of action (20-60 minutes). There is no known toxic dose of naloxone in humans and it has been used in doses up to 4 mg/kg intravenously.

Clinical Effects of Carfentanil/Etorphine:

CNS: Sedation, lethargy, miosis, syncope, seizures, coma respiratory depression and arrest. RAPID LOSS OF CONSCIOUSNESS LEADS TO CESSATION OF BREATHING.

CV: Hypotension

GI: Nausea, vomiting

Carfentanil/etorphine emergency kit:

Whenever carfentanil/etorphine is used the antidote should be readily accessible. This kit will contain the following:

Naloxone 1 mg/ml, 10 ml multi-dose vials (#10) Five additional bottles will be kept in the large animal (beige and brown) immobilization kit.
23 ga. IV butterfly (#3)
35 ml syringe (#2)
10 ml syringe (#2)
20 ga. needle (#4)
Tourniquet (used to help establish IV access), alcohol swabs, gauze pads, tape, carfentanil/etorphine protocol, Hennepin County Poison Center sticker

In addition, the portable oxygen tank (orange case) complete with human mask, demand valve resuscitator, and nasal airway will be available whenever narcotics are being used.

TREATMENT OF HUMAN EXPOSURE TO CARFENTANIL OR ETORPHINE

DO NOT LEAVE THE PATIENT UNATTENDED! The veterinarian or animal health technician shall be in charge until the health and safety officer or paramedics arrive.

1. Designate people by name to:
a. Call for help. This individual will immediately call 911. Tell them there has been an narcotic overdose involving a fast acting, extremely lethal agent and request immediate emergency medical transport. Give them the exact location of the victim and tell them to which entrance the ambulance should respond. This person should then radio the health and safety officer and the switchboard giving them the same information, then proceed to meet the ambulance at the requested entrance and escort them to the patient location. All extraneous radio communications should cease.

b. Get emergency kit and oxygen case. This person will:

1. Draw up 30 ml (three bottles, 30 mg total) of naloxone into a 35 ml syringe using a 20 ga. (pink) needle.
2. Draw up 10 ml (one bottle, 10 mg) of naloxone into a 12 ml syringe using a 20 ga. needle.
3. Ready oxygen for use.

c. Monitor the victim's pulse and respiration rate.

d. Get water--cool or room temperature, not hot.

2. Place victim on side to prevent aspiration and obstruction of airway by tongue. Elevate legs.

3. If the exposure was via the oral, ocular, or dermal routes, flush the narcotic exposed area with copious amounts of water. Do not use hot water. Avoid self-contamination.

4. Initiate respiratory support as needed. Tilt the head back and pull the jaw forward. Remove any foreign material. Give 1 breath every 5 seconds. Use mouth to mouth resuscitation until the oxygen tank with the face mask and a demand valve resuscitator can be applied. Oxygen flow rate should be set at 6-10 liters/min.

5.Establish IV access, place one 23 ga. IV butterfly, tape in place. If the patient is unconscious, and IV access cannot be quickly established, proceed immediately to step 8.

6. If the patient is awake and talking--observe only.

7. If the patient is losing consciousness, unable to walk or follow commands, administer 30 ml of naloxone IV push (slowly, to effect) utilizing the prefilled syringe and butterfly.

8. If IV access is not immediately available and the patient is symptomatic, give 10 ml naloxone into any visible vein or IM into the shoulder or thigh. (If IM, divide dose and give 5 ml in each of two sites.) Then proceed again with attempts to place the catheter.

9. Continue to repeat naloxone doses (10-30 ml) until the patient wakes up and is able to talk. Multiple doses may be required.

10. Make sure 911 has been called and immediately transport to the emergency room according to the protocol established by ALF ambulance.

11. Send this protocol and all unopened vials of naloxone with the patient to the emergency room. Accompany victim to the hospital if possible.

12. Designate someone to call the Hennepin Regional Poison Center (347-3141) to page Dr. Ling (336-0716), Dr. Keyler (643-7836), and the Medical Toxicologist on call.
APPENDIX 2

GUIDELINES FOR HANDLING ETORPHINE AND CARFENTANIL—MINNESOTA ZOOLOGICAL GARDEN

General

These agents will be handled only by the veterinarians and animal health technicians. All legal restrictions and requirements regarding ordering, storage, use, inventory, record keeping, and disposal of these drugs will be strictly observed.

The veterinarian shall regularly review and rehearse safety and emergency procedures with staff.

The protocol for accidental exposure to narcotics will be kept with the immobilization and emergency kits at all times.

The emergency kit and oxygen tank must be readily accessible whenever these drugs are used. Never handle these drugs unless there is another person with you who is trained in CPR, is familiar with the narcotic treatment protocol, and has had experience giving IV injections. This includes cleaning darts!

Plan immobilizations whenever possible to occur when first aide personnel are on duty and the public is not present. If public is present, barricade and secure the area around the animal to be immobilized.

Before an Immobilization

Before beginning an immobilization using these agents, the veterinarian will contact the first aide officer on duty and inform them when and where the procedure will take place.

All personnel involved in an immobilization should be made aware of the potential danger of the drugs. No unnecessary personnel should be in the immediate vicinity.

Immobilizations should be done in an area with running water. If this is not possible, a bucket or jug of fresh water should be readily available.

When handling these agents, always wear goggles or safety glasses and a mask, or a face shield. Always wear gloves, preferably two pair. A long sleeved shirt or coveralls and long pants should be worn when possible.

The drugs are to be drawn up into the syringe just prior to use to avoid filled syringes laying around. Always draw up the animal’s reversal agent and label it before handling the immobilization agent. Record the name and amount of the immobilization agent before vials are opened.

Dart loading should be done slowly and methodically. Interruptions should be avoided. Stand well away from other people. Never over-pressurize the bottles containing these drugs. Keep the bevel of the syringe or dart needle facing away from you at all times. Use pliers to replace or remove needle caps.

Loaded darts that are transported should be capped and placed in a secure, impenetrable, unbreakable, leak-proof container that is clearly marked "carfentanil, etorphine". Place all materials contaminated with these drugs in a similar container, until they can be properly rinsed and disposed of in a sharps container.
Note: Avoid using etorphine and carfentanil in blowpipe syringes if at all possible. If you do use them in blowpipe syringes, always place a needle guard or syringe case over the needle when pressurizing the dart. Stand well away from other people and face away from them in case the dart flies off of the pressurizing syringe, or the syringe is dropped. A full face shield should be worn during loading and firing blowdarts. Use a hand held pump rather than a blow pipe.

During an Immobilization

Treat all guns, darts, syringes as if they were loaded firearms.

All personnel should be behind the person administering the drugs and out of the path of the projectile or of a possible ricochet or spray.

Goggles or a face shield should be worn when darts are fired.

A staff member trained to used dart projectors would do so only after the dart is made up and loaded into the projector by the veterinarian. The veterinarian must ensure safe handling of the projector and will remain in direct supervision during this time.

The location of any darts in an animal or its pen should be monitored. All persons involved in handling the immobilized animal should wear gloves. Identify the area of patient which has had contact with the drug and warn everyone to avoid touching this area. Rinse the area with water after the dart has been removed.

Only the veterinarian who is wearing gloves and eye protection should remove the dart from the animal. All other personnel should stand well away when the dart is removed. It is preferable to cover the dart as it is removed in case there is residual drug left in the syringe under pressure.

Used darts shall be stored in an impenetrable, non-breakable, leak-proof container labeled "carfentanil, etorphine".

Note: Hand injecting these agents can pose considerable risk. All personnel restraining the animal should wear full face protection and gloves. Use a luer-lock syringe and be prepared for the animal's response.

After an Immobilization

When not in use these drugs must be immediately returned to a class five safe. Make certain their use has been properly recorded.

Cleanup should be done immediately after an immobilization. Two trained staff members must be present and the emergency kit readily accessible. Contaminated syringes, needles, darts, and other materials will be handled only by the veterinarians or animal health technicians. Pressure should be released from all darts before cleaning takes place. Use pliers to remove needles. All contaminated equipment should be triple rinsed while wearing gloves, goggles, and a face mask or face shield. Disposable equipment should be placed in the sharps container after cleaning. All empty drug bottles will be triple rinsed by the veterinarian and disposed of in the sharps container.

First aide personnel should be informed when the procedure is over, the darts have been cleaned and the drugs have been returned to the safe.
LITERATURE CITED

MANUAL RESTRAINT SYSTEMS FOR THE MANAGEMENT OF NON-DOMESTIC HOOFSTOCK

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Numerous authors have discussed the need for effective restraint when working with non­
domestic species.1,3,8,10 Early approaches relied primarily on manual restraint, however the
stress and trauma experienced by both the animals and handlers frequently led to less than
optimal results. The advent of effective chemical restraint in the 1950's and 1960's,(and the
substantial developments in chemical restraint since that time ) has reduced the untoward
effects of restraint procedures, and has allowed the development of modern zoological
medicine and research.

In recent years a number of needs have developed (physiological research programs, disease
surveillance, economic limitations, decreased availability of chemical agents and game
ranching operations) which have led to major developments in manual restraint systems,
especially for procedures involving non-domestic hoofstock.

Manual restraint systems have historically been used in the management of some species.
Modified versions of commercial cattle chutes have been utilized for water buffalo, Bubalus
bubalus2; bison, Bison bison9; banteng, Bos javanicus5; and gaur, Bos gaurus.7 These systems
allowed blood and tissue collection, reproductive procedures and medical treatment.
However, when these systems were used for deer, antelope or equid species, problems and
injuries have occurred.

In recent years a number of restraint devices have been developed which take into account
the size, conformation, and behavior of the animals upon which they will be used. Like the
modified cattle chutes, these devices have their origins in commercial ranching operations
(deer, as opposed to cattle) and have been adapted for zoological applications.

Regardless of the specific restraint device used, a system must be in place to allow efficient
transfer of the animal between its holding enclosure and the restraint device. In an ideal
situation, the restraint device and transfer alleys should be integrated into the design of a
holding facility. A number of principles should be considered in this design:

* The walls of all holding and transfer areas should provide visual barriers to improve
control of the animal, to prevent unwanted visual stimulation, and to prevent
attempted flight through the walls or gates when the animal is stimulated.

* Transfer gates between holding pens, or from pens to transfer alleys, should be
placed in corners to produce a funnel effect.

* Doors between holding pens and transfer alleys should be placed, hinged and sized
such that the door can swing and be secured across the transfer alley to form a
barricade which restricts the animal's movement within the facility.
* In large holding facilities, long transfer alleys should contain turns to prevent the animal from building up speed and momentum during transfer.

* Where possible, catwalks should be provided (on the outside of the pen/alley) to allow operator access for control.

Restraint Devices

In its most basic form, an effective restraint device should limit movement in three planes: forward—backward, side to side, and up and down. Three basic designs have been employed to accomplish this task: lateral squeeze chutes, drop-floor cradles and horizontal-flip devices. Each of these will be discussed below, along with variations on each system.

Lateral Squeeze Chutes

In its most basic form, a lateral squeeze chute consists of a fixed side wall, a mobile side wall, which can be moved toward and away from the fixed wall, and front and rear gates for entry and exit. Once the animal enters the chute and the entry gate is closed, the mobile wall is advanced toward the fixed wall effectively squeezing the animal laterally. Removable panels are typically provided in both side walls to allow access to the animal. These devices have been used successfully on species ranging from small deer and antelope to giraffe. However, they generally have several deficiencies:

1) Animals can still move front to back, and may jump or sit down unless restrained very forcefully.
2) Wide sets of horns and antlers often limit the amount of lateral pressure that can be applied.
3) Access to the front and rear of the animal is often limited.

One variation that restricts upward movement is having the mobile wall hinged so that the top is able to move farther inward than the bottom creating a triangular inner space.

Another common variation of this system is the "rotunda squeeze". In this system, the animal is moved into a "round-house" or "interchange" with two walls that hinge off of a center pole. Once the animal has entered the structure, one wall is fixed, and the second wall is swung around to restrain the animal against the fixed wall. The advantages of this system are:

1) The wedge shaped area formed between the walls more effectively limits the front-to-back movement of the animal.
2) The "round-house" or "interchange" may facilitate the transfer of animals between holding pens or transfer alleys.

Drop-floor Cradle

Drop-floor cradle-type restraint systems have their origins in the deer farming industry of New Zealand. These devices operate on the principle that if an ungulate is held off of its feet it will often stop struggling and if it does struggle it will not be able to use its feet or
legs for leverage. The side walls of a drop-floor cradle system typically form a wedge (narrower at the floor, wider at the top). Once the animal is moved into the device, the floor is lowered either by gravity or hydraulically, and the animal's body is suspended in the wedge formed by the side walls. In most cases the animal is actually suspended at the shoulders and hips and only moderate pressure is placed on the abdomen or chest walls. Once the animal is suspended, operators are frequently needed to restrain the animal's head to prevent self-inflicted trauma and to apply pressure on the back to prevent dorso-ventral flexion of the trunk. Aside from providing more effective restraint than lateral squeeze chutes, drop-floor cradles provide several other advantages:

1) Once the floor has been lowered, and the animal is suspended by the side wall wedge, its feet protrude from the bottom, allowing good access for hoof care.
2) Since the animal cannot use its legs for leverage, it cannot move front-to-back. This allows both the front and rear doors to be opened if necessary for access to the head, neck and rump areas.

A commercially produced drop-floor cradle system is currently available in the United States (The Tamer, Fauna Research Inc., Red Hook, New York). This system is currently in use in several zoological institutions (St. Louis Zoo, San Diego Zoo, Oklahoma City Zoo, Fossil Rim Wildlife Center) and has been used successfully in several thousand procedures with zoological specimens.

**Horizontal-Flip Restraint Devices**

Restraint devices that rotate the animal horizontally (onto its side) have recently been developed at the Orana Park Wildlife Trust in New Zealand (Garland, personal communication). This device takes advantage of some of the principles involved in both lateral squeeze chutes and drop-floor cradles, and adds several new features. Unlike most other restraint devices, this system loads from the side with one side wall swinging into place as a push wall. Once the animal is loaded, the side wall is closed. The side walls (which are covered on their interior surface with concave pads to conform to the animal's body) are moved together with a ratchet system to restrain the animal. When the animal is restrained (as in the lateral-squeeze system) the entire restraint changer is rotated 90 degrees along its long axis. When the animal's feet leave the ground, it will generally stop struggling. The animal's head can then be easily restrained (it is now at operator's level) to prevent self-inflicted trauma. Doors at the bottom of the chamber can be opened to allow greater access to the feet, legs and ventral areas of the animal. A major drawback of this system is its limited access to the posterior of the animal. 6

**Conclusion**

The concept of "manual restraint" has been expanded to include the use of runways and chutes to facilitate the management of non-domestic hoofstock. 6 Recent developments in these restraints have:

1) Increased safety for both animal and operator.
2) Improved the degree of restraint possible without extreme stress.
3) Allow for flexibility in the size and type of animal to be restrained.
4) Decreased reliance on chemical restraint for routine procedures.

LITERATURE CITED

BIOTELEMETRY IN ZOO AND WILDLIFE HEALTH CARE

Robert A. Cook, VMD*

The advent of radio-tracking technology in the study of free-ranging wildlife has provided conservationists with invaluable field data on the movements of individual animals. As technology has progressed it has been possible to expand radio-tracking techniques to also include the radio transmission of other biological data, such as body temperature and heart rate. The veterinarians' role in biotelemetry studies are multi-fold:

To assist in the safe and proper placement of monitoring equipment.

To identify important health questions which can be answered using remote monitoring systems.

To assist in data collection and analysis.

A multi-disciplinary approach was elected in formation of The Biotelemetry Studies Unit of NYZS/The Wildlife Conservation with the assistance of a start-up grant awarded by the Institute of Museum Services. A biotelemetry laboratory was constructed in the Wildlife Health Center, guided by Drs. Fred Koontz, William Karesh, and Robert Cook. The facility located on the grounds of the International Wildlife Conservation Park/Bronx Zoo is unique in that it provides a sight for controlled study of new technologies prior to general application. Curators, clinical veterinarians, field veterinarians, and field biologists together design, implement and analyze the results of biotelemetry projects. Those projects which are in progress or completed include:


ASSISTED INTUBATION TECHNIQUES IN LLAMAS (Lama glama): (RETROGRADE INTUBATION)

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Objectives

In the llama whenever general anesthesia (GA) is indicated, endotracheal intubation is recommended for two main reasons. First, pulmonary physiologic changes that occur during GA leads to a decrease in functional residual capacity and closure of small airways which can lead to life threatening hypoxemia. Second, and more importantly is that even after 24-48 hours fasting the ‘C-1’ compartment of the complex stomach still contains ingesta. The normal cycle of C-1 is to move ingesta cephalad for re-chewing, making the animal at risk for pulmonary aspiration.

In the llama model endotracheal intubation is difficult for multiple reasons (Fig. 1):
1) Narrow space between rami of the mandible.
2) Inability to open mouth widely (Fig. 1A)
3) Prominent elevation on the dorsum of the caudal aspect of the tongue (Fig. 1B)
4) An elongated soft palate that may be situation either ventral (stippled) or dorsal to the epiglottis (Fig. 1C).

Three methods of endotracheal intubation are currently used:
1) Blind oral intubation
2) Laryngoscopy using a straight blade.
3) Bougie (stylet)

Of all three techniques, the latter (3) is generally preferred for other applications. All three of these methods tend to be less than ideal. We propose retrograde intubation as a novel approach to the intubation of this species.

Methods

I Model:
Llama (Lama glama): The decapitated head (neck length 2 feet) of a five year old male llama which had died within 24 hours and kept refrigerated overnight.
II Instruments:
1) Cook Retrograde Intubation Set (#C-Retro-11.0-70-38E-110).
   Contents:
   a) 6 cc syringe
   b) 18 gauge angiocatheter (5 cm long)
   c) J-tip guidewire, length 110 cm, width 0.97 mm
   d) Teflon guide catheter, length 70 cm, width 11 Fr
2) Miller #5 straight laryngoscopic blade
3) Endotracheal tube, length 50 cm, ID 12 mm

III Procedure:
The head of the llama was hyperextended and the 18 gauge angiocatheter was introduced into the ventral surface of the trachea (Fig. 2). The entrance site was approximately 8-10 cm distal to the thyroid cartilage and midline to the trachea. The angiocatheter was first entered at a 90° angle to the long axis of the trachea. Once in the trachea (verified by aspirating for air with the 6 cc syringe), the angle was changed to 45° (where the tip of the needle is pointing cephalad). The needle was removed and the plastic catheter was left in place (again, catheter tip facing cephalad). The J-tip guidewire was advanced through the angiocatheter into the trachea and advanced cephalad. Because in the llama the soft palate lies dorsal to the epiglottis the guidewire came out easily through the nostril (our goal was to intubate orally, Fig. 2). The procedure was repeated as above but before inserting the guidewire a Miller #5 straight blade was introduced orally over the tongue in order to lift the soft palate and block the nasal passage so that the guidewire would exit from the oral cavity (Fig. 3). Once this technique was established the guidewire could be passed easily out through the oral cavity (Fig. 4). With the guidewire pulled out of the oral cavity to an appropriate length, the angiocatheter was removed and a hemostat was clamped to the guidewire at the level of the neck (to anchor the wire). The teflon guide catheter was then placed over the guidewire and advanced until resistance was met (the distal tip of the guide catheter was now flush against the internal surface of the trachea at the entry point of the guidewire, Fig. 5). The guidewire, threaded within the guide catheter, was then used as a guided stylet for the endotracheal tube. The endotracheal tube was then easily placed into the trachea (Fig. 6). Once the endotracheal tube was in place, the hemostat was removed and the guidewire and guiding catheter were removed by pulling both simultaneously out through the mouth (Fig. 7). The endotracheal tube was then advanced to an appropriate depth. This procedure took us less then 1.5 minutes. Post intubation the trachea and larynx were opened and inspected showing no major trauma to any structure.

Discussion
The guidewire initially exited out of the nasal cavity. It is possible that this could be capitalized upon for nasal intubation. However, the nasal cavity tends to be narrow (permitting only small tubes, increasing airway resistance), and is prone to hemorrhage. By including the Miller#5 straight blade this tendency was eliminated, allowing the guidewire to exit out of the oral cavity as desired. This technique is simple, fast, effective, atraumatic, and requires little training.
Conclusion

Retrograde intubation is an effective method of intubation for this species. In the opinion of the authors, this technique is equally applicable in other species where direct, oropharyngeal visualization of the larynx for intubation is often difficult and time consuming. For example, this technique is used frequently in humans and in laboratory rabbits. Retrograde intubation may in the future be considered the intubation technique of choice for:
1) animals that are difficult to intubate normally through the oropharyngeal route (e.g., orangutan, warthog, koala)
2) in those animals with airway pathology that leads to distortion of the anatomy and/or mass effect.

REFERENCE LITERATURE

Camelidae/General Veterinary:

Human:
Figure 1. Mid-sagittal view of llama head and neck (esophagus removed). A - narrow oral aperture. B - elevated dorsal-caudal aspect of tongue. C - Elongated soft palate lying on either the dorsal or ventral (stippled) surface of epiglottis. D - Epiglottis.

Figure 2. J - tip guide wire travelling cephalad through the angiocath into the trachea and inadvertently exiting out of the nasal cavity.
Figure 3. Miller #5 blade inserted into oral pharynx, lifting (arrow) the soft palate cephalad (before advancing the J - wire) in order to block the nasopharynx.

Figure 4. J - wire travelling through 18 gauge angiocath and now exiting out of the oral cavity. Miller #5 blade still in place blocking the nasopharynx.
Figure 5. Teflon guiding catheter threaded over guidewire. The guiding catheter ends up flush with the trachea. Note: 18 g angiocath has been removed and the guidewire clamped with a hemostat (not shown).

Figure 6. Endotracheal tube threaded over entire structure (guide wire and guide catheter), and advanced into trachea (note: hemostat at the point still clamped to guide wire).
Figure 7. Hemostat is unclasped. The guidewire and guide catheter are removed simultaneously (arrow). The endotracheal tube is advanced to desired depth.
COMMON DISEASES AND TREATMENTS IN MACROPODS

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Introduction

The Family Macropodidae comprises many species of Kangaroos and Wallabies. They range in size from small rufous hare-wallabies (Lagorchestes hirsutus) to large red kangaroos (Macropus rufus). These animals are generally quite disease resistant in the wild but in captivity are susceptible to a number of disease entities.

Diseases in the Wild

By far the most common problem Macropods face in the wild is trauma. Trauma can result from motor vehicle accidents (MVA's), dog attacks, cat attacks or shooting accidents. Treatment for trauma is extrapolated from companion animals but it should be remembered that Macropods only have two weight bearing limbs and therefore treatment should be aimed at providing early weight bearing or secondary problems can result. Cat attacks prove very difficult to manage as the majority of Australian marsupials lack any innate immunity against Pasteurella spp. and succumb from septicaemia despite aggressive therapy. Tetanus and coccidiosis both occur in the wild but are more common in captivity.

Anesthesia

There are a number of agents suitable for anesthetising macropods e.g. ketamine 5mg/kg /xylazine 5mg/kg, ketamine 10mg/kg /diazepam 0.5mg/kg, etorphine 0.1mg/kg /acepromazine 0.4mg/kg. I prefer to induce anaesthesia with zoletil (zolazepam/tiletamine) - either 10-20mg/kg intramuscularly or 2.5-5mg/kg intravenously. Macropods are notoriously difficult to intubate and as such I don't try, preferring to maintain anaesthesia via a mask using oxygen and halothane. Zoletil plus local anaesthesia is useful for minor surgical procedures, e.g. abscesses, castrations, suturing.

Diseases in Captivity

(i) Tetanus is common in captive macropods. Clinical signs are as for companion animals, i.e. muscle rigidity, hyperaesthesia and convulsions. Sometimes animals are found dead in good condition with no post-mortem pathology. Treatment includes sedation using diazepam (1mg/kg), high doses of penicillin and tetanus antitoxin. Keeping the animal in a dark, quiet environment aids treatment. Prevention is by the use of tetanus toxoid or five in one vaccine (multivalent clostridial vaccine).
(ii) **Lumpy jaw** is caused by bacteria invading the alveolus of molar teeth and subsequently spreading to adjacent bone and/or soft tissue. Predisposing conditions include poor diet, overcrowding, poor husbandry. Treatment is almost always unrewarding. Prevention involves avoiding predisposing factors.

(iii) **Strongyloides** infection of the stomach. This species of nematode which has not been named can cause hypoproteinaemia leading to death. Clinical signs include loss of weight, dependent edema and lethargy. Diagnosis can be difficult as it involves a Baermann technique which relies on active larvae separating themselves from the faecal mass, but can be confirmed by histopathology after post-mortem. Treatment is with the appropriate anthelmintic. I prefer Ivermectin at 200ug/kg subcutaneously or orally. Benzimidazoles are also effective but mebendazole can be toxic in certain species, e.g. Red Legged Pademelons (*Thylogale stigmatica*).

(iv) **Toxoplasmosis** caused by *Toxoplasma gondii* can cause sudden death in macropods. Most Australian marsupials have no natural antibody to this protozoan parasite. Animals are generally found dead and diagnosis is made by histopathology. Sometimes animals are lethargic but ante-mortem diagnosis can be difficult. Antibody levels are not always diagnostic. Treatment can be tried with sulphonamides and anti-malarial drugs but is generally unrewarding. Vaccination with *Hammondia hammondi* oocysts by the oral route may offer partial protection against clinical effects.

(v) **Capture myopathy** is generally a sequel to extended capture procedures or confining nervous animals in small enclosures. Animals become lethargic, stiff, exhibit torticollis and opisthotonus followed by paralysis and death. Treatment includes fluids, sedation, antibiotics, corticosteroids and high doses of Vitamin E. Prevention is always preferable and involves quick capture techniques and use of short and long acting neurolept analgesics, e.g. Trilafon (perphenazine enanthate), Modecate (fluphenazine decanoate), Valium (diazepam) or Stresnil (azaparone). I now prefer to immobilize larger macropods by projectile syringe rather than run the risks of capture myopathy with manual capture.

**Diseases of Orphaned Macropods**

The diseases seen in hand-reared orphaned Macropods are generally attributable to failure of passive transfer of maternal antibody. As the gestation period of these animals is extremely short and young are born in an altricial state it seems logical that this group of animals acquire their immunity through their dam’s milk.

**Coccidiosis** is common in hand-reared Eastern Grey Kangaroos (*Macropus giganteus*) and Western Grey Kangaroos (*Macropus fuliginosis*). Clinical signs include diarrhea, dysentery, anorexia, leading to dehydration and death. Treatment is generally unrewarding but coccidiostatic drugs such as amprolium, sulphonamides and Zoaquin.
(Diiodohydroxyquinoline) can be used. Newer coccidiocidal drugs, e.g. Baycox (toltrazuril) 10mg/kg orally are having better success. Plasma transfusions are being trialled as a preventative measure with good results.

**Candidiasis** or thrush caused by *Candida albicans* causes diarrhea in orphaned, hand-reared Macropods. Diagnosis can be made by gram stains of fecal smears. Nilstat (nystatin) 5,000 i.u./kg TID for three days is successful in treating this disease.

**Pneumonia** is common in hypothermic orphaned Macropods. The causative bacteria is generally gram negative, e.g. *E. coli* or *Klebsiella* spp. My treatment of choice is amikacin sulphate 10mg/kg BID for five days which is generally effective.

**Kangaroo Pox** caused by a pox virus causes wart-like lesions on the extremities. These are generally self-limiting and require no treatment.

**Cloacal prolapses** and **intestinal intussusceptions** occur sporadically. Prolapses are self-evident and respond to a purse-string suture left in place for approximately three days. Clinical signs of intussusceptions include severe depression and frank blood from the rectum. Treatment generally requires resection and anastomosis of the affected bowel. The cause of these problems is unknown.

I have presented only the common diseases seen in macropods in Australia. Limitation of time precludes me going into any more detail.
USE OF TILETAMINE-ZOLAZEPAM IN THE IMMOBILIZATION OF MARSUPIALS

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A number of injectable anesthetic and tranquilizing agents have been employed to immobilize marsupials, both in captive and free-ranging settings. This report will deal with the use of the combined tiletamine-zolazepam anesthetic (Zoletil or Telazol) either as the sole agent or in conjunction with other agents in the immobilization of a variety of marsupial species in both captive and free-ranging settings.

Anesthetic and medical records from marsupial species held at the Royal Melbourne Zoo, Healesville Sanctuary, Werribee Zoological Park and the San Diego Zoo were examined. In addition, reports from biologists whose work involved immobilization of marsupials under field conditions were solicited. Immobilizations were performed for a variety of reasons, including capture of presumed healthy animals for identification and obtaining biological data, immobilization of animals for treatment of illness or injuries, immobilization of animals for transport or transfer and immobilization of animals for surgical procedures. In some cases the tiletamine-zolazepam combination was the sole immobilization agent, while in other cases it was used in conjunction with isoflurane gas inhalation anesthetic or supplemented with intravenous ketamine hydrochloride anesthetic. In addition, the tiletamine-zolazepam combination was used in conjunction with short (azaperone) and long-acting (perphenazine enanthate) neuroleptic agents in an attempt to reduce problems associated with the transfer of animals, especially from large open paddocks into smaller enclosures.

The tiletamine-zolazepam combination appears to be a good injectable agent for immobilization of marsupials. The rapid induction time, good muscle relaxation and analgesia (with appropriate dose) and smooth recovery make it an ideal agent for use under field conditions or as an initial immobilizing agent to be used in conjunction with isoflurane gas inhalation anesthesia for prolonged procedures. While Holz (1992) has reported mortality in squirrel gliders (Petaurus norfolcensis) associated with 10 mg/kg tiletamine-zolazepam immobilization, we have seen no mortalities associated with the use of this drug combination in 15 other species of marsupials. There was some variation in response between species, in the response of different individuals of the same species and in individual reaction to the same dosage on different occasions. The level of relaxation, depth of anesthesia and occurrence of undesirable reactions such as excessive salivation, response to noxious stimuli and muscular activity while immobilized appeared to be influenced by several factors, including dose, success of initial injection and time allowed after injection before handling the animal.
Our experience with the use of tiletamine-zolazepam combination as an injectable immobilizing agent has led us to the following conclusions:

1. Previously published doses and doses extrapolated from eutherian mammals of comparable size appear to be higher than are actually needed for immobilization of marsupials.

2. Undesirable reactions such as excessive salivation and prolonged recovery times are seen more frequently with higher doses of tiletamine-zolazepam. Response to noxious stimuli, hyperexcitability and muscular activity while immobilized may occur if handling is attempted before induction is complete.

3. The time from injection to initial effect with this drug combination can be quite brief (1-3 minutes). Animals can be safely handled almost as soon as they go into lateral or sternal recumbency. However, waiting at least 15 minutes after initial injection before handling the animal appears to minimize responses by the animal to stimuli and allow the animal to reach a deeper level of anesthesia with the same dose of tiletamine-zolazepam than if handled earlier.

4. The use of isoflurane/oxygen inhalation anesthesia, administered either by facemask or endotracheal tube intubation, is a useful adjunct to tiletamine-zolazepam immobilization. This is particularly useful if the animal is required to undergo extensive surgical procedures or prolonged examination procedures.

5. Supplementation with intravenous ketamine hydrochloride at 1-3 mg/kg was useful under field conditions to achieve greater depth of anesthesia or for prolonged procedures. No significant adverse effects were seen with the use of supplemental intravenous ketamine hydrochloride.

6. Atropine at 0.02-0.04 mg/kg IM was useful to control excessive salivation seen either as idiosyncratic response to tiletamine-zolazepam combination in some individuals or in association with higher dosages of tiletamine-zolazepam.

7. Recovery time was prolonged and animals showed a greater tendency to struggling during recovery when higher doses of tiletamine-zolazepam were used. Use of isoflurane inhalation anesthesia in addition to the initial tiletamine-zolazepam immobilization did not seem to influence the recovery time or character. When immobilized with tiletamine-zolazepam combination at or near the "target" dose, recovery in most animals was uneventful and complete within 1-2 hours post-initial injection.

8. Use of neuroleptics (azaperone at 1 g/kg IM and perphenazine enanthate at 0.5-3 mg/kg IM) in animals immobilized with tiletamine-zolazepam combination appeared to be effective in reducing the incidence of self-induced injury, stress and attempts to escape associated with transfer-translocation of animals from large or free-range type enclosures to smaller enclosures.
9. The "target" doses listed in the accompanying table represent the preferred
dose of tiletamine-zolazepam for most procedures. For simple procedures such as transfers
between enclosures, immobilization to permit crating an animal for shipment or minor
procedures such as bandage changes, lower doses may be used with good results and the
advantage of more rapid recovery times.
### Tiletamine-Zolazepam Combination Immobilization in Various Marsupials

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Immob.</th>
<th>Dose Range (mg/kg)</th>
<th>Target Dose (mg/kg)</th>
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</thead>
<tbody>
<tr>
<td>Red kangaroo <em>(Macropus rufus)</em></td>
<td>63</td>
<td>3.1-20.0</td>
<td>7-10</td>
</tr>
<tr>
<td>Eastern grey kangaroo <em>(Macropus giganteus)</em></td>
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AUCKLAND ZOO'S VETERINARY INVOLVEMENT IN THE KAKAPO RECOVERY PROGRAM

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Introduction

The Kakapo, *Strigops habroptilus*, a large, flightless, nocturnal parrot, is one of the rarest and most endangered species in the world. Endemic to New Zealand, the Kakapo was once widespread on the North, South & Stewart Islands. Now approximately 50 birds are known to survive on four islands Codfish Island, Maud Island, Little Barrier Island and Mana Island.

The decline of the Kakapo is due to several factors. The introduction of mammalian predators including the feral cat, mustelids and three species of rat have had the greatest effect. Habitat destruction and competition from introduced herbivores have also contributed. It is not known if introduced avian diseases have had an effect in the past on the decline of the bird.

In 1989 New Zealand's Department of Conservation (DOC) initiated a detailed recovery plan for Kakapo in an attempt to halt its decline. The plan is updated regularly following meetings of the Kakapo Recovery Team. The plan includes several conservation objectives - those which involve Auckland Zoo relate to the development of techniques using captive management with other native parrot species, the Kaka (*Nestor meridionalis* / *Nestor septentrionalis*) and Kea (*Nestor notabilis*) and the artificial incubation rearing of Kakapo chicks. As a result, prior to 1991, Auckland Zoo's curatorial and keeping staff developed suitable skills which have been used on Kakapo should any captive management be required.

Over recent years the Kakapo breeding success has been very poor. In 1991, 2 male chicks were parent raised on Little Barrier Island. This was the first time breeding had occurred since 1981. It was thought that the newly instituted practice of supplementary feeding on the island had played a significant part. After three infertile eggs had been transferred to the Zoo in January/February 1991, a fourth fertile egg arrived which was incubated and hatched artificially. The chick died suddenly at 4 days old.

In March 1992, over a period of one month, three malnourished chicks were flown from Codfish Island to be handraised at the Zoo. Of the three one survived and has been relocated to Maud Island, a sanctuary off the north coast of New Zealand's South Island.

This year, 1993, has seen very little breeding activity. A sole fertile egg hatched by the parent disappeared after three days.

This paper describes Auckland Zoo's veterinary involvement with Kakapo since 1991 which has included attention to both captive and wild birds.
**Disease Prevention**

As the captive management of Kakapo was seen as a very high priority by the Zoo a separate incubation/brooder facility was organized in the veterinary facility. A 24 hour surveillance was maintained each time eggs or chicks were brought to the Zoo.

Advice was given to the curator and keeping staff involved on the following topics:

i) hygiene standards - overalls/gowns to be worn, cover over shoes. Strict personal hygiene, disinfection types and use.

ii) normal physical characteristics of nestlings

iii) signs and causes of ill-health

iv) outline of possible treatments should ill-health occur

**Treatments (including surgery and anesthesia)**

Very few veterinary treatments have been performed on Kakapo.

The Zoo's first involvement was on 25 May 1992 when the first Kakapo brought to the Zoo at the age of 59 days (45 days after arriving) accidentally ingested a soft feeding tube whilst being fed. Immediate attention was required when the tubing couldn't be removed from the conscious bird. The bird, weighing 1273 g was masked down using Isoflurane (Forane, Abbott) and intubated using a 3.5mm endotracheal tube. Internal oesophageal and crop laparoscopic examination revealed a large quantity of food in the crop. This was removed by suction using plastic tubing and a syringe and repeated flushing using warm water whilst the birds head was raised to avoid regurgitation. By using the 3 mm diameter rigid laparoscope (Storz, Germany) the tubing could then be seen and removed with long crocodile forceps. Apart from transient melaena the bird recovered uneventfully.

This same chick continued to develop normally except for the occasional regurgitation of small quantities of partially digested food. The frequency of this behavior prior to and after the incident did not change.

Early on the 7th June, the bird aged 72 days, suddenly and briefly showed signs of respiratory embarrassment which included mouth breathing with its head tilted upwards. Normal behavior followed which included the ingestion of normal quantities of food. The signs then reappeared six and a half hours later. Treatment was instituted and was based around the differential diagnoses of acute Aspergillosis (the birds were kept on a natural leaf mold substrate) and inhalation pneumonia. It included oxygen therapy via a brooder box, nebulization with enrofloxacin (Baytril 2.5%, Bayer NZ Ltd), amphotericin B (Fungizone, Squibb) and levamisole (Nilverm, Pitman-Moore NZ Ltd). In addition the chick was injected with enrofloxacin and flunixin meglumide (Finadyne, Schering Plough Pty Ltd). The bird progressively worsened despite treatment until it collapsed. An air sac tube was inserted under isoflurane anaesthesia. It however died fifteen minutes later. A detailed necropsy was performed early next morning.
As aspergillosis was a possible differential diagnosis, the leaf mold substrate was removed immediately and prophylactic treatment was instituted for the two remaining chicks, which included initially 5 fluorocytosine (Alcobon, Roche) and then itraconazole (Sporinox Janssen-Cilag) (both in feed) and vitamin A (Vetade, Veterinary Ethicals Ltd).

On 13 June the second Kakapo aged 65 days and weighing 1278g became agitated at periods throughout the day and was reluctant to eat during the evening. Treatment at this stage included enrofloxacin (Baytril Bayer NZ Ltd 2.5% ) and flunixin meglumine (Finadyne, Schering Plough Pty Ltd) by injection. Forced expiration and tail bobbing were noted later that evening. The next day respiration appeared worse; treatment was continued. Normal periods of activity were noted although there was a reluctance to eat. Blood samples were taken for hematology and biochemistry but unfortunately the chick died very suddenly that evening. Necropsies of this and the previous chick are discussed below.

The third Kakapo at this stage was fed less Roudybush formula decreasing both volume and concentration. In addition after consultation with other veterinarians, it was decided to treat it with cephalixin (Ceporex, Pitman-Moore NZ Ltd) and continue the itraconazole. She was also treated with praziquantel (Droncit, Bayer NZ Ltd) as she had passed an as yet unnamed cestode. This bird survived to weaning and is now self supporting within a captive environment on Maud Island.

On the 18th March 1993 I was requested by the local conservancy of DOC to attend an injured Kakapo on Little Barrier Island which is 25 minutes flying time by helicopter from Auckland. The male bird was found by one of the DOC scientists on the island near to the "track and bowl" of another male. The bird was brought down to the ranger's house where it was examined. There was trauma to the right upper eyelid (a triangular piece of eyelid was missing) and corneal lacerations were noticed. On top of the head there was an area of feather loss approximately 5cm in diameter, with two smaller areas 2cm diameter of full thickness skin loss. The injuries appeared to be 2-3 days old. The wounds were cleaned and debrided.

The bird was treated with injectable and oral antibiotics, injectable anti-inflammatories, vitamins and subcutaneous fluids. The eye was treated with antibiotic cream twice daily. Treatment was continued for 5 days as the wild bird could be held with a minimum amount of stress in a tuatara enclosure. After five days the bird was re-examined and released. Using radio-telemetry techniques the bird was caught again one week later. The injuries had greatly improved.

On the 21st March I was asked to attend another Kakapo on the island. This male bird was found on examination by the DOC scientists to have its transmitter harness growing into the cranial aspect of both wings. It was thought surgery maybe required and so a surgery table plus portable anaesthetic machine was organized on top of the mountain where the bird was normally located during the booming season. However, the bird was eventually caught using a baited trap four days later (the transmitter harness had been lost). Deep, clean wounds

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4cm x 1cm were seen on the cranial aspect of both wings. The wounds were cleaned and debrided and a tail transmitter was attached. The booming activity of the males seemingly caused the harness to grow into the skin. Both birds are doing well.

Necropsies

Four necropsies have been performed on Kakapo since 1991. The first necropsy of the four day old chick was performed by a local pathologist. A diagnosis of inhalation pneumonia with a pure growth of *Klebsiella ozaenae* on cloacal swab and a combined growth of two *Klebsiella* species, *ozaenae* and *pneumoniae* was found on the liver surface.

Of the other three Kakapo two involved the handraised nestlings and the other one was an adult male found dead on Little Barrier Island in November 1992. Detailed necropsies were performed and included taking measurements of morphometric parameters, weights and dimensions of organs.

All three birds were in excellent condition. The first chick had evidence of very hemorrhagic lungs with small green plaques of material in the opening of the syrinx. Histopathology revealed an acute necrotizing pneumonia associated with the inhalation of food particles.

The second Kakapo has a similar gross picture with congested hemorrhagic lungs but also included thickening of the crop mucosa and the gizzard had a piece of twig which had penetrated the koilin layer causing localized inflammation in the muscularis. Histopathology revealed an acute aspiration pneumonitis and intra-pulmonary hemorrhage with a focal chronic granulomatous air sacculitis and pneumonia.

It was considered that the twig may have contributed to the regurgitation and inhalation of food although this remains speculative.

Of these latter two necropsies, the cause of the inhalation pneumonia is not entirely clear. Frequency of regurgitation was increased with the use of a more concentrated feeding formula and this may have increased the likelihood of inhalation.

The fourth Kakapo, a male found dead on Little Barrier Island was in excellent pre-booming condition, weighing 2.76 kg. It had many superficial injuries to the legs, on the right and left body walls and on the neck. Histopathology revealed edema and hemorrhage in the parabronchi and air capillaries and congestion throughout both lungs which was consistent with acute stress and compatible with the history of fighting between males.

Data Collection

Throughout the period of the Zoo's involvement with Kakapo, it has been important to develop a baseline data bank of information. Detailed records have been kept on the handraising procedure, behavioral characteristics, weight gain, respiratory rates and food preferences. In addition crop and cloacal swabs were taken daily in their first week, every
second day the next week and every third day the following week. The swabs were gram stained and cultured and were taken to monitor any changes in the gastrointestinal flora. ²

Few opportunities have arisen to obtain blood samples for serum biochemistry and hematology. However a detailed proposal by 3 veterinarians and 1 DOC scientist to obtain normal baseline data from wild Kakapo when there is a prior management opportunity is currently under consideration by DOC. The collection of blood samples for hematology and biochemistry and surveillance serology on certain diseases (e.g. chlamydiosis, yersiniosis and Newcastle disease) is recommended.

Cloacal, choanal and crop swabs could be taken to look at normal gastrointestinal tract flora and faecal samples for parasitology. The birds could also have a full veterinary examination.

In this way a useful data bank of information could be collected which would be of great benefit to the future captive management of these birds.

Liaison

Throughout the period of the Zoo’s involvement with the Kakapo recovery programme veterinary input has been sought on a variety of topics. These have included treatments, feeding regimes, necropsy and histopathology findings. Liaison with DOC staff, veterinary specialists at the local animal health laboratory and at Massey University Veterinary school, staff at Auckland Zoo, the Kakapo sponsors, Comalco, and the press have all been required at various stages. In addition veterinarians and pharmacologists overseas have been contacted.

A key role of the veterinarian has been the interpretation of clinical and pathological data to non-veterinarians involved in the programme.

Conclusion

Over the past three years veterinary input into many aspects of the Kakapo Recovery Programme has increased markedly. DOC is also increasingly seeking required veterinary input and advice for other endangered species. To date these have included the Tuatara (Sphenodon punctatus), the Takahe (Notornis mantelli), the Stitchbird (Notiomystis cincta), the New Zealand Short-tailed Bat (Mystacina tuberculata), Little Blue Penguin (Eudyptula minor), North Island Brown Kiwi (Apteryx australis mantelli), Kaka (Nestor meridionalis septentrionalis), and the Striped dolphin (Stenella caeruloalba).

In the future there are likely to be great opportunities for considerably more specialized veterinary input.
ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. R. Jakob-Hoff, Senior Curator at Auckland Zoo for help in preparation of this paper. Thanks also to Massey University Veterinary School and Auckland Animal Health Laboratory, to DOC staff, to Dr. Diane Fraser for reviewing this paper, to Mick Sibley, Curator Zoological Projects at Auckland Zoo and Maria Boyland and Barbara Bates, veterinary assistants at Auckland Zoo for their support. Karen Searle and Helen Maybury's skill in typing this paper are greatly appreciated.

LITERATURE CITED

OPPORTUNISTIC INFECTIONS, CANCER AND HEMATOLOGIC DISORDERS ASSOCIATED WITH RETROVIRUS INFECTION IN THE KOALA

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The population of free-roaming koalas (Phascolarctos cinereus) is slowly declining as a result of habitat loss and reduced fecundity in metapopulations due to infection with Chlamydia psittaci. Additional documented causes of mortality due to disease include bacterial pneumonia and neoplasms of the lymphoid organs and skeletal system.

A review of the pathology records of the Zoological Society of San Diego (ZSSD) revealed the most prevalent causes of mortality in the koala colony to be neoplasia and severe debilitating disease due to opportunistic infection. Neoplasms included multicentric lymphoma, osteosarcoma of the long bones and osteochondroma-like tumors of the skull. Pneumonia, as a result of infection with Bordetella bronchiseptica, Klebsiella pneumoniae and Cryptococcus neoformans, with dissemination of the latter to the central nervous system, was documented. Lymphoid depletion and/or pancytopenia, either idiopathic in nature or due to overwhelming infection, was also diagnosed.

The possibility existed that these diseases were indirectly the result of modulation/suppression of the immune system by an unknown infectious agent. In order to investigate this possibility, primary peripheral blood lymphocyte (PBL) cultures were established. Cultures were monitored visually for cytopathology and supernatants were assayed for Mg²⁺ and Mn²⁺-dependent reverse transcriptase activity. Reverse transcriptase activity was not detected in any of the cultures from any of the animals tested; however, electron microscopic examination of fixed cells from the majority of the koalas tested revealed virus particles, compatible in size and morphology with a retrovirus. Attempts to immortalize the koala primary PBL cultures or adapt the virus to a continuous marsupial cell line for the purpose of propagating virus to be used as an antigen target for antibody detection have been unsuccessful. As a result, several pairs of degenerate oligonucleotide primers were used in the polymerase chain reaction (PCR) in an attempt to detect active virus infection. With genomic DNA from fresh PBL, fresh buffy coat cells or cultured PBL as the target template, PCR amplification of viral DNA was achieved with one of the pairs of primers. This DNA fragment, representing a portion of the reverse transcriptase region of the polymerase gene, was molecularly closed and sequenced. Computer analysis of the sequence confirmed it to be that of a retrovirus.

Retrovirus infection has been detected by PCR in the PBL of the majority of the animals in the ZSSD koala colony. Correlation of PCR-based detection with electron microscopic examination of cultured PBL is nearly 100 percent. Therefore, PCR offers a rapid, sensitive and less costly technique for detection of active infection. Types of diseases experienced by koalas in which retrovirus infection has been detected include those documented in the
pathology records and discussed above. In addition, a number of virus-infected koalas in the colony have remained relatively healthy, a few with positive Cryptococcus spp. nasal cultures of a transient Cryptosporidium infection.

These results provide a basis and reagents for investigating additional factors influencing population dynamics of metapopulations of free-roaming koalas. Retrospective molecular epidemiological studies of specific causes of reduced fecundity and/or mortality in wildlife is becoming more practical due to the ability to detect the presence of infectious agents in a wide variety of tissues and body fluids with PCR. A number of opportunistic infections have been documented in several species of both captive-raised and free-roaming marsupials. The results described in this abstract should serve as a foundation for pursuing studies directed at identifying underlying factors involved with these diseases.
Small populations are vulnerable to extinction from disease and other factors. We investigated the role of disease in the extinction of a wild population of a marsupial, the Eastern Barred Bandicoot (EBB) (*Perameles gunni*), as a basis for management recommendations. A serosurvey was conducted for exposure to the protozoan parasite *Toxoplasma gondii* in 55 captive and 5 free-ranging EBB using commercially available direct agglutination (DAT) and modified agglutination tests (MAT). Serologic cut-offs used were DAT >512 classified as positive, and titers 64 <DAT <256 classified as suspect. We found 8% of the EBB were seropositive, and 33% were suspect for toxoplasmosis. Seropositive status was significantly associated with age (*P* <.04) and being wild caught (*P* <.023)(as opposed to captive born). Seropositive and suspect EBB were also more likely to have eye pathology (*P* <.017) and lens abnormalities (*P* <.007).

Higher geometric mean DAT titers (*X*=69) were seen in EBB with ocular pathology vs. those without ocular pathology (*X*=31) (*P* <.04). However, seropositive status did not account for all instances of ocular pathology, suggesting either that the test used is not sufficiently sensitive at low antibody levels or that other mechanisms are involved. All females in the >2.5 y age class were seronegative, still reproducing, and responsible for substantial lifetime reproductive contributions to captive EBB populations. Infection with *T. gondii* probably occurs after weaning in EBB, but infection may not result in clinical disease.

Computer simulations using Vortex 5.1 indicate that although captive populations appear to be secure, mortalities from *T. gondii* can increase the probability of extinction and decrease heterozygosity of wild EBB populations. This indicates that toxoplasmosis could have contributed to the decline of wild EBB populations, and could limit reintroduction.
efforts. Therefore, management strategies designed to limit environmental burdens of *T. gondii* by eliminating cats from EBB environments and food sources, as well as by minimizing stress levels, should benefit recovery efforts.
SEROLOGICAL RESPONSES OF TAMMAR WALLABIES (*Macropus eugenii*) TO INOCULATION WITH AN ATTENUATED STRAIN OF *Toxoplasma gondii*

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Toxoplasmosis is a significant disease of Australian marsupials which frequently causes mortality in captive and free-ranging populations.\(^5\)\(^8\) It is thought that marsupials are more susceptible than eutherian mammals to toxoplasmosis due to a reduced evolutionary exposure to the parasite as felids have been present in Australia only since European settlement.\(^5\) The disease occurs in two forms in Australian marsupials: (i) Acute toxoplasmosis often presents as outbreaks of sudden death. If clinical signs are seen they reflect respiratory and/or nervous system pathology. (ii) The second type of disease caused by *Toxoplasma gondii* results from reactivation of latent infection. It is well known that this parasite can form tissue cysts in animals surviving initial infection that can reactivate at a later time and cause clinical disease which normally presents as neurological deficits. Outbreaks of the disease assumes particular importance for rare and endangered marsupials especially in captive breeding programs and in the management of small free-ranging populations in remnant habitats. An effective vaccine to protect against this disease would have enormous benefit in both these situations.

Over the last ten years a number of different types of vaccine against *T. gondii* have been developed and trialed in mainly eutherian species. Killed whole tachyzoite vaccines were found not to protect lambs from congenital infection when vaccinated ewes were challenged with live oocysts.\(^12\) Reddacliff (1993) used a *Toxoplasma*-related protozoan *Hammondia hammondi* in Tammar Wallabies as a possible vaccine. He found that although *Hammondia* may offer protection against disease caused by initial infection it did not prevent the formation of tissue cysts. Key antigens from the cell membrane of the *Toxoplasma* organism have been used as immunogens\(^1\) with some promise. Unfortunately this type of vaccine known as iscoms are not readily available as yet. Frenkel (1990) produced a temperature sensitive mutant (ts-4) strain of *T. gondii* which confers protective immunity in mice. Opossums inoculated with this vaccine developed a serological response but were not subsequently challenged with virulent *T. gondii*.\(^9\) This trial gives no real indication of the safety of the ts-4 strain in Australian marsupials as clinical toxoplasmosis is not reported in opossums.\(^3\) Unfortunately the strain can be difficult to transport and two attempts to import the vaccine into Australia have been unsuccessful due to its death before arrival. In New Zealand, a vaccine containing of live tachyzoites of a *T. gondii* strain has been shown to prevent *Toxoplasma* abortion in ewes and does.\(^2\) This vaccine is now commercially available in New Zealand and Great Britain for use in sheep and goats. (*Toxovax; AgResearch, Wallaceville, New Zealand*). This mouse-adapted strain (S48) which can be grown in tissue
culture, fails to produce tissue bradycysts in inoculated hosts and does not lead to oocyst formation when fed to cats. In this presentation, we report the findings of an pilot study to assess the safety of the New Zealand S48 vaccine in tammar wallabies (Macropus eugenii).

Tammar wallabies from a large breeding colony were bled in the two to three months prior to the commencement of the trial. Six animals were selected based on a seronegative reaction to direct (DAT) and modified (MAT) agglutination tests performed according to the manufacturers instructions (bioMerieux Marcy l'Etoile, 69620 Charbonnieres les Bains, France). The direct and modified agglutination tests have been used successfully in the serodiagnosis of acute toxoplasmosis. Prior to administering the S48 vaccine, the selected animals were again bled and randomly allocated into three groups of two. Each animal in Group 1 received 250,000 tachyzoites, Group 2 125,000 tachyzoites/animal and Group 3 62,000 tachyzoites/animal by intramuscular injection. Ten days post-vaccination one animal from Group 1 and both animals from Group 2 died without any prior signs of illness. At this time a Group 3 animal was so ataxic and depressed that it was euthanized. No clinical abnormalities were seen in the other two animals.

Blood samples collected from animals at 0, 7 and 12 DPI (days post inoculation) were tested by the agglutination test (see table 1). It appears that the two surviving animals (7788 & 7312) had become naturally exposed and seroconverted in the 12 week interval between the preliminary bleeding and the commencement of the study. At the time of inoculation with the S48 vaccine both animals had high DAT and MAT titres suggestive of an IgG response specific to Toxoplasma. These two animals (one given 250,000 and the other 62,000 tachyzoites) survived the inoculation. In contrast the other four wallabies were still seronegative at 0 DPI. By 7 DPI low DAT titres suggestive of an early IgM response were evident and by 12 DPI all four animals had high DAT titres and negative MAT results consistent with recent exposure to Toxoplasma antigen. The vaccination did not alter DAT or MAT titres in animals 7788 and 7312.

Smears were taken from the site of inoculation, liver, spleen and lung of the three dead and one euthanized wallabies. All samples tested positive for the presence of Toxoplasma tachyzoites using a Toxoplasma specific fluorescent antibody test. Composite tissues from each animal were then inoculated into the peritoneal cavity of mice. Mice commenced dying on 3 DPI and large numbers of Toxoplasma organisms were present in their abdominal cavities. Paraffin-embedded sections were prepared routinely for light microscopy from formalin-fixed tissues and stained with haematoxylin and eosin. Lesions were similar in all 4 wallabies. Necrotic lesions were found in spleen, gut associated lymphoid tissues, and in muscle at the vaccine injection sites. Lungs had extensive alveolar edema and congestion, moderate acute interstitial pneumonia and some focal necrosis. In the liver there was moderate to severe, acute hepatitis and necrosis. Kidneys had severe, focal, mainly perivascular, interstitial nephritis, with some associated necrosis. There was focal necrosis in the adrenals and one wallaby only had some tiny necrotic foci in the heart. Tachyzoites were observed, both free and clumped within cells, within and surrounding the necrotic areas of all tissues. There were no lesions in the brains. The extensive necrosis of lymphoid tissues
was similar to previous experimental acute toxoplasmosis in macropods. The severe perivascular inflammation and necrosis in liver and kidney has not previously been reported for toxoplasmosis in macropods.

The death or severe illness in the four *Toxoplasma*-naive tammar wallabies indicates that the S48 strain is capable of inducing acute toxoplasmosis leading to mortality. This finding is both a demonstration of the susceptibility of macropods to toxoplasmosis and a measure of the pathogenicity of the S48 strain. The two animals that were seropositive at the time of inoculation survived suggesting that prior natural exposure was protective against such a lethal challenge. The results suggest the S48 strain of *T. gondii* is unsuitable for use as a vaccine in macropods. Some indication is given that protective vaccination against toxoplasmosis is possible by the observation that wallabies surviving field exposure to *T. gondii* survived subsequent inoculation with the virulent S48 strain.

**LITERATURE CITED**

Table 1: Serological results for Tammar Wallabies inoculated with the S48 *Toxoplasma* vaccine strain at 0, 7 and 12 days post-inoculation (DPI)

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TOXOPLASMOSIS IN THE ZOOLOGICAL PARK

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Toxoplasma gondii is a protozoan parasite that infects all species of mammals and birds. The disease, toxoplasmosis, is a zoonosis. Cats (felidae) are the only definitive hosts of T. gondii, and the only animal that passes the oocyst in the feces. Oocyst are spherical to subspherical (11-14 X 9-12 µm) without a micropyle. They sporulate to contain 2 sporocysts with 4 sporozoites in each sporocyst. No stieda body is present on sporocysts. All mammals and birds may be intermediate hosts of T. gondii and have the parasite in their tissues.

The oocyst passed in feces of felidae (cats) must sporulate in the environment to become infective. Under favorable conditions oocysts sporulate to the infective stage in 24 to 96 hours and remain viable for several months. Cats pass oocysts in the feces for approximately 7 to 21 days. Unless severely immunosuppressed or stressed, oocysts will not be passed again. A few cats may have diarrhea during this phase of the infection, but most cats are entirely asymptomatic. Animals are infected when they ingest the sporulated oocyst in food or water. The oocyst excysts in the small intestine and sporozoites invade the cells of the body and multiply asexually as tachyzoites. Cells are destroyed as the tachyzoites multiply. When the cells rupture, tachyzoites are released to invade other cells. Tachyzoites in tissues are banana-shaped and measure 5-8 x 1-2 µm. Eventually the asexual multiplication slows, and the organisms multiply slowly as bradyzoites. Bradyzoites are smaller than tachyzoites and occur together in tissue cysts in muscles and organs including the brain where they remain for the life of the host. These animals are immune to further infection and disease by T. gondii unless severely stressed or immunosuppressed. All carnivorous animals and birds in the zoological park can become infected with T. gondii by ingesting the tissue stages in meat (tachyzoites or more commonly bradyzoites in cysts). Animals that do not eat meat may become infected by accidentally ingesting or inhaling (and then ingesting) oocysts from an environment contaminated by cat feces. The feral cat population of a zoological park disperses oocysts into the environment that will persist for months. Caged cats may also pass oocysts after eating raw meat or rats containing the tissue cysts. Insects, animal handlers, and rodents may mechanically transfer oocysts from one area to another. Animals may become infected by eating rodents in the zoo; however, rodents and other animals do not pass oocysts in the feces.

Although clinical disease is not always apparent, many animals in zoological collections are infected each year by T. gondii. The pathophysiology of the infection in the intermediate host is the same whether the infection is acquired by eating oocysts or tissue stages in meat. The ingested zoites invade cells and multiply as tachyzoites which rupture the cells releasing tachyzoites to invade other cells. This disruption of cells is responsible for the illness seen in toxoplasmosis. Bradyzoites are formed in tissue cysts in muscles and organs including the brain where they remain for the life of the host. After bradyzoites are formed, illness will no longer be apparent in most animals. Many mild cases of toxoplasmosis in animals and birds in the zoological park go undiagnosed and resolve without treatment. Most animals
develop immunity after an initial infection and will not suffer from the disease again unless severely stressed or immunosuppressed. These animals can not transmit the infection unless they are eaten.

Although clinical disease is not always apparent, there are many reports of toxoplasmosis from zoological collections. These reports detail illness and death in marsupials, monkeys, lemurs, and pallas cats after *T. gondii* infection. Cats usually do not display any illness during the intestinal phase when oocysts are produced. Usually by the time antibodies against *T. gondii* can be measured in a cat, oocyst shedding has stopped. If a cat is serving as an intermediate host, lethargy, respiratory distress, CNS problems or lymphadenopathy may occur. Marsupials are particularly susceptible, and illness and death are the usual sequelae of infection.

We have experienced 3 epizootics of toxoplasmosis in the marsupial colony at the Knoxville Zoological Park. The potaroos, wallabies, gray and red kangaroos that were infected became lethargic, developed respiratory distress and died within a few hours after the onset of the lethargy. On necropsy examination the animals had acute interstitial pneumonia, multifocal granulomatous encephalitis and meningitis, necrotizing hepatitis, and granulomatous necroulcerative gastroenteritis. Tachyzoites and areas of inflammation and necrosis were seen in the eyes, heart, kidneys, lymph nodes, muscles and pancreas. Animals that survived the epizootic developed antibodies against *T. gondii*, but the immunity produced in marsupials does not appear to be protective for long periods of time. All of these animals eventually died from a clinical syndrome resembling toxoplasmosis. We have also observed lethargy, respiratory distress, CNS signs, and ocular problems in binturongs, lemurs, and red pandas. Two lemurs from the zoo have died from *T. gondii* infection. Lemurs that survive primary infections appear to develop a stable immunity to *T. gondii*. Most hoofed stock at a zoological park will become infected with *T. gondii*, and species that are naive or phylogenetically distant to domestic ungulates may develop illness. However, abortion is a common sequelae when a female (particularly ovines and caprines) is infected for the first time during pregnancy. Subsequent pregnancies will not be affected. We have observed anorexia, diarrhea, and dyspnea in cassowaries and rheas infected with *T. gondii*, and ocular and CNS signs in an ostrich.

Diagnosis of *T. gondii* is difficult because oocysts are only found in the feces cats. *T. gondii* antibodies are present in the blood on serologic examination, and tissue stages may be found throughout the body by histopathologic analysis of tissue sections from animals that die of toxoplasmosis. They are particularly common in the brain, lungs, lymph nodes, and muscles of some animals. Specific immunoperoxidase staining of tissues is very helpful. A positive titer for *T. gondii* antibodies indicates that the animal has been infected with the organism, but it does not prove that the organism is responsible for a particular disease syndrome. Many animals have *T. gondii* antibodies, but show no signs of disease. We have serologically confirmed *T. gondii* infection with no signs of disease in African elephants, hyenas, Asian lions, African lions, Siberian tigers, black leopards, jaguars, a snow leopard, a cheetah, a serval, cougars, lynx, bobcats, lesser pandas, opossums, goats, black bears, white-tailed deer,
raccoons, coatis, a celebes crested ape, Diana monkeys and other primates. That does not mean that these species will never have illness from *T. gondii* infection, but that most of time illness will be slight and a lasting immunity will develop.

Toxoplasmosis is zoonotic, but animals must be eaten by another bird or mammal to transmit the disease. Only cats pass the oocyst in the feces. Humans, become infected like other animals by ingesting sporulated oocysts from the environment or the tissues stages in poorly cooked meat. Just as in other animals infection of the fetus with subsequent abortion or other complications may occur in women that are infected for the first time during pregnancy. The young, the infirm, or the immunosuppressed may experience illness from the infection (respiratory distress, CNS signs, myositis, ocular lesions). Toxoplasmic encephalitis is now a common cause of encephalitis in humans in the U.S., and frequent causes intracerebral mass lesions in patients with AIDS.
CRYPTOSPORIDIUM SPP. AND CRYPTOSPORIDIOSIS OF DOMESTIC AND WILD ANIMALS: LIFE HISTORY, DIAGNOSIS, AND MANAGEMENT STRATEGIES

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Introduction

Organisms of the genus Cryptosporidium are small coccidian parasites that infect the microvillous region of epithelial cells lining the digestive and respiratory organs of vertebrates. Recognized and named over 80 years ago by the American parasitologist, E. E. Tyzer, these small (2 to 6 um, depending on stage of life cycle), obligate intracellular protozoans remained until recently nothing more than a biomedical curiosity. Before 1980, infections with species of Cryptosporidium were considered rare in animals and in humans they were believed to be the result of a little-known opportunistic pathogen of immune deficient individuals outside its normal host range. Beginning in 1982, our concept of these protozoan parasites changed; they are now considered to be important, widespread causes of diarrheal illness in man and in some domesticated and wild animals.

The role of Cryptosporidium parvum as an enteropathogen of humans is now well established. In immunocompetent persons, C. parvum may cause a short-term (3 to 20 day) diarrheal illness that resolves spontaneously. However, in the immunocompromised patient, cryptosporidiosis often causes a life-threatening, prolonged, cholera-like illness. At the time of this writing, no effective therapy for cryptosporidiosis has been identified; thus, the finding of this parasite in the immunocompromised host, especially patients with the acquired immune deficiency syndrome (AIDS), usually carries a poor prognosis.

Cryptosporidium spp. are also important pathogens of many wild and domesticated vertebrates, including captive and free-ranging wildlife. Following brief discussion of the taxonomy and life history of Cryptosporidium spp. this necessarily brief communication will address the pathogenesis, management and diagnosis of cryptosporidiosis in animals. A more thorough understanding of Cryptosporidium spp. and cryptosporidiosis can be obtained from a recently published book (General References, 1) and several reviews listed in the General References 2-5.

The Etiologic Agents of Cryptosporidiosis

Small protozoans assigned to the genus Cryptosporidium have a close taxonomic relationship to the other true coccidia, Isospora belli, Sarcocystis spp., and Toxoplasma gondii, that infect human beings and to Eimeria spp. and Isospora spp. that infect other mammals and birds. These organisms are obligate intracellular protozoans assigned to the phylum Apicomplexa, class Sporozoasida, subclass Coccidiasina, order, Eucoccidiorida, and suborder Eimeriorina (the true coccidia). At least two valid species of Cryptosporidium infect mammals (C. parvum infecting the small intestine and C. muris infecting the stomach). On the basis of oocyst morphology, C. parvum, not C. muris, is associated with all well-documented cases of cryptosporidiosis in mammals. Thus, at the time of this writing, the species with oocysts
measuring 4 to 5 μm that produces clinical illness in humans and other mammals should be referred to as *C. parvum*. If there is not enough morphologic, life-cycle, and/or host specificity data to relate it to Tyzzer's original description of *C. parvum*, the parasite producing clinical illness in mammals should be referred to as *Cryptosporidium* sp.

In addition to *C. parvum* and *C. muris* there are at least 6 other valid species of *Cryptosporidium* infecting vertebrates (Table 1). The listing in Table 1 will undoubtedly become larger as valid new species of *Cryptosporidium* are described from vertebrate hosts.

Table 1. Recognized species of *Cryptosporidium* infecting vertebrates. These are species that the author believes to be valid based on published studies.

<table>
<thead>
<tr>
<th>Species</th>
<th>Host(s)</th>
<th>Oocyst Size (μm)</th>
<th>Site of Infection, Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. parvum</em></td>
<td>mammals</td>
<td>5.0 x 4.5</td>
<td>small &amp; large intestine, diarrhea; biliary tree, cholecystitis; upper respiratory tract, bronchitis</td>
</tr>
<tr>
<td><em>C. muris</em></td>
<td>mice, cattle</td>
<td>7.4 x 5.6</td>
<td>digestive glands of stomach, poor weight gain</td>
</tr>
<tr>
<td><em>C. sp.</em></td>
<td>guinea pigs</td>
<td>approx. 5.0</td>
<td>small &amp; large intestine, diarrhea</td>
</tr>
<tr>
<td><em>C. baileyi</em></td>
<td>poultry</td>
<td>6.2 x 4.6</td>
<td>respiratory epithelium, pneumonia &amp; airsaculitis; cloaca &amp; bursal epithelium, poor weight gain?</td>
</tr>
<tr>
<td><em>C. meleagridis</em></td>
<td>poultry</td>
<td>5.2 x 4.6</td>
<td>small intestine, diarrhea</td>
</tr>
<tr>
<td><em>C. sp.</em></td>
<td>quails</td>
<td>approx. 5.0</td>
<td>small intestine, diarrhea</td>
</tr>
<tr>
<td><em>C. serpentis</em></td>
<td>reptiles</td>
<td>6.0 x 5.4</td>
<td>gastric mucosa, gastric hyperplasia and anorexia</td>
</tr>
<tr>
<td><em>C. nasorum</em></td>
<td>fishes</td>
<td>not available</td>
<td>intestine, anorexia and emaciation?</td>
</tr>
</tbody>
</table>

**Life Cycle**

Studies of different isolates (calf and human) of *C. parvum* in suckling mice revealed that the life cycle of this parasite (Fig. 1) is similar to that of other true coccidia (eg. *Eimeria* and *Isospora* spp.) infecting mammals in that it can be divided into six major developmental events: excystation, the release of infective sporozoites; merogony, the asexual multiplication within host cells; gametogony, the formation of micro- and macrogametes; fertilization, the union of micro- and macrogametes; oocysts wall formation, to produce an environmentally resistant stage that transmits infection from one host to another; and sporogony, the formation of infective sporozoites within the oocyst wall. The life cycle of human and calf isolates of *C. parvum* differs somewhat from that of other monoxenous (one host in life cycle) coccidia such as *Eimeria* and *Isospora* spp., parasites usually presented as the "typical coccidia". Each intracellular stage of *C. parvum* resides within a parasitophorous vacuole confined to the microvillous region of the host cell, whereas comparable stages of *Eimeria* or *Isospora* spp. occupy parasitophorous vacuoles deep (perinuclear) within the host cells. Oocysts of *C. parvum* undergo sporogony while they are within the host cells and are infective when released in the feces, whereas oocysts of *Eimeria* or *Isospora* spp. do not sporulate until they are passed from the host and exposed to oxygen and temperatures below...
37° C. Studies, using experimentally infected mice, have also shown that approximately 20% of the oocysts of \textit{C. parvum} within host enterocytes do not form a thick, two-layered, environmentally resistant oocyst wall. The four sporozoites of this autoinfective stage are surrounded only by a single unit membrane. Soon after being released from a host cell, the membrane surrounding the four sporozoites ruptures and these invasive forms penetrate into the microvillous region of other enterocytes and reinitiate the life cycle. Approximately 80% of the oocysts of \textit{C. parvum} found in enterocytes of suckling mice were similar to those of \textit{Eimeria} and \textit{Isospora} spp. in that they developed thick, environmentally resistant oocyst walls and were passed in the feces. Thick-walled oocysts are the life cycle forms that transmit the infection from one host to another. The presence of autoinfective, thin-walled oocysts and type I meronts that can recycle are believed to be the life cycle features of \textit{C. parvum} responsible for the development of severe infections in hosts exposed to only a small number of thick-walled oocysts, and for persistent, life-threatening disease in immune deficient persons who are not exposed repeatedly to these environmentally resistant forms. Light microscopic and ultrastructural features of some of the developmental stages of \textit{Cryptosporidium} in enterocytes of the experimentally infected host are shown in Figs. 2 and 3. Details of the ultrastructure of \textit{Cryptosporidium} spp. can be found in general reviews on \textit{Cryptosporidium} listed under General References.

**Clinical Features and Pathogenesis**

**Mammals infected with \textit{C. parvum}**

*Clinical features:* The most common clinical feature of cryptosporidiosis in immunocompetent and immunocompromised humans is diarrhea, the symptom that most often leads to diagnosis. Characteristically, the diarrhea is profuse and watery; it may contain mucus, but rarely blood and leukocytes. Both the duration of symptoms and outcome typically vary according to immune status of the patient. Severely immunocompromised persons such as AIDS patients usually experience a prolonged, life-threatening illness, whereas most immunocompetent persons experience a short-term illness with complete, spontaneous recovery. In AIDS patients, \textit{C. parvum} also infects the gall bladder and biliary tree epithelium and can cause acalculous cholecystitis. There are several reports of tracheal and bronchial infections resulting in bronchitis.

Severe, profuse diarrhea is also the most common clinical feature of cryptosporidiosis most domesticated and wild mammals, especially animals less than 4 weeks of age. Calves, sheep, goats, deer, horses and a number of exotic ruminants have been reported to have severe cryptosporidial diarrhea. The main presenting sign of cryptosporidiosis in the young of these animals is profuse diarrhea with shedding of infective oocysts (up to $10^7$/g of feces). Varying degrees of dullness, anorexia, fever, and loss of condition can occur. Similar clinical features have been reported in non-human primates infected with \textit{C. parvum}. 

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Diarrheal illness due to cryptosporidiosis has been reported, but is less common in dogs, cats, raccoons, rabbits, pigs and rodents. It appears that these mammalian hosts may often have asymptomatic infections of *C. parvum* and that they may serve as potential reservoir hosts.

**Pathogenesis:** At present, the pathophysiologic mechanisms of *Cryptosporidium*-induced diarrhea are poorly defined. Studies in germ-free calves monoinfected with *C. parvum* suggest that malabsorption and impaired digestion in the small bowel coupled with malabsorption in the large intestine are major factors responsible for diarrhea in calves with cryptosporidiosis. Similar malabsorption, attributed to parasite-induced villus damage, has also been reported in a neonatal pig model. This malabsorption and impaired digestion may result in an overgrowth of intestinal microflora, a change in osmotic pressure across the gut wall, and an influx of fluid into the lumen of the intestine. Malabsorption and impaired digestion have also been reported in humans infected with *C. parvum*. The secretory (often described as cholera-like) diarrhea common to most immune deficient patients with cryptosporidiosis suggests a toxin-mediated hypersecretion into the gut; however, the authors are not aware of reports documenting such a toxin. Definitive, systematic studies are needed to determine the mechanisms by which *C. parvum* and its metabolites or toxins may alter normal intestinal function of a susceptible animal model.

**Calves infected with *Cryptosporidium muris***

**Clinical Features and pathogenesis:** A species of *Cryptosporidium* whose oocysts are morphologically identical to those of *C. muris*, described by Tyzzer in 1912, is the agent of a disease known as abomasal (gastric, peptic gland) cryptosporidiosis. This organism produces lesions of dilation of peptic glands in the abomasum of calves and adult cattle. Infected cattle may shed oocysts in their feces for many (4-6 or more) months. A severely affected abomasum may weigh 2-3 times more than an abomasum from a non-infected bovine of the same age and size. Histologic examination of such heavy infections often reveals that most peptic glands contain numerous developmental stages of the parasite; one report described all glands as being full of developing parasites. In such severely parasitized cattle lower weight gains (a decrease of up to 33%) have been noted when compared to non-infected penmates.

The prevalence and distribution of *C. muris* in cattle and its overall role as an agent causing economic loss to the bovine industry remain undefined. The parasite has been reported from calves and/or adult cattle in Washington state, Idaho, Nevada, California, and Alabama. Using an acid-fast staining technique with a sensitivity of approximately 86% of that provided by Sheather's sugar flotation, Anderson reported that 54 of 1216 (4.4%) feeder cattle in eight lots in Idaho had *C. muris* oocysts in their feces. Using the same diagnostic procedure approximately 1.6% of the 12,069 samples obtained from calves and cows in 21 dairy herds in Idaho, Nevada, and California contained *C. muris* oocysts. Additional studies are needed to more clearly define the impact of *C. muris* on beef and dairy production. Additional features of *C. muris* infections in cattle can be found in General References 1,
Birds infected with *Cryptosporidium* spp.

Species of *Cryptosporidium* have been observed in the enteric, respiratory, and renal epithelium of numerous species of birds (For list of species see General References 1, 5). Within the enteric tract, cryptosporidia have been reported in the salivary glands, proventriculus, small intestine, cecum, colon, cloaca, and bursa of Fabricius. In the respiratory tract and oculonasal tissues, parasites have been observed in the nasal chamber, palatine cleft, turbinates, infraorbital sinuses, conjunctiva, larynx, trachea, all levels of the bronchi, and airsacs. Within the excretory tract, developmental stages of *Cryptosporidium* sp. have been found in the ureters, collecting ducts, collecting tubules, and distal convoluted tubules. At the time of this writing, there are three recognized species of *Cryptosporidium* infecting birds, however, there are undoubtedly other species that have not been described.

*Cryptosporidium baileyi* infecting chickens and turkeys: *Cryptosporidium baileyi* is apparently restricted to the respiratory tract and distal gastrointestinal tract (cecum, distal colon, cloaca, and bursa of Fabricius). Respiratory disease is observed most commonly and is the more severe than intestinal disease. Clinical signs consist of respiratory distress characterized by depression, rales, coughing, sneezing, and/or dyspnea. The lumens of organs of the respiratory tract contain a mucocellular exudate consisting of exfoliated epithelial cells, macrophages, heterophils, lymphocytes, plasma cells and developing parasites. Accumulation of this exudate results in both pneumonia and airsaculitis. Additional details of the clinical features and pathogenesis of *C. baileyi* infections can be found in General References 1 and 5.

*Cryptosporidium meleagridis* infecting turkeys: Intestinal cryptosporidiosis may result in moderate to severe diarrhea, mortality, or lack of observable clinical signs. Postmortem examination of *Cryptosporidium*-infected turkeys with diarrhea revealed a small intestine that was pale and contained cloudy, mucoid exudate. Villi were moderately atrophic, crypts were hyperplastic, and infected segments of the small intestine contained a mixed mucosal inflammatory infiltrate (Goodwin et al. 1988. Avian Dis. 32, 63.) Additional details of the clinical features and pathogenesis of *C. meleagridis* infections can be found in General References 1 and 5.

*Cryptosporidium* sp. infecting quail: Natural or experimental cryptosporidiosis (intestinal and respiratory) has been reported from bobwhite and common quail. Clinical signs and pathogenesis is similar to that reported above for *C. baileyi* infections in chickens, however, infection in quails is not due to *C. baileyi*. Intestinal disease usually results in high mortality in birds from hatch to 12 days. Diarrhea appears as a clear to brown fluid, or may be white
and watery. Villous atrophy, sloughed villi, or detached enterocytes are prominent histopathologic features. Additional details of the clinical features and pathogenesis of *Cryptosporidium* sp infecting quails can be found in General References 1, 5 and in several papers (Hoerr et al. 1986. Avian Dis. 30:421; Ritter et al. 1986. Avian Dis. 30:603; Guy et al. 1987. Avian Dis. 31:713.)

**Reptilian Cryptosporidiosis**

Most cases of cryptosporidiosis in reptiles appear to be a problem in captive animals. In snakes, *Cryptosporidium* sp. has been reported to infect boids, colubrids, elapids, and vipers, localizing in the gastric mucosa. Although oocysts may be passed in the feces for many months or even years, cryptosporidiosis is most often diagnosed because of a clinical problem. Clinical signs may include anorexia, persistent or intermittent postprandial regurgitation, lethargy, firm midbody swelling, and progressive weight loss. Microscopic lesions consist of hyperplasia and hypertrophy of gastric glands, concomitant atrophy of the glandular cells, edema of the submucosa and lamina propria, reduction in lumenal diameter, and inflammation of the gastric mucosa.

**Management of Cryptosporidiosis**

At the time of this writing, no specific anti-*Cryptosporidium* drug has been identified for use in humans or animals. Of the more than 90 therapeutic and preventive modalities tested for efficacy against cryptosporidiosis in humans and animals, few have been reported to have any effect on the severity and duration of disease. The antacoccidial agents lasalocid and halofuginone lactate have been reported to be moderately effective in ruminants (calves and lambs) when administered at near toxic doses. In the absence of an effective treatment, management to prevent outbreaks and supportive measures for infected animals appears to be the only logical ways to intervene.

Measures for controlling outbreaks of calf cryptosporidiosis on the farm as outlined by Angus (General Reference 1, chapter 5) may also be applicable to some captive animals. These measures (see below) are equally applicable to other enteric infections, which is important because multiple agent outbreaks including *Cryptosporidium* are usually more common than monoinfections with this parasite. Aided by the knowledge that oocysts are environmentally resistant appear to be refractive to commercial disinfectants (not true for bacterial and viral enteropathogens) and that clinical disease can be deferred by individual penning from birth in sterilized pens or hutches, the following principles based on the experiences of veterinary scientists should be adopted:

1. Calf rearing should be on an "all-in, all-out" basis.
2. Individual pens should be stringently cleaned and disinfected between batches of calves.
3. Calves should be born and raised in a clean, dry environment.
4. Ideally, newborn calves should be penned individually for 2-3 weeks.
5. Sick calves should be removed immediately from the company of healthy calves.
6. Healthy calves should have different attendants than those of sick calves.
7. Attendants should keep boots, protective clothing, etc. as free from feces as possible.
8. Utensils should be heat sterilized, if possible, daily.
9. Vermin, farm dogs, and cats should be controlled.
10. Colostrum management and nutrition should be satisfactory.
11. Appropriate prophylactic measures against other agents, such as rotavirus or ETEC-K99+ vaccines, should be employed.

Even on the best managed farms, all of these control measures may not be possible. Adopting these measures to the management of wild captive animals may be even more difficult. In general, any procedure that reduces fecal-oral contamination should be adopted. Maintaining animals, especially the young, in warm, dry, clean environments is important. Also, if an animal becomes infected, it is important to isolate it from non-infected animals and to make sure that it receives adequate fluids and nutrients.

Identification of Oocysts and Endogenous Stages

(Adapted from: Current, W. L. Techniques and laboratory maintenance of Cryptosporidium. in, Dubey, J. P., R. Fayer, and C. A. Speer, eds. Cryptosporidiosis in Man and Animals. CRC Press, Inc. 1990, pp. 31-49 - Reproduced with permission)

Before 1980, human cryptosporidiosis was diagnosed by histologic examination of biopsy of the intestinal mucosa. Routine hematoxylin and eosin can be used to visualize the developmental stages of the parasite as dark, basophilic, spherical bodies 2 to 5 mm (depending on stage in life cycle) in the brush border of mucosal epithelium. Transmission electron microscopy can be used to confirm the diagnosis. Such invasive, expensive, and time-consuming procedures are no longer required for the diagnosis of cryptosporidiosis since a variety of techniques have been developed to identify Cryptosporidium spp. oocysts in feces and other body fluid specimens. Oocysts, usually released in large numbers from the infected epithelium, can be identified in stool specimens representing a sampling of the entire intestinal tract or in sputum or respiratory aspirates representing a sampling of the entire respiratory tract. Biopsy specimens may be of some value in determining the site of infection and lesions associated with the parasite.

A. Preservation and Storage of Specimens Containing Oocysts

Preservation of Specimens: Most diagnostic specimens examined for oocysts will be stool samples; however, other fluids such as sputum should be handled the same way. For the diagnosis of cryptosporidiosis, stool specimens should be submitted as fresh material or in 10% formalin or sodium acetate-acetic acid-formalin (SAF) preservatives. If parasites are not going to be used for subsequent experimental purposes, fixed specimens are recommended because of biohazard considerations. Fresh or preserved stool specimens can be examined as wet mounts or they can be concentrated or stained as outlined below to aid in the visualization of Cryptosporidium spp. oocysts.

Storage of Specimens: Potassium dichromate solution (K₂Cr₂O₇, 2 to 2.5% w/v in water) is used routinely as a storage medium to preserve oocyst viability, it is not a fixative. When stored at 4°C in K₂Cr₂O₇, Cryptosporidium spp. oocysts remain viable for at least three
months and some may retain infectivity for up to 12 months.\(^1\) Since the percentage of viable oocysts begins to decrease after three months of cold storage, it is advisable to generate fresh oocysts every 3 to 4 months. Some researchers prefer to store oocysts of Cryptosporidium spp. in balanced salt solutions supplemented with antibiotics because chromium is an environmental contaminant requiring special disposal and handling procedures. Hank's balanced salt solution (HBSS) containing penicillin, 10,000 U; streptomycin, 10 mg; Fungizone, 0.05 mg; and Mycostatin, 500 U/ml is recommended for the storage of Cryptosporidium spp. oocysts and also for the storage of sporocysts of Sarcocystis spp.

B. Identification of Oocysts (Fig. 4)

Two general approaches have been used widely to identify Cryptosporidium spp. oocysts in stools or body fluid specimens. Concentration techniques are used routinely in the research laboratory whereas staining techniques, before or after concentration of oocysts, are used more often in the clinical microbiology laboratory.\(^3\)\(^-\)\(^4\) Because Cryptosporidium spp. oocysts are similar in size and shape to some yeast cells, considerable experience is required to obtain accurate results with concentration and/or staining techniques. Useful stool concentration techniques include flotation of oocysts in Sheather’s sugar solution, in zinc sulfate solution (1.18 or 1.2 sp. gr.), or in saturated sodium chloride solution (1.27 sp. gr.). Stool concentration techniques using sedimentation include formalin-ether and formalin-ethyl acetate. Some studies have found no differences among these methods, whereas others have found formalin-ether sedimentation and sodium chloride flotation to be the most sensitive procedures.\(^4\)\(^-\)\(^5\) Some researchers feel that the Sheather’s sugar flotation gives results equal to or better than those obtained with formalin-ether or formalin-ethyl acetate sedimentation.\(^6\)\(^-\)\(^8\) Sheather’s sugar flotation, perhaps the most widely used concentration technique, is outlined below.\(^9\)\(^-\)\(^10\)

Sheather's Sugar Flotation:

1. If the stool specimen is not fluid, it may be necessary to dilute it with water before straining it through cheesecloth to remove large particulate material. Mix thoroughly approximately 1 ml of fecal (sputum) suspension with 10 ml of Sheather’s sugar solution (sugar, 500 g; water, 320 ml; phenol, 6.5 g) in a 15 ml centrifuge tube. A cap should be placed on the tube to prevent aerosol formation.

2. Centrifuge at 500 xg for 10 minutes, obtain a sample from the surface of the flotation medium (this can be done easily by using a wire loop or Pasteur pipet), place it on a microscope slide, add a coverslip, and examine microscopically (see 3).

3. Sheather’s sugar flotation combined with phase-contrast microscopy is an excellent procedure to identify oocysts and to distinguish oocysts from contaminating yeast cells.\(^1\)\(^-\)\(^1\)\(^0\)\(^-\)\(^1\)\(^1\) C. parvum oocysts appear as bright, birefringent, 4.5 to 5.5 mm spherical bodies containing 1 to 4 dark granules. With good-quality, phase-contrast, oil immersion objectives the four sporozoites surrounding the centrally located oocyst residuum can often be seen. Oocysts of other species of Cryptosporidium; eg., C. muris or C. baileyi, will be of a different size and shape but will otherwise appear
similar to *C. parvum*. Contaminating yeast cells do not appear as bright, birefringent bodies, do not contain dark granules or sporozoites, and sometimes have small budding cells on their margin.

When viewed with brightfield microscopy, oocysts are often difficult to distinguish from yeast cells. When viewed with brightfield microscopy, *C. parvum* oocysts appear as 4.5 to 5.5 mm translucent, spherical bodies containing 1 to 4 dark granules. With some microscopes, these oocysts often appear light pink. Yeast cells do not have the characteristic granules and are not pink in color. After approximately 15 minutes in the hyperosmotic Sheather's solution some oocysts begin to collapse and the percentage of collapsed oocysts increases with time. Collapse of the oocysts usually involves a folding in of the oocyst wall along the oocyst wall suture.

**Acid-Fast Staining:**

Most recommended stains for *Cryptosporidium* spp. oocysts cannot be performed on stools preserved in polyvinyl alcohol (PVA) fixative. The routine stains (trichrome, iron hematoxylin) used for stool diagnosis of other parasites are not helpful for the identification of *Cryptosporidium* spp. oocysts. The most widely used staining techniques for demonstrating *Cryptosporidium* spp. oocysts in fecal specimens are based on the acid-fast staining properties of the oocyst wall. Several acid-fast stains have been used in hot or cold procedures and modified by incorporation of dimethyl sulfoxide. It appears that reported differences in acid fast stains and methods represent personal preference rather than marked differences in sensitivity and specificity. The most commonly used acid-fast procedure is the modified Kinyoun cold technique outlined below.

1. With a wooden applicator stick or a disposable culture loop, spread the specimen on a microscope slide and allow to air dry.
2. Fix in absolute methanol for 5 to 10 min and air dry.
3. Cover the smear with modified Kinyoun acid-fast stain (fuchsin-carbol, Harleco, Gibbstown, NJ) for approximately 2 min.
4. Rinse briefly in tap water (some prefer 50% ethanol), cover the smear with decolorizing solution (10% H$_2$SO$_4$) and incubate for approximately 1 min and then rinse briefly in tap water.
5. Cover the smear with counterstain (light green SF yellowish stain, Harleco; or methylene blue, 0.3 g/100 ml deionized water), incubate for approximately 1 min, rinse in tap water, air dry, and observe microscopically.
6. For optimal staining with this procedure, staining, decolorizing, and counterstaining times are determined empirically. Also, it is important that fecal smears are not too thick to ensure adequate penetration of the reagents.
7. When stained properly, oocysts are 4.5 to 5.5 mm, red, spherical bodies against a green or blue (depending on counterstain) background. Oocysts stain red with varying degrees of intensity whereas most fecal debris and yeast cells take up the color of the background stain.
Negative Staining:

The negative staining procedure originally described by Heine is also useful for detecting oocysts in fecal specimens. This procedure has the advantage of requiring less preparation time and fewer steps than the Kinyoun technique but does not result in the production of a permanent slide. The procedure works best on fresh fecal specimens but it will give satisfactory results with specimens fixed in 10% formalin. The author does not know if specimens preserved in other fixatives lend themselves to negative staining. A modification of the original negative-staining procedure is outlined below.

1. With a wooden applicator stick, mix thoroughly on a microscope slide an equal volume (5 to 10 ml) of feces and modified Kinyoun's carbol fuchsin stain. (The stain is prepared by dissolving 4.0 g of basic fuchsin in 100 ml deionized water plus 20 ml 95% ethanol. After the stain is dissolved add slowly 8 ml of liquified phenol.)

2. As soon as the smear has air dried, add a drop of immersion oil directly to the smear. Place a coverslip on top of the immersion oil, and observe with a brightfield microscope.

3. The characteristic, bright, birefringent appearance of Cryptosporidium spp. oocysts against a dark staining background is due to an oocyst wall that is not stained during this procedure and that is impervious, preventing entry of the stain and loss of water from the interior of the oocyst. Fecal debris, yeast, and bacteria all stain darkly. Often the stain collects around the outside of the oocyst as the smear dries, resulting in dark rings around bright oocysts. After approximately 15 min in immersion oil, the oocysts begin to collapse.

Immunofluorescent Antibody Stains to Detect Oocysts:

Most humans and animals with cryptosporidiosis pass enough oocysts in their stools so that most of the concentration and/or staining techniques described in the literature (several are outlined above) are adequate for detection and diagnosis. However, more sensitive techniques are sometimes needed to detect oocysts in specimens that contain few parasites and large amounts of debris. These specimens include fecal samples from asymptomatic carriers or filtrates from surface or drinking water samples. Both polyclonal and monoclonal antibodies specific to C. parvum can be used in direct or indirect immunofluorescent assays to detect oocysts in these specimens. An immunofluorescent diagnostic kit is available commercially (Merridian Laboratories, Cincinnati, Ohio) which utilizes a monoclonal antibody specific to the oocyst wall of C. parvum. Polyclonal antibodies can also be used as described below.

Preparation of Polyclonal Antisera:

1. For immunizing rabbits, suspend oocysts (10⁸/ml) in 2 mM TRIS buffer (pH 8.0) containing 10 mM phenylmethyl-sulfonylfluoride (PMSF), and disrupt by 3 freeze-thaw cycles, involving incubation in liquid nitrogen for 10 min followed by incubation at 37°C until the suspension is thawed. Disruption of oocysts and sporozoites can be monitored microscopically.
2. Inoculate rabbits every 4 weeks with approximately $10^7$ disrupted oocysts. Choices concerning the use of adjuvants and route of administration are numerous and a matter of preference. Intravenous administration of disrupted oocysts without adjuvant and subcutaneous boosting (2 to 3 times) with the antigen mixed in Freund’s incomplete adjuvant has been used in our laboratory results in sera with high titers of antibody specific to Cryptosporidium.

Indirect Immunofluorescent Antibody Procedure Using Polyclonal Antibodies:

1. With a wooden applicator stick or a disposable culture loop, spread the specimen on a microscope slide and allow to air dry. To save reagents, 12-well multi-spot microscope slide (Shandon, Inc. Pittsburgh, PA, Catalog No. 99910090) can be used. Approximately 4 ml of specimen can be placed in each well, air dried and processed as described below.

2. Fix the smear in cold 95% ethanol for 5 min and then incubate in phosphate buffered saline (PBS, pH 7.2) at room temperature for 2 min.

3. Cover the smear (4 ml if 12-well slides are used) with rabbit antiserum (diluted to appropriate concentration in PBS) and incubate for 30 min at $37^\circ$C. The rabbit antiserum can be diluted 1:100 to 1:1000, depending on titer.

4. Wash 5 times by incubating in PBS for 3 min.

4a. If background fluorescence is a problem, one can use an appropriate blocking agent at this point to prevent non-specific binding of the FITC-conjugated antibody added in step 5. Appropriate blocking agents include 1% bovine serum albumin or 1% gelatin, both in PBS.

5. Cover the smear (4 ml if 12-well slides are used) with goat anti-rabbit IgG conjugated to FITC and incubate for 30 min at $37^\circ$C. Addition of 0.01% (w/v) Evans blue stain to the diluted FITC-conjugated antibody helps distinguish oocysts from fecal debris and yeast cells. Oocysts will fluoresce bright green against a dark background and contaminating debris and yeast cells will appear red as a result of the Evans blue counterstain.

C. Detection and Identification of Endogenous Stages

Routine histologic methods can be used to detect endogenous stages of Cryptosporidium spp. in specimens of mucosal epithelium, but identification of different life cycle stages can be difficult. Transmission electron microscopy facilitates identification of developmental stages of Cryptosporidium spp. that reside within parasitophorous vacuoles confined to the microvillus region of epithelial cells. To identify stages of Cryptosporidium spp. in fresh mucosal scrapings, cultured cells, or the chorioallantoic membrane of chicken embryos, a light microscope equipped with high quality phase-contrast or Nomarski interference contrast (NIC) optics is best. The author prefers NIC optics for this purpose. If NIC or phase-contrast optics are not available, one can use brightfield microscopy to identify some of the developmental stages of Cryptosporidium spp. in stained mucosal smears.
GENERAL REFERENCES AND RECOMMENDED READING


OTHER GOOD REVIEWS


REFERENCES FOR IDENTIFICATION OF OOCYSTS AND ENDOGENOUS STAGES


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Figure 1. Diagrammatic representation of the proposed life cycle of *C. parvum* as it occurs in the mucosal epithelium of an infected mammalian host. Developmental stages of *C. parvum* corresponding to some stages in this life cycle diagram are shown in light and electron photomicrographs contained in Figures 2 and 3. After excysting from oocysts in the lumen of the intestine (a), sporozoites (b) penetrate into host cells, and develop into trophozoites (= uninucleate meronts) (c) within parasitophorous vacuoles confined to the microvillous region of the mucosal epithelium. Trophozoites (uninucleate meronts) (c) undergo asexual divisions (merogony) to form merozoites. After being released from type I meronts, the invasive merozoites enter adjacent host cells to form additional type I meronts (recycling of type I meronts) or to form type II meronts (f). Type II meronts do not recycle but enter host cells to form the sexual stages, microgamonts (g) and macrogamonts (h). Most (approximately 80%) of the zygotes (i) formed after fertilization of the microgamont by the microgametes (released from microgamont) develop into environmentally resistant, thick-walled oocysts (j) that undergo sporogony to form sporulated oocysts (k) containing four sporozoites. Sporulated oocysts released in feces are the environmentally resistant life cycle forms that transmit the infection from one host to another. A smaller percentage of zygotes (approximately 20%) do not form a thick, two-layered oocyst wall, they only have a unit membrane surrounding the four sporozoites. These thin-walled oocysts (l) represent autoinfective life cycle forms that can maintain the parasite in the host without repeated oral exposure to the thick-walled oocysts present in the environment. The life cycle of *C. baileyi*, infecting chickens, differs from the one shown in that this parasite has an additional type (type III) meront that is derived from type II merozoites. (Drawing by Kip Carter, University of Georgia. Reprinted with permission from W. L. Current and B. L. Blagburn. 1990. *Cryptosporidium* infections in man and domesticated animals. pp. 155-185. In, P. L. Long (ed.), *Coccidiosis of Man and Domestic Animals*, CRC Press, Boca Raton, Florida).
Figure 2. Scanning electron micrograph showing numerous developmental stages of *Cryptosporidium* in the microvillous region of the intestinal mucosa. Each parasite is contained within a parasitophorous vacuole that bulges out from the microvillous region of the enterocyte. Some merozoites of a mature type I meront (M) are exposed as a result of a portion of the parasitophorous vacuole membrane being removed during processing. Arrows point to craters in the mucosal surface formed by empty vacuoles that remain after the parasites were released.
Figure 3. A. Transmission electron micrograph of developmental stages of *C. parvum* within parasitophorous vacuoles bulging from the microvillous region of ileal enterocytes of an experimentally infected mouse. Macrogametes (one labeled MA) contain the characteristic amylopectin granules near the center and wall-forming bodies near the periphery. One trophozoites (T) (uninucleate meronts) and one meront (M) with budding merozoites can be seen. B. Light photomicrograph of a histologic section (stained with hematoxylin and eosin) of small bowel biopsy obtained from an immunocompromised patient with persistent cryptosporidiosis. Three of the numerous developmental stages of *C. parvum* within the brush border of the enterocytes are denoted by arrowheads.
Figure 4. Composite line drawings of oocysts of *Cryptosporidium muris* (larger oocyst on left) and *C. parvum*. Note the four sporozoites and large central residuum surrounded by the oocyst wall. Within the oocyst wall is a suture (dotted line) that opens up during excystation to release the infective sporozoites.
A 20 YEAR RETROSPECTIVE STUDY OF CAUSES OF MORTALITY IN A COLONY OF TITI MONKEYS (Callicebus spp.)

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Introduction

Titi monkeys (Callicebus spp.) are small arboreal primates of South America. On the basis of cytogenetics, biogeography and considerations of tegumentary coloration, the genus was recently reclassified in 13 species and 16 subspecies. Titi monkeys are distributed over most of the tropical forests of the Amazon and Orinoco basins, parts of the Atlantic forest, as well in southern Brazil, Bolivia, and Paraguay. One species, C. personatus, is found in only the coastal forest of southeastern Brazil. This species and the subspecies C. p. personatus, C. p. nigrifrons, and C. p. melanochir, are listed among the most endangered neotropical primates, due to the rapid destruction of the remaining Atlantic rain forest, and an illegal trade.

Previous field studies have established that Callicebus sp. has a monogamous family unit of social organization with parental care by the adult male. The three most studied species, C. moloch, C. torquatus and C. personatus, defend virtually exclusive territories utilizing loud intergroup vocalizations (dawn calls), usually with adult male and female duetting. Titi monkeys have a frugivorous diet with supplements of leaves, flowers and insects.

Since 1972, the California Regional Primate Research Center (CRPRC) has maintained a breeding colony of Callicebus spp. for behavioral studies. Founder animals were obtained from captive populations (Tulane Primate Center, USA and Gottingen, West Germany) and from the wild (Bolivia and Peru). This study was undertaken to determine the causes of mortality in the CRPRC colony during a 20 year period.

Material and methods

Survey data included individual animal records, pathology records and population data from the CRPRC computer database system. Pathology records were reviewed, and pathological findings were tabulated by organ system. Monkeys that died in quarantine were considered as a separate group because they were not exposed to CRPRC management.
A total of 177 titi monkeys medical records were examined. Of these, 28 were stillbirths, 13 newborn (less than 24 hours), 32 neonates (1 to 30 days), 12 juvenile (>30 days to 1 year), and 92 adults (77 from the colony and 13 from the quarantine).

Results

The colony mortality rate is documented in Figure 1. The peak mortality in 1976 and 1977 are related to quarantine deaths in 1976 and post-quarantine deaths of wild caught animals from the same shipment. Mortality rates declined after 1990 when the titi monkey colony was moved to a new indoor facility.

Stillbirths (28 monkeys): Diagnosis of stillbirths included prematurity (17.85%), abortion of unknown cause (17.85%), septic abortion from placentitis or maternal sepsis (7.14%), trauma (7.14%) and undetermined due to autolysis, no lesions or no necropsy performed (50%).

Newborn (13 monkeys): Causes of newborn deaths were identified as maternal neglect (38.46%), prematurity (7.69%), septicemia (15.38%), twinning (15.38%) and undetermined (23.07%).

Neonatal death (32 monkeys): Diagnoses were maternal neglect (6.25%), sepsis (18.75%), environmental exposure (3.12%), trauma (3.12%), colitis (3.12%), isoerythrolysis (3.12%) and undetermined (18.75%). The following bacteria were cultured from the septic cases: Staphylococcus aureus, Streptococcus viridans, Corynebacterium sp, Citrobacter intermedius, Proteus spp., and E. coli.

Juvenile mortality (12 monkeys): Causes of death were environmental exposure (8.3%), gastrointestinal disorders (58.3%), and undetermined (25%). Most significant post mortem findings were gastrointestinal diseases, such as typhlocolitis, ulcerative gastritis, proctitis, colitis associated with Entamoeba histolytica, and ulcerative enterocolitis with invasive trichomonads. Interstitial pneumonia was present in one animal secondary to sepsis. Additional findings were cholangiohepatitis and pancreatic nematodiasis caused by Trichospirura leptostoma (3 animals).

Quarantine (13 monkeys): One of thirty animals imported from West Germany in 1978 died from presumptive metabolic or electrolyte imbalance. Incidental findings were hepatic trematodiasis and an anoplocephalid cestode in the duodenum.

Sixteen of 24 wild caught Bolivian monkeys died in quarantine in 1976 due to a herpesvirus outbreak of unknown origin. Clinically, animals were obtunded and dehydrated. They developed ulcerative lesions on the oropharynx and had purulent nasal and ocular discharge with upper respiratory signs. The most remarkable pathological features were necrotizing ulcerative dermatitis, cheilitis, stomatitis, glossitis, esophagitis, and necrotizing gastro-enterocolitis with intranuclear inclusion bodies resembling herpesvirus. Herpesvirus was isolated in cell culture, but was not characterized.
One of 12 wild-caught Peruvian monkeys died during shipment in 1990. It was apparently severely immunocompromised with candidiasis of the tongue and esophagus and had *Morganella morganii* septicemia terminally. Three other animals in this shipment had subclinical *Plasmodium brazilianum* infection.

**Adults** (77 monkeys):

**Gastrointestinal Disorders:** Oral and esophageal candidiasis were an incidental finding in 3 animals. Twelve adult monkeys had varying degrees of parasitic glossitis, stomatitis, esophagitis and pharyngitis associated with *Gongylonema spp*. The majority of this group were first (N = 7) and second (N = 3) generation animals from the CRPRC colony or first generation (N = 2) from the captive group of West Germany.

**Bacterial enteritides:** Two animals had acute hemorrhagic gastritis/gastroenteritis as consequence of *Klebsiella pneumoniae* sepsis. The food was considered the source of *Klebsiella sp.* and possibly contributed a toxin which caused the stomach lesion and allowed bacterial septicemia to occur. Enterocolitis with septicemia was caused by *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. Bacteremia caused mesenteric and hepatic abscesses in titis. Although the relationship between *Aeromonas hydrophila* and diarrheal disease in non-human primates is unclear at present, this organism was cultured from two animals with gastrointestinal disease. Other microorganisms isolated from GI disorders were *Pseudomonas aeruginosa*, *Campylobacter spp*, *Klebsiella spp.*, and *Proteus mirabilis*.

**Parasitic enteritides:** In nine animals, amoebae and trichomonad organisms were found in areas of acute mucosal necrosis and thrombosis in the submucosal, lamina propria, and occasionally in the crypts of large intestine. In most cases, they were considered secondary invaders rather than primary pathogens. Giardiasis was found in two wild caught animals originally imported from Bolivia. Acanthocephalasis was associated with perforation of the small bowel and focal peritonitis in a titi monkey recently imported from Bolivia. Some of the seven cases of intestinal cestodiasis were identified as *Bertiella sp.*, *Atriotaenia sp.* and a possible *Mathevotaenia sp.*

**Hemorrhagic enteritis syndrome:** Hemorrhagic enteritis syndrome was often associated with weight loss, severe anemia, hypoproteinemia and hyperfibrinogenemia. It has been an important cause of mortality in the past, and has been associated with several enteric pathogens such as *Yersinia spp* (3), *Proteus vulgaris* (1), *Aeromonas hydrophila* (1), *Entamoeba histolitica* (1). No pathogens were identified in 5 cases. Two cases had extensive superficial necrosis morphologically similar to the lesions of swine dysentery. However, no silver positive spiral bacteria were identified.

**Liver:** Significant hepatic lesions were pigmented hepaticopathy (hemosiderosis)(N = 8), chronic periportal hepatitis (N = 4), hepatic lipidosis (N = 2), hepatic cestodiasis (similar to *Mesocestoides*)(N = 2), cholecystitis (N = 2 and one with cholelithiasis), and cholangitis. Hepatitis (N =6) and cholangiohepatitis (N =1) were secondary to sepsis from herpesvirus infection, *Yersinia spp*, *Staphylococcus aureus*, *Streptococcus viridans*, and *Klebsiella sp.*
Pancreas: Chronic pancreatitis and interstitial pancreatic fibrosis due to *Trichospirura leptostoma* were seen in 27 animals. The majority (70.37%) were from the same original group and none were wild-caught. This lesion had a strong familial prevalence with 9 from first generation (including several siblings pairs) and 5 from second generation animals.

Respiratory system: Interstitial pneumonia secondary to sepsis was a complication of gastroenteritis (*Y. enterocolitica* and hemorrhagic enteritis syndrome) or other infections (e.g. measles, valvular endocarditis). Suppurative pneumonia was also associated with sepsis due to enterocolitis (*Y. enterocolitica* and *Pseudomonas aeruginosa*) and herpesvirus infection. Suppurative verminous pneumonia (due to *Filaroides* spp.) was an incidental finding in a herpesvirus-infected animal. Acute non-specific pneumonia, peracute *Klebsiella* spp. pneumonia, aspiration pneumonia, cholesterol-type pneumonitis were other findings.

Cardiovascular system: Important entities were congestive heart failure, vegetative endocarditis of aortic valves with sepsis and disseminated intravascular coagulation due to a coagulase-positive *Staphylococcus aureus* and *Streptococcus viridans*, cardiomegaly due to renal disease, and myocarditis due to sepsis. Spontaneous atherosclerosis associated with dietary-induced hyperbetalipoproteinemia was the cause of death in one animal and an incidental finding in nine others.

Urinary system: Membranoproliferative glomerulonephritis (*N* = 9) and/or chronic interstitial nephritis (*N* = 4) were the most prevalent lesions, followed by glomerulosclerosis, hemoglobinuric nephrosis and renal infarction. End stage renal disease and polycystic kidneys were diagnosed in two adult males imported from West Germany.

Other interesting post-mortem findings: Although not a cause of mortality, dermatitis (seborrhea sicca) was a common finding and probably was associated with environmental factors. A case of colonic and splenic amyloidosis was found in a 29 year old male. Rat bite fever caused by *Streptobacillus moniliformis* was identified in one animal that presented with acute arthritis of the stifle. Congenital abnormalities noted included patent ductus arteriosus, triluminal tracheal, and a tracheal ring malformation. Neoplasia was not a major problem in *Callicebus* spp. Three males greater than 20 years old had thyroid adenoma and one of these three also had a pheochromocytoma and one a cholangiocarcinoma. Environmental stress leading to hypo and hyperthermia seems to contribute to morbidity and mortality. Although *Callicebus* spp. are tropical species, animals did not tolerate the excessive dry heat of the California valley.

Conclusions

This study has demonstrated that *Callicebus* spp. are susceptible to many of the diseases reported in other new world primates including Callithrichidae and Cebidae. Vermin control is essential for prevention of infection such as *Yersinia* spp, *Streptobacillus moniliformes*, *Trichospirura leptostoma*, *Mesocestoides*-like and *Gongylonema* spp. in captive titi monkeys. Our data suggests that mortality decreased dramatically with the improvement of husbandry, especially housing.
ACKNOWLEDGMENTS

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SELECTED LITERATURE

"MORTALITY RATE 1972 - 1992"

FIG. 1 Mortality Rate: Number of animal deaths per year divided by the average number of animals in the colony each 12 month period.
BILIARY CYSTS AND HEPATIC PROLIFERATIVE LESIONS IN CAPTIVE WILD FELIDS

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Hepatic and renal diseases have been observed in high prevalence in both wild caught and captive born non-domestic felids. Single or multiple non-parasitic intrahepatic cysts have been considered an incidental post-mortem finding in older domestic cats. Similar lesions have been seen in exotic felids including leopard, jaguar, lion, tiger, mountain lion and cheetah at the Veterinary Medical Teaching Hospital (VMTH), University of California, Davis. Because of these observations, a retrospective study was conducted to determine the significance, prevalence, etiopathogenesis and external factors associated with intrahepatic cysts of suspected biliary origin in felids and to differentiate these cysts from cystic hyperplasia of biliary epithelium, proliferative hepatocellular lesions, and primary and metastatic hepatic neoplasia in captive wild felids.

A questionnaire that included questions regarding diet, steroid therapy and relatedness of animals (see addendum) was sent to selected zoos, wild animal parks or other institutions housing wild cats in several countries in North America, Europe and South America. Necropsy reports, sometimes accompanied by paraffin blocks or slides, were contributed by the collaborators. In addition, 195 non-domestic feld necropsy reports, clinical data, and slides from the VMTH archives from 1970-1993 were examined. To date, necropsy data on 740 animals have been received from 14 collections. Of these, 33 large cats (18 males and 15 females) have had hepatic cysts or neoplasms. Species affected were Acinonyx jubatus, Felis concolor, Neofelis nebulosa, Panthera leo, Panthera onca, Panthera pardus, Panthera tigris. Ages ranged from 4 months to 26 years with an average age of 16 years. Six females (5 lions and 1 leopard) had been given progesterone or melengestrol acetate implants, and one male lion was given intramuscular testosterone.

The cysts in the liver generally were described as thin-walled, solitary or multiloculated with a "cellophane-like" appearance and filled with clear to pale yellow fluid. Some cysts contained brown or bloody fluid or blood clots and some contained milky to purulent material or were frank abscesses. Microscopically, these cystic structures were lined by a simple squamous or single layer of low cuboidal secretory epithelium that appeared to be of bile duct origin. Cysts containing opaque fluid were consistent with infection of
preexisting cysts. *Pasteurella sp* was cultured from one abscess in a lion. For hemorrhagic cysts, it was sometimes difficult to determine if these had a vascular or biliary cyst origin. In some cases, the walls of the cysts were highly vascularized, and there was hemorrhage from these vessels into the wall and lumen of cysts, suggesting that the blood-filled structures were originally biliary cysts.

The pathogenesis of these cysts in non-domestic felids still remains uncertain. There is neither clear association with contraceptive steroids nor association with other liver diseases such as veno-occlusive disease or cirrhosis. Age is definitely a predisposing factor, because most of our animals were older or truly geriatric. The congenital nature and genetic factors are still in debate. Solitary cysts were found in a 4 month-old tiger cub and in a 4 year-old adult tiger. Also, one lion sibling pair and one leopard sibling pair had multiple cysts, and one of each pair also had a biliary carcinoma. However, in a sibling pair of cougars the male, but not the female, had multiple cysts and a bile duct adenocarcinoma with metastasis. Interestingly, many cats had originated from a common source, although several animals were housed in different zoos.

Animals with biliary cysts from this study were asymptomatic. However, in humans some cysts can be large enough to have caused abdominal pain and distension or to have been easily palpated. Hepatic insufficiency due to these cysts in humans is uncommon, and routine serum chemistries were usually not helpful in the diagnosis. However, jaundice and increased liver enzymes occasionally occurred in the most severely affected people. In our cats the diagnosis was usually established by radiography, ultrasonography or at necropsy.

Polycystic liver disease has also been described in bears, white-tailed deer, golden hamsters, *cpk* mutant laboratory mice, farm animals, non-human primates such as squirrel monkeys, and humans. At the VMTH, similar lesions have also been seen in a llama and a dingo. It is believed that these cysts generally arise as congenital abnormalities of the intra and extra hepatic biliary system. In humans, there is an association between intrahepatic cysts and autosomal dominant polycystic kidney disease in adults (ADPKD) and autosomal recessive polycystic kidney disease in infants (ARPKD). Additional predisposing factors in humans include gender (females greater than males), age, multiple pregnancies, cirrhosis, and the administration of contraceptives. Despite the pathologic resemblance of our cases to the hepatic cysts seen in ADPKD and ARPKD, only two cats in our study had evidence of renal cystic disease.

Biliary hyperplasia, biliary cystadenomas and carcinomas, and a hepatocellular carcinoma were found in addition to biliary cysts in big cats in this survey. In some species, biliary cystadenomas are considered the third most frequent primary liver tumor after hepatocellular carcinoma and hemangiosarcoma. Distinguishing a true bile duct adenoma from developmental or congenital cysts of the bile ducts is difficult. Biopsy and immunostaining techniques are of great importance to differentiate primary malignant tumors of hepatocyte or biliary epithelial origin from biliary hyperplasia, biliary cysts, or metastatic tumors. For the cases in this study, histopathology, immunohistochemistry and ultramicroscopic morphology will be applied to aid in the differential diagnosis of the hepatic lesions.
Other significant and interesting post-mortem findings besides biliary cysts and/or liver tumors in this survey included: veno-occlusive disease in lions, leopards, one tiger and one cheetah. Membranoproliferative glomerulonephritis, renal papillary necrosis, renal calculi and chronic interstitial fibrosis, discospondilitis, degenerative joint disease, osteoarthritis, pheochromocytoma, generalized amyloidosis, endogenous lipid pneumonia, thyroid cysts and parovarian cysts were also found in the majority of the animals.

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LITERATURE CITED

SURVEY OF HEPATIC CYSTS IN EXOTIC FELIDS IN CAPTIVITY

1. How many wild felids do you have in your collection now?

2. How many have you had since 1975?

3. Which species?

4. How many animals of each species have died and been examined post mortem since 1975?

5. Were any of these diagnosed as having:
   a. Liver disease?
   b. Biliary cysts?
   c. Biliary tumors?
   d. Renal cysts?

6. Were any of the animals with biliary or renal cysts, biliary cystadenoma or adenocarcinoma related?

7. Did relatives of any of the animals with these lesions die without evidence of biliary cysts?
FOR EACH ANIMAL WITH BILIARY CYSTS AND/OR HEPATIC TUMORS,
PLEASE ANSWER THE FOLLOWING:

Animal # or name:

1. Pathology 
   (which institution performed the biopsy/necropsy):

2. Species and subspecies:

3. Age:

4. Sex: M F  
   a. If female: how many pregnancies?  _ _ _ _  Contraception? __
   b. If male: contraceptive procedures? ______________


7. Date of death:

8. Diet:  
   a. whole prey? _______________ What type? ______________
   b. meat? _______________ What type? ______________
   c. "home made" prepared diet? ______________
   d. commercially prepared diet? ____________ brand(s) ________

9. Treatment history:
   a. antibiotics? ________
   b. hormones? __________
   c. tranquilizer? _________
   d. other ____________________
10. Pathology: (If possible, please attach reports)
   a. Were cysts: single? _______ multiple? _______
      localized? _______ disseminated? _______
   b. Was bile duct patent? _______
   c. Was there hepatitis or chronic liver disease?
   d. Were there renal cysts?
   e. Chronic renal disease?
   f. Were there proliferative or cystic lesions in any other organs?

Thank you very much for your participation in this survey. Please indicate names of clinicians, pathologists and institutions to be acknowledged in the publication of this research.
LINGUAL SPIRURIDS AND PASTEURELLOSIS IN GOELDI'S MONKEYS (*Callimico goeldi*)

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In July 1992 a group of four Goeldi's monkeys (*Callimico goeldi*), one male and three females, at the National Zoological Park rapidly became severely depressed and anorectic. One was found collapsed. Within 24 hrs, three of the animals were dead.

Gross examination of the carcasses revealed no significant internal lesions, although increased hyperemia of the lips was noted in two animals. *Pasteurella multocida* was isolated from heart blood of the first two monkeys to die.

Histologic examination of the tissues indicated an acute bacteremia with fibrinous thrombi and bacteria in the pulmonary vessels. Neutrophils were transmigrating vessel walls, and there was necrosis of adjacent pulmonary tissue. Similar changes were noted in the liver of one animal. Frequently the centers of lymph node follicles were hypocellular and lymph vessels contained bacteria, neutrophils, debris, and fibrinosuppurative thrombi. Lymphoid depletion was also present in the splenic white pulp. In the deep glossal mucosa, multiple cross sections of metazoan parasites were noted associated with a mild to moderate inflammation consisting of focal aggregates of plasma cells and microabscesses. In one monkey there were small colonies of bacteria in the glossal epithelium and perivascularly in the interstitium of the glossal musculature. The parasites had a thick cuticle, lateral alae, coelomyarian musculature and high numbers of larvated eggs. These morphological characteristics are consistent with a spirurid nematode.

The surviving female had hyperemic gingivae and marked gaseous gastric distension. Gastric washes were unremarkable. The animal recovered with fluid therapy and a nine day course of ceftiofur (10 mg i.m., b.i.d.) (Upjohn, Kalamazoo, Minnesota, 49001, USA). Nine days later, tongue scrapings were positive for spirurid eggs, and the monkey was treated with injectable ivermectin (0.2 - 0.4 mg/kg) (Ivomec; MSD Agvet, Division of Merck & Co., Inc., Rahway, New Jersey, 07065, USA). Scrapings were again positive five days later and ivermectin treatment was repeated. The monkey was returned to the exhibit 20 days after the death of the other monkeys.

One month later the surviving monkey suddenly became weak and dull, and died during treatment. Necropsy findings, both gross and microscopic, resembled those of the three cases described above. *P. multocida* was isolated from the liver. No change in the appearance of the glossal nematodes was discerned to suggest any alteration in parasite viability with anthelmintic treatment.

Subsequently, hypersalivation was observed in two other Goeldi's monkeys in January 1993 that had recently transferred to the exhibit from the Conservation Research Center, in Front Royal. One of these monkeys had swollen lips, an hyperemic oropharynx, and a mucoid exudate and roughened surface on the tongue. Tongue scrapings from both animals yielded low numbers of spirurid eggs similar in appearance to those seen on histology in the
previously described group. The nematode infection in these two monkeys was initially treated twice with injectable ivermectin (0.2 - 0.4 mg/kg). However, the facial swelling did not reduce until oral mebendazole (70 mg/kg) (Telmin; Pitman-Moore, Inc., 421 E. Hawley Street, Mundelein, Illinois, 60060, U.S.A.) was administered. No parasite eggs have been recovered from recent tongue scrapings.

Retrospectively, the keepers had noted an increased level of salivation in the group of animals for approximately three years, which had been investigated in February 1992. Clinical records indicate the occasional presence of spirurid-like eggs in feces of the Goeldi’s monkeys from the exhibit, and these monkeys were subsequently treated with ivermectin.

Lingual spirurids are commonly reported in wild caught non-human primates, but are generally considered to be an incidental finding unassociated with disease. *Gongylonema pulchrum* and *Spirura guianensis* are the spirurids most commonly found on histology in sections of primate tongue and esophageal epithelium. *G. pulchrum* has a wide host range, and the body is completely located in the host mucosa. *Spirura* spp. are usually found more superficially in the epithelium and consequently these nematodes are often visible on gross post-mortem. There is less inflammatory infiltrate associated with *Spirura* spp. infestations, but frequently some fibrosis occurs. In the cases described, the nematodes were deep in the mucosa and inapparent on necropsy examination. Thus *Gongylonema* spp. is the more likely parasite agent.

Cockroaches and dung beetles are common intermediate hosts for *Gongylonema* and *Spirura* spp., and the small primates in the collection have been observed ingesting these insects. Recently, an increased population of arthropod pests has been recognized in the building in which these monkeys were housed, and we speculate that the increased numbers with possibly an increased concentration of the intermediate stages may have precipitated the cases described.

*P. multocida* commonly causes respiratory infection in rodents, but has been isolated in the normal naso-oropharyngeal flora of many domestic species. It is infrequently associated with septicemia and hemorrhagic foci in primates, although the pathology records at the National Zoological Park indicate isolation of the bacteria from septicemic golden lion tamarins and black-tailed marmosets in association with tooth abscesses.

In conclusion, the presence of oropharyngeal spirurid infection may not be as innocuous as previously thought. The parasites were associated with inflammation and increased salivation, and possibly resulted in bacterial infection in immunosuppressed hosts. Alternatively, spirurid migration may provide a direct route of entry for bacterial pathogens.
The Galapagos tortoise (*Geochelone elephantopus*) is one of only two living species of giant tortoise and is endangered. Despite the fact that a number of zoos have held Galapagos tortoises, few have been able to establish self-sustaining populations and little has been published on the diseases of Galapagos tortoises.

The Zoological Society of San Diego (ZSSD) has long held a large and self-sustaining population of Galapagos tortoises. A review of the pathology records of the ZSSD revealed 54 necropsy accessions since 1964. The most significant cause of mortality has been gastrointestinal disease due to bacterial, nematode, and amoebic infections, as well as sand ingestion (Anderson and Rideout, personal observation). No neoplasms were identified until 1983.

Since 1983, 9 galapagos tortoises have died. Eight of the nine had gross evidence of necrotizing enterocolitis, but in the four cases that had histologic evaluations, the underlying disease process was lymphoma of the gastrointestinal (GI) tract. Two of the affected animals were juveniles (less than 7 years of age) and two were adults (20 years of age or older). Although it has been difficult to follow animals shipped to other institutions, one animal that was hatched at ZSSD and shipped as a juvenile to another institution died several months after arrival from a similar lymphoma (case 5). Individual cases are identified in Table 1.

In all 5 cases, the lymphomas appeared to be centered in the GI tract. The most extensive involvement occurred in the small intestine and colon, but ranged from the oral cavity to the terminal colon. In the early stages of involvement (cases 1 and 2), the lymphoid cells were invasive, but not destructive. When mucosal ulceration occurred, it was accompanied by secondary bacterial invasion with associated acute necrotizing inflammation. More advanced cases (cases 3, 4, 5) had widespread highly invasive and destructive infiltrates with a prominent vascular orientation. Hematologic dissemination to other viscera occurred in four cases (cases 1, 3, 4, and 5). The vascular orientation and destructive behavior was maintained in the visceral metastases.

The origin of the neoplastic cells is open to speculation, but based on the histologic distribution, the neoplasia may have arisen in the lymphoid tissue of the GI tract. The phenotype of the cells is also not clear. Morphologically they resemble medium to large lymphocytes, but further characterization is not possible at this time due to a lack of suitable reagents (P. Kline and E. R. Jacobson, personal communication).
The young age of three of the five affected animals, and the apparent clustering of the cases, raises the possibility of an infectious etiology. Preliminary ultrastructural examination in case 4, however, failed to reveal any evidence of viral particles. A non-productive viral infection has not been ruled out. The possibility of an inherited component also exists, since all 3 animals of known parentage were offspring of a single female, and all affected tortoises of known sex were male. There was no known history of exposure to carcinogenic agents. Additional studies to clarify the etiology of these lymphomas are in progress.

TABLE 1: Individual Tortoise Data.

<table>
<thead>
<tr>
<th>Tortoise #</th>
<th>Age</th>
<th>Sex</th>
<th>Birth Date</th>
<th>Death Date</th>
<th>Dam #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7 yrs</td>
<td>M</td>
<td>7/06/76</td>
<td>6/16/83</td>
<td>128</td>
</tr>
<tr>
<td>2</td>
<td>21 yrs</td>
<td>M</td>
<td>8/09/63</td>
<td>5/20/84</td>
<td>128</td>
</tr>
<tr>
<td>3</td>
<td>22 yrs</td>
<td>M</td>
<td>8/09/63</td>
<td>12/25/84</td>
<td>128</td>
</tr>
<tr>
<td>4</td>
<td>5 yrs</td>
<td>M</td>
<td>5/29/87</td>
<td>3/24/92</td>
<td>unk</td>
</tr>
<tr>
<td>5</td>
<td>3 yrs</td>
<td>unk</td>
<td>8/06/85</td>
<td>2/ ?/88</td>
<td>unk</td>
</tr>
</tbody>
</table>

AGE = Rounded to the nearest year.
SEX: M = Male; unk = unknown.

LITERATURE CITED

Mycobacterium bovis INFECTION IN U.S. DEER AND ELK FARMS

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Historical Background

The United States Department of Agriculture (USDA) started a tuberculosis eradication program in 1917. At that time, tuberculosis caused more losses to the cattle industry than all other infectious diseases combined. Early estimates indicate that approximately 5 percent of all the carcasses inspected had lesions of tuberculosis. At that time, approximately 25 percent of the tuberculosis cases in children were due to Mycobacterium bovis the etiological agent of bovine tuberculosis. Since 1917, State and Federal governments have spent over 1 billion dollars to eradicate TB in cattle. Today bovine TB is very rare in cattle with an incidence of less than 1 per 100,000 animals. The virtual eradication of bovine tuberculosis in the United States has been a significant step in protecting the health of its citizens.

The eradication of M. bovis from cattle herds has not been easy. Wisconsin for example began area testing in the early 1920's and continued it until 1966. Every cattle herd in the state was tested at least once (often 4 or 5 times) during this period of time. Area testing was replaced with slaughter surveillance in 1966 because the incidence of TB had dropped to such a low level it was no longer cost effective to continue area testing.

Three significant events have occurred during the Wisconsin TB eradication program. The first event occurred in the 1930's when Herman Buneson MD, Chairman of the Chicago Board of Health said that no milk could enter Chicago unless it originated from herds that were test negative for tuberculosis. The Northern Illinois Dairy Cooperative did not take him seriously and eventually went bankrupt because it was unable to sell milk to the Chicago market. Wisconsin took him very seriously and eventually became the major supplier of milk to the Chicago market. This one event ensured that Wisconsin would become the nation's dairy state. The second major event occurred in the 1940's when Wisconsin became one of the first states to institute a policy of whole herd depopulation for herds known to be infected with M. bovis. This policy cost the cattle industry millions of dollars because the indemnity offered at that time covered very little of the losses actually incurred by the producer. The third major event happened in 1986 when Wisconsin was declared tuberculosis-free by the United States Department of Agriculture.

Tuberculosis-free status is very important for interstate commerce since animals from TB-free states can move interstate without a tuberculosis test. In addition, TB-free accreditation is required by many countries for international shipment of live animals, semen and embryos. For these reasons the cattle industry in the United States has a keen interest in keeping the industry free of bovine tuberculosis. Seventy years of dedicated effort by veterinarians and the livestock industry as well as hundreds of millions of dollars have been invested to rid the country of bovine tuberculosis.
Current Situation

Commercial deer and elk farming is a relatively new industry in the United States. This industry is faced with many of the same problems faced by the cattle industry over 70 years ago when they had to be regulated for the first time. USDA-APHIS first became aware of a tuberculosis problem in cervidae in late 1990. Tuberculosis investigations of captive deer and elk herds began following reports from Canada that some Montana elk herds may have sold *M. bovis* infected animals. Since that time, bovine tuberculosis has been confirmed in Montana herds epidemiologically linked with the original Canadian herd. Agriculture Canada has spent in excess of 11 million dollars since 1990 depopulating herds in Ontario and Alberta to ensure the eradication of TB (personal communication, Eric Broughton, Agriculture Canada). The Wisconsin Department of Agriculture became aware of TB in late 1990, when tuberculosis was found in a red deer herd. The *M. bovis* infection was traced to an elk purchased from another state. The Wisconsin herd has been quarantined and the quarantine will not be lifted until the department has sufficient testing evidence to conclude that tuberculosis has been eradicated from the herd.

Since 1990, 8 states have discovered *M. bovis* in at least one cervidae herd. In response to the tuberculosis problem many states have enacted rules to regulate the intrastate and interstate movement of cervidae. There is tremendous variability in the interstate rules for shipment of animals. Wisconsin for example, requires that the herd be tested for tuberculosis before any animal can enter the state. This interstate testing requirement was made for 2 reasons. The original *M. bovis* infected elk imported into Wisconsin was test negative for TB (caudal fold test) and a negative TB test on an individual animal has a very low negative predictive value (<50%) when the prevalence of the disease is unknown.

Last year, New York State had bovine tuberculosis transmitted from a captive deer herd to a dairy herd. This outbreak of tuberculosis caused 23 dairy herds to be quarantined in New York and Pennsylvania and caused New York State to lose its accredited TB-free status. This outbreak has cost the New York and Pennsylvania cattle industries millions of dollars due to the increased costs of testing and lost markets. As a consequence of this outbreak, the New York Legislature appropriated $650,000 to the Division of Animal Industry, and instructed the Division to test all the cervidae herds within 2 years. To date, 7 herds out of 156 tested have been confirmed to be infected with *M. bovis*. This relatively high prevalence rate (4.5%) has serious implications for the livestock industry in New York (personal communication, John Huntley, State Veterinarian, New York).

Wisconsin has embarked on a program to test all the commercial deer and elk herds in the state. To date, 15 herds in addition to the herd quarantined for tuberculosis have been tested, and all have been test negative for tuberculosis. The Division of Animal Health is in the process of testing the remainder of the herds, but is severely hampered by the lack of facilities at many deer herds to conduct the tuberculosis test. To address the facility problem the Governor's proposed 1993-95 biennial budget would require persons raising commercial deer (elk, red, fallow and sika deer) to provide confinement facilities acceptable to the department to conduct a tuberculin test.
Mycobacterium bovis diagnostics

No herd is considered to be infected with *M. bovis* unless the organism is isolated by bacteriological culture. Unfortunately, bacterial isolation requires the submission of tissues from affected animals. Most of the bacteriological culturing for *M. bovis* in the U.S. is performed at the National Veterinary Services Laboratory located in Ames, Iowa. Most of the TB-infected animals are identified through the use of diagnostic tests since there is very little slaughter surveillance of deer and elk in the U.S. Most bacterial isolates thought to be *M. bovis* by standard biochemical methods are confirmed with a commercial gene probe (Gen-Probe, Inc., San Diego, CA). This probe classifies isolates in the Mycobacterium tuberculosis complex. Interestingly only recently has it been possible to distinguish between *M. bovis* and *M. tuberculosis* based on a recently published polymerase chain reaction technique. ⁷

Since it was first developed by Robert Koch over 100 years ago; the intradermal skin test has been the mainstay of tuberculosis testing. Tuberculin is prepared by harvesting the supernatant of broth cultures of *M. bovis* and precipitating the supernatant with ammonium sulphate.¹ The end product is sterilized and standardized for potency and is known as purified protein derivative or P.P.D.¹ In animals 5,000 I.U. of tuberculin is given intradermally with reading by observation and palpation 72 hours later. This is an extremely useful test when herds or populations of animals are tested to determine if they are infected with *M. bovis*. The test is not very useful on individual animals to determine infection status or in herds to eradicate *M. bovis*.² ³ The test has been reported to have a sensitivity and specificity of 85% and 95% respectively in cattle.² In 1973, the comparative cervical test (CCT) was developed as an aid to diagnose bovine TB. The CCT is used in conjunction with the intradermal PPD test, with administration if an animal was positive to the PPD test. The test has a sensitivity and specificity of 74% and 97% respectively, and has been shown to be effective in detecting infected cattle in herds with a high prevalence of disease.⁸ Unfortunately, the proportion of false positives increases with a decrease in prevalence within the herd.⁸ A new test for TB diagnostics in cattle is the gamma interferon assay developed in Australia by Paul Wood. This assay detects bovine gamma interferon produced by lymphocytes sensitized to *M. bovis*.¹¹ This assay is currently under field evaluation by USDA-APHIS. This test may be useful as a rapid screening test to test populations of animals before they can enter the country or state.

*M. bovis* testing is much more difficult in deer and elk herds for 2 reasons. The first reason is that diagnostic test accuracy (sensitivity and specificity) for the cervical tuberculin test is not well established in North American herds. New Zealand data indicates there can be a high number of false positives with the cervical skin test in some herds.⁵ Field experience in Wisconsin has shown approximately 50% of the herds tested have false positive test results ranging from as low as 5% to as high as 25% of the animals tested. The CCT has worked quite well to sort out herds with a number of cervical skin test responding animals. Also, commercial deer and elk are much more difficult to handle than cattle. Dairy and beef cattle have been domesticated for thousands of years and are amenable to handling with a minimum of equipment. This is not the case with commercial deer and elk. Elk in particular cannot be handled unless tranquilized or restrained in a chute.

²²⁶ 1993 PROCEEDINGS AMERICAN ASSOCIATION OF ZOO VETERINARIANS
The cervical skin test and the comparative cervical test are the only tests officially approved for use in deer and elk in the United States. The commercial deer and elk industry is quite interested in the Blood Tuberculosis Test or BTB developed by Dr. Frank Griffin at the deer research laboratory in New Zealand. The BTB test is composed of three components: an ELISA and lymphocyte stimulation assay and a measure of inflammatory mediators.\(^5\) All three factors are used to determine the \textit{M. bovis} status of individual animals and herds. The BTB test has been reported to be more sensitive and specific than either the cervical or the CCT test in New Zealand.\(^5\) Currently, there is a joint Agriculture Canada and USDA-APHIS study to evaluate the efficacy of the BTB test in North American deer and elk herds. Preliminary results have not been made available at this time. Limited experience in Wisconsin has indicated that the BTB test is more specific (\(p < .001\)) than the cervical skin test and has similar test sensitivity.\(^9\)

**Conclusion**

The commercial deer farming industry has tremendous growth potential. However, the industry is facing the same growing pains the nation’s cattle industry faced over 70 years ago when it had to confront bovine tuberculosis. When Federal Uniform Methods and Rules for TB eradication in cervidae become official, the industry will be helped by a program to certify herds as tuberculosis-free. Animals from tuberculosis-free herds would not require testing for intrastate movement. Tuberculosis-free certification will help the industry market animals for breeding stock and will enhance their value. Testing of herds on a state-wide basis in states such as New York and Wisconsin will also provide assurance to the cattle industry that the disease is being aggressively eradicated in those states. Eliminating the tuberculosis cloud hanging over this industry should help with the continued growth and development of this industry.

**LITERATURE CITED**

Tuberculosis in captive, exotic and native hoofstock is most frequently caused by *Mycobacterium bovis* and occasionally by *M. tuberculosis*. For public health and regulatory reasons, infections caused by the agents listed above must be differentiated from infections caused by other mycobacterial species, such as *M. avium* and *M. paratuberculosis*, which are also isolated with some frequency from ungulates in zoos.

Most mycobacterial species cause granulomatous lesions which cannot be differentiated grossly or by histopathology. Thus, the definitive diagnosis of tuberculosis still relies upon the isolation and identification of *M. bovis* in tissues of affected animals. Surveillance, which is the basis of the current federal TB Eradication Program, is a powerful method to monitor tuberculosis, and should include complete necropsy of all animals that die and culture of a representative sample of lymph nodes from all regions of the body. Sampling of widely distributed nodes may be important in the detection of other mycobacterial species and thus differentiation from possible *M. bovis* infection.

Antemortem tests such as the tuberculin skin test (ST), the enzyme linked immunosorbent (ELISA) and the lymphocyte stimulation test (LST) have been used as supplementary tests, but the results must be interpreted with caution due to the diversity of tests used and lack of validation in most zoo ungulate species. For example, currently over 12 protein antigens, plus numerous polysaccharide and cell wall antigens, have been described and used in ELISA with varying serologic reactivities within both infected and uninfected individuals. To validate the accuracy of tests in individuals requires that test systems and protocols be standardized within a species, then evaluated in the species in known infected and uninfected individuals. True infection status must be determined by confirmatory culture of a comprehensive set of samples obtained at necropsy, or possibly by antemortem biopsy of infected animals. A system established by the zoologic community for centralized reporting of results and serum banks would enhance efforts to validate and improve the utility of antemortem tests for tuberculosis in zoo ungulates.

The standard national and international test used to screen for tuberculosis in humans and animals is the tuberculin skin test (ST) which measures delayed-type hypersensitivity to a protein derivative (tuberculin) prepared from *M. bovis, M. avium*, or *M. tuberculosis*. The site of injection varies with the species and can have an effect on test reaction. A survey of tuberculin skin testing techniques used at different zoos revealed substantial variability in the type of tuberculin used, frequency of testing and disposition of reactors.

False positive skin tests (lack of specificity) have been reported due to infection with other mycobacterial species. The comparative skin test (CST) increases the specificity of the ST by testing reactor animals with both *M. avium* and *M. bovis* PPD antigens and comparing the relative response but at the expense of a lowered sensitivity. False negative skin tests (lack of sensitivity) have been reported in animals with disseminated or advanced tuberculosis.
The ELISA test has been used in camelids,9,22 bovids10 and captive reared cervids19 for detection of antibodies to *M. bovis*. Skin test negative (anergic) animals with advanced, disseminated disease generally respond with higher ELISA values than animals with more localized infections.10 Multiple test systems and antigens have been described, but standardization of test procedures is necessary for comparison of results within species. The use of highly purified antigens in both ELISA and skin tests shows potential for increasing specificity of tuberculosis diagnosis but at the cost of decreasing sensitivity.4 Different interpretations of ELISA cutoff values may be necessary when applied to infected versus noninfected herds.9 Researchers have reported an increase in ELISA values after skin testing in *M. bovis* infected domestic cattle,10 captive reared deer6 and llamas.18 The response does not occur in uninfected controls. Thus, this combination of tests shows some promise in increasing the accuracy of the individual tests.

Cell mediated immunity to *M. bovis* infection develops earlier than humoral immunity. In an attempt to detect subclinically infected animals with greater accuracy than is provided by the ST, alternative tests of cell mediated immunity to *M. bovis* have been developed. The lymphocyte stimulation test (LST) has been used in New and Old World Camelids to detect *M. bovis* infection and to differentiate *M. bovis* from *M. avium* infection.11 The LST test requires viable cells and suffers from large within test and between test variability making interpretation difficult.

In cattle, specific induction of gamma interferon in whole blood cell cultures exposed to PPD *M. bovis* correlates well with the LST,24 and may be a more sensitive measure of the cell mediated immune response to *M. bovis* infection than the ST.25 Preliminary studies of lymphokines produced in response to *M. bovis* infection have been reported in cervids3 but not in other species.

Griffin *et al* reported increased sensitivity and specificity over ST tuberculosis testing in deer by using a combination of ELISA, LST, fibrinogen, plasma viscosity, differential white cell count, and hematologic values.8 The use of multiple tests to measure both cellular and humoral immunity may improve accuracy of tuberculosis diagnosis in the herd, but requires further development and validation in each species. The standard in the individual animal remains culture and isolation of *M. bovis* from tissues, which is reliable across all species.

A gene amplification technique, termed polymerase chain reaction (PCR) has been widely used in diagnostic microbiology to amplify the DNA of infectious agents and enhance detection. The application of PCR to aid in the rapid diagnosis of *M. tuberculosis* and *M. bovis* has been also reported.5,16 Cousins *et al* used primers derived from a *M. bovis* secretory protein, (MPB70), to amplify a 372 bp DNA fragment, and found the technique to be very sensitive and highly specific.5 The primers used positively identified 84 strains of *M. bovis* tested and did not react with 24 other species of mycobacteria, or strains from other genera including *Rhodococcus equi*, *Nocardia asteroides*, *Actinomyces bovis*, and *Actinobacillus lignieressi*. The technique was sensitive enough to detect a single viable cell in serially diluted samples. However, the primers used by Cousins *et al* were not capable
of discriminating between \textit{M. bovis} and other members of the \textit{M. tuberculosis} complex. Plikaytis \textit{et al.},\textsuperscript{10} using primers derived from both IS6110 and GroEL to amplify the DNA fragments, found it possible to differentiate \textit{M. tuberculosis} and \textit{M. bovis}.

PCR technology has been applied to clinical samples such as sputum\textsuperscript{1,17} and lymph node biopsies\textsuperscript{15} for human tuberculosis diagnosis. When compared to culture as the standard for diagnosis, PCR has been reported to be as sensitive\textsuperscript{1} as or possibly more sensitive than culture\textsuperscript{17} in detecting \textit{M. tuberculosis} in sputum samples. Further refinement of PCR, and possibly immunohistochemistry, applied to various tissue preparations would provide critically valuable methods for differentiating \textit{M. bovis} infection when histopathologic lesions are compatible, but growth or confirmation by culture is unsuccessful. DNA probes and PCR techniques have already been used to identify \textit{M. avium} spp. and \textit{M. paratuberculosis} in both humans\textsuperscript{13} and domestic animals.\textsuperscript{23,26} The technique has the advantages of high sensitivity and specificity, and, like culture, can be used across species. Application of DNA probes and PCR techniques to clinical samples collected by biopsy or tracheal washes, can be an aid in rapidly differentiating mycobacterial infections in animals identified by immunologic tests as possibly infected.

\textbf{LITERATURE CITED}


American Association of Zoological Parks and Aquariums (AAZPA) accredited zoos have not been immune to the devastation arising from outbreaks of mycobacteriosis, be it in amphibians, reptiles, birds or mammals. Such discoveries have led to drastic measures to eradicate the diseases, in most cases promoted by conscientious clinical veterinarians, veterinary pathologists, curators and animal care staffs retained by these parks. As licensed animal exhibitors, all parks holding species of bovidae and cervidae are regulated by the United States Department of Agriculture (USDA) as well as the individual state veterinary agencies. The primary concern of these agencies is with a single species of infectious organism, \textit{Mycobacterium bovis}, and with a single goal, to keep this organism from contaminating the domestic livestock industry. While AAZPA accredited zoos have never been the source for major contamination of domestic livestock, they have none the less been implicated via a poorly delineated definition of a "zoo". A "zoo" as defined in a 1991 USDA sponsored International Conference on Bovine Tuberculosis in Cervidae included game farms, animal dealers, roadside menageries, unaccredited zoos, as well as accredited zoos.\textsuperscript{1} It is with this awareness that the present problems of mycobacteriosis in cervidae are approached.

\textbf{Historical} \textit{Mycobacterium bovis} Outbreaks in Cervids:

1969- A zoo collection is exposed via an \textit{M.bovis} infected axis deer (\textit{Axis axis}).\textsuperscript{2}

1974- infection in 130 sika deer (\textit{Cervus nippon}) and fallow deer (\textit{Dama dama}) in southern California.\textsuperscript{3}

1978- Multiple large scale outbreaks of farmed deer in New Zealand.\textsuperscript{4}

1981- Cluster of outbreaks in South Dakota in cervids believed to have transferred infection to bison.

1984- 24 infected bison herds disclosed in ten states. The source was believed to be infected elk purchased from a menagerie/farm in Iowa.

1990- 34 elk herds in Alberta, Canada were infected from a source believed to be a privately owned Montana elk herd. \textit{This led to the 1990 closure of the canadian border to llamas and cervidae from the United States}. It was at this same time that USDA officials became more concerned with the cervid farming industry.\textsuperscript{5}
1991- Trace back from an infected Montana elk herd found that animals were purchased in 1987 from a Nebraska game ranch which had also sold elk to a game ranch in Colorado. All of these animals were confirmed as infected. In addition captive elk at two Oklahoma ranches were infected.\(^1\)

1991- Bovine tuberculosis confirmed in a total of 13 captive cervid herds located in eight states: Colorado, Montana, Idaho, Nebraska, New York, Oklahoma, Texas, and Wisconsin. Cattle herds which were apparently infected by association with tuberculous cervids resulted in the loss of Accredited-Free status for the State of New York.\(^6\)

**Regulatory fact finding**

Tuberculosis in captive cervids, exotic species and other ranched animals was judged to be a threat to domestic livestock, native wildlife and to the public health of US citizens. A number of presentations, conferences and hearings proceeded the preliminary changes to the regulations governing the interstate shipment of cervidae. These included:

1. **International Conference on Bovine Tuberculosis in Cervidae.** 16-17 July 1991. Hosted by the USDA, this 2 day conference was designed to assist the USDA in determining the extent of the TB problem and solicit solutions from a broad group of invited participants including animal industry, state regulators, federal government researchers, epidemiologists and regulators. The American Association of Zoo Veterinarians (AAZV) was well represented and a working group was assigned to Tuberculosis in Zoo Ungulates.\(^1\)

2. **United States Animal Health Association (USAHA) Meeting.** 25 October-1 November 1991. The USAHA is composed primarily of cattle industry representatives, government regulatory personnel, and to a lesser extent other farmed livestock. It serves as a forum for discussion between the agricultural industry and the regulatory agencies which govern them. AAZV has a presence in this organization, primarily to represent the interests of AAZPA accredited zoo animals.\(^5\)

3. **Cervid TB Meeting hosted by the Wisconsin Department of Agriculture.** June 1992. Representatives from the cervid industry, state agriculture departments, AAZPA accredited zoos, federal researchers and epidemiologists presented information to a number of state veterinarians.

4. **USAHA Meeting.** 31 October-6 November 1992. Draft regulations governing the testing, interstate movement and disposition of cervidae was presented. No reference was made within these documents to animals held by AAZPA accredited zoos.\(^7\)

**Regulations governing the testing and interstate movement of cervidae**

As of the time of this manuscript preparation (April 1993) there is no finalized official document outlining the regulations governing testing and movement of cervidae. This leaves
the state veterinary medical officers (VMOs) without a reference for evaluating what test to perform on which types of animals. Thus, it is important to check with each state which is destined to receive animals from an AAZPA accredited zoo cervid herd as well as the state VMO within your state. Most states have adopted the interim guidelines established by the USDA in response to the 1990 border closure with Canada. These guidelines include:8

1. Intradermal injection of 0.1 ml of USDA contract PPD Bovis tuberculin in the midcervical region with reading by observation and palpation at 72 hours, plus or minus 6 hours. This test is referred to as the Single Strength Cervical TB Test (SSCT) Many states require that the administering accredited veterinarian receive special instruction on the proper administration of this test.

2. The injection site measuring approximately 2 1/2 inches must be clipped using electric clippers with a fine blade. The intradermal injection is made in the center of the clipped area.

3. Reading of the cervical tuberculin test is by palpation and the guidelines give a specific description.

4. Any response, irrespective of size, detected by palpation or visually will be considered a suspicious test and the responding animal classified as suspect.

5. All responses must be reported.

6. Suspect animals shall either be held for a comparative cervical test (CCT) no less than 90 days following or sacrificed and receive a complete necropsy.

7. Suspects which require the CCT will have the test performed and read by a State or Federal Veterinary Medical Officer.

8. The CCT is applied exactly as for cattle except that all animals having equal sized avian and bovine responses or predominantly bovine responses shall be classified as Positive.

9. Sacrificed animals will receive a complete necropsy by or in the presence of a VMO. Tissue samples for histopathology and culture shall be sent to the National Veterinary Services Laboratory in Ames, Iowa.

10. Lymph nodes for submission must include portions of: mandibular, retropharyngeal, parotid, mediastinal (middle and caudal), tracheobronchial (right and left), and hepatic.
Representatives of the Animal Health Committee of the AAZPA and Infectious Disease Committee of the AAZV have been working with the USDA and state veterinarians in an attempt to demonstrate the unique situation under which zoo cervidae are maintained. A proposal of exemption from uniform industry standards has been set forth. This can only be successful if zoo veterinarians have a herd surveillance program in place. The USDA has been positive in verbal review of the outlined proposal. The draft regulations for the cervid industry excluded AAZPA accredited zoos. However, without written regulations governing AAZPA accredited zoos, the VMOs must each make their decisions on testing procedures independently. Work continues to better define this situation.

Most state VMOs are unaware of the animal health precautions and procedures which are standard in most AAZPA zoos. It is in the best interests of every zoo veterinarian to form an open and positive relationship with the state and federal VMOs. Invite them to review preventive health procedures and necropsy protocols. Proving that there is a preventive health care program, complete necropsy exams, good quality animal identification and record keeping, as well as trace back capability will all be looked on as positive aspects of the zoo health care program. When the situation arises where the status of a rare or endangered cervid is in question, a good herd surveillance program may be viewed as valuable documentation in lieu of accurate intradermal skin testing procedures.

REFERENCES

8. Interim guidelines for Cervid TB testing.
THE ROLE OF THE NATIONAL CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC) IN THE IMPORTATION OF NONHUMAN PRIMATES 1993 UPDATE

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It has long been recognized that nonhuman primates (NHPs) can carry and transmit diseases to human contacts. Indeed, because of the phylogenetic closeness, many human pathogens can be transmitted to and studied in nonhuman primates far better than in any other species. This has led to a great demand for nonhuman primates in research, and has contributed to the establishment of captive breeding centers where many of these species who are suffering loss of natural habitat are preserved and propagated.

At present, many NHPs in the United States are wild-caught, rather than captive-bred. The speed and efficiency of modern transportation systems allows wild-caught NHPs to arrive in the United States only a few weeks after capture. Many of these animals are from areas where infectious agents not frequently encountered in the United States are found. For example, some species may be asymptotically infected with viruses that are highly pathogenic for humans, such as herpes B virus. In addition, NHPs may be exposed to human-origin infectious agents such as tuberculosis and shigellosis while still in their country of origin.

Role of the CDC's division of quarantine

Although regulation of these activities was first prescribed in 1948, the Division of Quarantine (DQ) has recently assumed an increasingly important role in regulating the importation and quarantine of NHPs in order to prevent the introduction and transmission of serious human pathogens. This role has required restricting the uses for which NHPs may be imported. In particular, it has necessitated the implementation of a special permit process for importation of rhesus, cynomolgus, and African green monkeys; the registration of approved quarantine facilities; the inspection of arriving shipments of these NHP species at ports of entry into the United States; and the supervision of post-inspection quarantine procedures.

Prior to 1975, regulation of NHPs from endemic or epidemic yellow fever areas was of primary concern. In 1975, regulations were promulgated to address emerging concerns regarding rare hemorrhagic fevers, tuberculosis, and other zoonotic diseases of new or resurgent importance. Since 1975, importers of nonhuman primates must be registered with CDC, and are required to report the presumptive or confirmed diagnosis of specific diseases of potential public health importance to CDC within 24 hours of recognition.
The Division of Quarantine maintains inspectors at 8 major ports of entry into the U.S.; these inspectors are charged with inspecting immigrants and refugees, dogs, cats, turtles, etiologic agents, and human remains upon their arrival at a port of entry, as well as nonhuman primates.

Filovirus in quarantined cynomolgus monkeys (1989/1990)

The imported filovirus events of 1989-90 amply demonstrated the need for these regulations. Four separate shipments of monkeys from the Philippines were found to be infected and had to be destroyed. The outbreak was originally thought to be caused by Ebola virus, a closely-related and rapidly fatal hemorrhagic fever virus known to have caused two human disease outbreaks in Africa with associated mortality rates of 70%-90%. By late 1990 it was apparent that this particular strain was not pathogenic for humans, but the investigation amply demonstrated the potential for the importation and rapid spread of such diseases.

To address this, specific disease control requirements, including requirements for the handling of all primates during transit were added to 1975 regulations. It was at this point that a special permit procedure for the importation of three of the most commonly imported research primate species (rhesus, cynomolgus, and African green monkeys) was instituted. These additional requirements remain in effect.

Transportation requirements

The 1975 regulations affected air carriers only in that: a) cargo shipments could only be accepted from registered importers and b) individuals could no longer import primates for use as pets. Since then, only occasional animals have had to be seized by inspectors at ports of entry, generally from private citizens who are unaware of these regulations. There were and are, of course, other regulations which address animal welfare and transport (IATA, USDA, USF&W, CITES, IHR, etc.)

Guidelines which were developed early in the filovirus investigation emphasized the importance of safe handling of primates during transit, including the containment of potential contamination, protection of other cargo, training and protective clothing for transport workers, limiting access to the animals, and expediting the clearance and transfer process. These measures are consistent with, and supplement, the existing IATA and other regulations. Although CDC has communicated with IATA during the investigation and upon implementation of guidelines, the responsibility for verifying arrangements for compliance has necessarily remained with the registered importer.

Surveillance (1990 - present)

In the course of the filovirus investigation and subsequent to it, the Division of Quarantine has monitored the handling of nearly 250 arriving shipments of nonhuman primates, involving over 43,000 individual animals. The vast majority of these shipments have been in compliance with IATA and CDC requirements. When deficiencies have been identified, the importers and the air carriers have worked together to resolve the issue. Fortunately,
with the exception of a number of tuberculosis-positive shipments, there have been no outbreaks of zoonotic disease associated with the importation and quarantine of nonhuman primates since 1990. So far, occasional lapses in procedure during transit are not known to have led to development of disease in humans, although one employee at an import quarantine facility did convert to a positive TB skin test during 1991 and was placed on preventive therapy, following exposure to a group of TB+ cynomolgus monkeys.

**Herpesvirus simiae**

Of great potential concern is the almost universal infection of rhesus and cynomolgus monkeys with *Herpesvirus simiae* virus, also known as "monkey B virus". Although causing only mild disease in these species, untreated human infections are generally fatal. Due to the ubiquitous nature of this virus in most macaque monkeys, it is assumed to be present in all of them. Testing for *Herpesvirus simiae* is therefore not part of quarantine testing, but exposure to the virus constitutes a major occupational hazard for animal handlers, both during and post-quarantine.

**Occupational health - Animal BioSafety Level 3**

All personnel involved with the handling of macaque species should routinely use Animal Biosafety Level 3 precautions (latex gloves, dust/mist "respirator" face mask, face shield or goggles, disposable coveralls or lab coat, metal-reinforced leather gauntlet gloves for animal handlers, and chemical restraint of all macaques prior to physically handling them). Because of *Herpesvirus simiae*, a special protocol for handling bite, scratch, or splash exposures should be in place at all facilities which house macaque species. At present only one laboratory routinely cultures and isolates *Herpesvirus simiae* under an NIH grant. Facility protocols for macaque exposure incidents should include provisions for sending human and animal specimens to Dr. Julia Hilliard at the Southwest Foundation for Biomedical Research (512/674-1410), according to the investigational protocols established. In addition, Dr. Louisa Chapman, of CDC's Division of Viral and Rickettsial Diseases, should be notified immediately at (404/639-3747).

**Proposed regulations**

While recent inspections indicate that disease control practices in the industry are much improved, it is important to note that the emergency measures implemented in response to the filovirus events of 1989-90 were never intended for permanent application. CDC has developed proposed new regulations and technical standards for the importation of nonhuman primates which will serve to codify the practices that have evolved over the past 3 years through government and industry cooperation. In an effort to incorporate input from the importer community, the proposed technical standards were distributed for comment to registered importers and the AAZPA in August, 1992. There will be another opportunity for input from a wider audience when The Notice of Proposed Rulemaking (NPRM) is published in the *Federal Register*.
Conclusions

Although there are numerous potential public health risks associated with the importation of nonhuman primates and other live cargo, such cargo can be imported safely when all parties involved follow established standards for worker protection. The Division of Quarantine appreciates the constructive working relationship that we presently enjoy with the current 45 registered importers (including 8 zoos), 11 special permit quarantine facilities, the commercial air carriers, IATA, and the other federal agencies involved. We thank you for the opportunity to participate in this meeting, and look forward to working with the zoological parks on future importations.
The process surrounding the safe and humane transportation of nonhuman primates is governed by several different agencies within the Federal and international regulatory system. Each agency has rules pertaining to their respective jurisdictional areas. These regulations are overlapping, contradictory and confusing to the exporter, importer and consignee, as a result of the multiple perspectives from which they originated. Sorting through the maelstrom of regulatory issues can cause the simple transfer genetically important nonhuman primates to become a nightmarish event.

The airline industry is currently a highly regulated body. Many of their rules and regulations pertaining to cargo are formulated by the International Air Transport Association (IATA). This organization has done a remarkable job of self regulating the industry. Animal shipments are governed by the IATA's Live Animal Board (LAB), which formulates rules governing how all animal cargo is handled. Within the European Community (EC) the IATA rules have been recognized as law. All member nations of the Convention in the Trade of Endangered Species of Flora and Fauna (CITES) have adopted the shipping regulations as formulated by IATA LAB. This makes these regulations one of the first pieces of industry generated rules to be recognized as international law. The IATA Live Animal Regulations (LAR) apply when no other rules or regulations are in place within the governance of any member nation. All nations have the opportunity to write their own rules, but IATA's LAR are intended to set minimal standards and fill the void when CITES member nations lack appropriate regulations.

The United States has multiple agencies writing regulations which cover the various aspects of importation and transportation of non human primates. The United States Department of Agriculture (USDA) has standards outlined in the Animal Welfare Act (AWA). The Department of Interior (DOI) has the Standards for the Safe and Humane Transportation of Wild Birds and Mammals. Centers for Disease Control (CDC) placed their "Interim Guidelines" and Special Permit Requirements for the Importation of Nonhuman Primates in place following the identification of a previously unidentified nonhuman primate filovirus. Lastly, the IATA LAR apply to all airlines from the time the consignment is accepted to the time it is transferred to the consignee.

Serious questions arise as to which rules apply during each stage of shipment and quarantine, who is responsible at each segment of the process and how long the regulations apply to an imported monkey.

Interior's regulations apply from the start of the shipment to arrival at the facility. They explicitly hold the importer responsible for assuring that the Standards are followed at all points along the way. Fish and Wildlife is also responsible for the enforcement of all CITES documentation and the Lacey Act. Both sets of regulations apply since all nonhuman primate species are listed in the CITES appendices. Some confusion exists with respect to
uniformity of container requirements during shipment. Feeding and watering during transit are addressed. All elements of the Standards are intended to enhance safety for the exotic species shipped for any purpose.

The Animal Welfare Act likewise applies at all points of transfer from the animal welfare perspective. The primary focus of the AWA is to assure that animals arrive in good condition and are not subjected to poor handling during shipment. The design of the shipping container is slightly different in the AWA from other rules. Feeding and watering requirements are addressed.

CDC has entered into the regulatory arena. CDC’s thrust is public health and preventing the introduction of zoonotic diseases which are currently absent from the US. Earlier regulations covering importation and quarantine poorly described the methods for assuring safety. Importation permits were issued without inspection of quarantine facilities and or procedures. The regulations as originally published lacked adequate description of CDC’s vision for controlling the introduction of foreign diseases.

Primate filovirus entered the US from a shipment of *Macaca fascicularis* from an exporter in the Philippines. It has antigenic similarity to Ebola virus found in Africa in the mid to late 1970s. It does not produce disease or fatality in man. It is fatal to nonhuman primates. It was detected during quarantine in a US facility. It was dealt with professionally and appropriately by the importer.

CDC feels that this outbreak justifies changing the importation and transportation standards for nonhuman primates. Public health is the issue. USDA deals with all agriculturally related problems such as foot and mouth disease, velogenic viscerotropic newcastle disease and tuberculosis. This is inaccurate; tuberculosis is zoonotic and is transmissible to man. It, therefore, falls under CDC jurisdiction in the interest of public health, at least for nonhuman primates. Does CDC express similar public health concerns over tuberculosis originating in the bovine species, birds, turtles?

What exactly has happened as a result of CDC’s renewed enthusiasm for primate diseases.
1) Quarantine facilities and programs are subject to inspection for licensing. The number of licensed importers fell from over 150 to under 40 today. Zoos are poorly represented in the number of remaining licensed quarantines. 2) Many airlines are hesitant to carry nonhuman primates for any reason. New York state continues to maintain an embargo on primates entering through their port. CDC has imposed clothing requirements, these dress requirements have individuals who come in contact with the shipping containers wearing: a) coveralls, b) boots, c) caps, d) masks, e) eye protection, and f) gloves which will prevent scratches or slivers. The vehicle for transporting the animals from the airplane to the quarantine is planeside. The inside of the cargo hold is disinfected. The pallet holding the animals is disinfected. Inspectors from the Public Health Service, Fish and Wildlife, USDA, Customs and Immigration may meet the airplane to oversee the procedures. Ground crews are held at a safe distance to minimize risk of exposure. The entire scene replicates our personal visual image of the release of the “Andromeda Strain”. 3) The number of airports, especially major international transfer points, equipped to handle nonhuman primates is
becoming fewer. 4) The facility requirements for an ongoing quarantine makes it very difficult to house multiple shipments at one time. 5) Quarantine regulations apply to all nonhuman primates even those originating in zoological collections of non-third world countries.

Proposed "Technical Standards" from CDC will increase the difficulty of importing animals whether for research, exhibition or breeding. Responsible animal management will not endorse faulty or poor disease control mechanisms during importation, but regulations which restrict importation by intimidating the carriers with potential and undocumented risks are likewise unacceptable.

Each set of regulations governing the transportation and importation of nonhuman primates is consistent with the mission and goals of the agency of origin. Conflicts arise in areas of overlapping responsibility and jurisdiction. Further problems arise due to poor interagency communication. This is especially true when international borders are crossed and an international agency, IATA, becomes involved with completely different purposes. For example, IATA is concerned with poorly trained ground crews (as far as live animals are concerned) feeding and watering animals as required by all US agencies. IATA is concerned with how containers are handled in transit, how leakage, spillage and other contamination is contained. Unfortunately the proposed IATA rule for absorbent material conflicts with CDC's for plastic sheeting. So the battle rages on. Poor communication. Poor determination of real risks. Little to no input from the industry importing nonhuman primates. There is little hope for change in the immediate future.
Almost at whim, human beings board planes, cars, boats and buses and travel to all parts of the globe. So, how complicated could the shipment of animals possibly be? Plenty! Need to move an animal tomorrow? Forget it.

Shipping animals is now a process of informing any number of agencies and ensuring that all of their protocols are followed. Preparation and knowledge of the subject are key elements to the science of shipping animals, but they are no substitute for the allowance of ample time to be certain that all of the variables line up correctly.

No two shipments are alike, conditions vary between destinations and regulations, weather, container specification, mode of transportation and veterinary requirements. Many different people become involved in the movement of animals, including among others curators, supervisors and keepers, veterinarians, crate builders, the airlines, as well as federal and local authorities. Many institutions have registrars, overlooking if not all, some of these requirements. The following outline is intended to help anyone who undertakes the task of organizing the transportation of animals, whether it be interstate or international.

First, there are five basic reasons why regulations have been imposed on animal shipping. These are as follows:

- Wildlife conservation
- Livestock health
- Animal welfare
- Human health
- Revenue and commerce

The government agency and/or applicable rulings which apply to these reasons include:

U.S. Department of the Interior (USDI)
Fish and Wildlife Service (FWS)

- Endangered Species Act (ESA), (FWS and NMFS)
- Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), (FWS)
- Marine Mammal Protection Act (MMPA), (NMFS and FWS)
- Migratory Bird Treaty Act (MBTA), (FWS)
- Lacy Act (Injurious Wildlife), (FWS)
- The Wild Bird Conservation Act (FWS)
U.S. Department of Agriculture (USDA)

--Animal Welfare Act (AWA), (APHIS)
--Animal Quarantine Regulations
  (quarantine, inspection and vaccination)

International Air Transport Association (IATA), regulations on animal shipping containers and their provisioning.

Department of Health and Human Service (CDC)

--Public Health Service Act

Department of the Treasury

--U.S. Customs

Many species are protected under more than one law. Aside from the list of agencies provided above, special attention has to be given to your state and local laws which may affect the activity you wish to undertake. For example, in New York the ruling agency would be the New York State Department of Environmental Conservation.

Paperwork required for all shipments:

Necessary shipping documents
AAZPA Health Certificate, USDA (APHIS) form No. 7020 (required only for mammals), airway bill, and at times a Letter of Acclimation (if a hardy animal is shipped during temperature extremes), container shipping labels and food and water instruction labels.

Convenience or courtesy documents
AAZK animal data transfer form, ISIS Specimen Report, ID request form, invoice (if sale) and medical records.

Domestic shipments out (in the mainland states and Alaska)
All of the above documents plus: Captive-bred wildlife requirements for endangered/threatened species and/or state and local requirements.

International exports
The documents are the same as above plus: an international health certificate signed by in-house veterinarian and USDA veterinarian at Port of Embarkation (replaces AAZPA Health Certificate), Customs Form 3177, and notification to USFWS for inspection.
International imports

Providing that the export country has sent the necessary paperwork, you are responsible for the following documents in order to clear the shipment: for birds and certain mammals (reservation and quarantine import permit), USFWS Form 3177, Customs Declaration of Free Entry Form 3321, Customs formal entry if animal’s value exceeds $1,000 (this is usually handled by a broker) and notification to USFWS for inspection and clearance.

In addition to the above paperwork, you must obtain appropriate permits if the species are listed under CITES, ESA or both. Table 1 is a guideline used by NYZS/The Wildlife Conservation Society to verify common requirements for the various mammalian orders in the collection.

Interstate shipments

Many states have special testing requirements for animals entering their state. Although these requirements were intended for the cattle/livestock industry, zoos, depending on the species, are not exempt. Many states also require entry permits prior to the transporting date. If you are confused and feel that your interpretation of the regulations is not necessarily what they intended, do not fear, you are not alone. Agency interpretation will vary from agent to agent and as new and sometimes temporary rules are imposed.

For example, state veterinary regulations can vary widely. Aside from the standard testing done on certain species of Perissodactyla and Artiodactyla, the regulations also require examination by a certified veterinarian and the assurance that the animal is free from infectious disease. Certain states have more involved regulations. For example, Arizona requires a TB test on all animals coming from a petting zoo. Georgia requires a TB test on elephants and primates, and Oregon requires TB and parasite tests on all primates.

Places such as Guam, U. S. Virgin Islands and Hawaii require entry permits for all animals plus shipments are subject to entry requirements deemed necessary by the state veterinarian. Hawaii, in particular, has special requirements for reptiles transported onto the Islands.

How does one avoid the confusion? One recommendation is to make an in-house shipping checklist outlining the various stages of animal transportation, as well as necessary documentation (Table 2). Maintain a phone list of state veterinarians to verify each state’s requirements. Always document everything said and done and by whom.

Mode of transportation is another restriction on animal shipments. Airlines may impose restrictions due to weather, crate size, total weight, aircraft size, type of animals (poisonous reptiles not accepted by certain airlines) and time of arrival. Cargo door dimensions vary on many domestic aircraft. For example, a crane crate if designed higher than 41 inches will not fit in any of the domestic commercial aircraft cargo doors.

Shipping large animals by air within the United States is at times impossible. A few airlines can handle such animals as adult gorillas or rhinoceros. ZANTOP and Federal Express can
handle gorillas and rhinos but only by special contract. The cost can be extraordinary and routes are very limited.

International shipments

International shipments require a great deal of planning and coordination between both parties, beginning with the permit process. While the application turnaround time in other countries may be short, the U.S. turnaround time is very lengthy. By the time the U.S. permit is acquired, the foreign permit may have expired, since the validation period is usually shorter.

At the initiation of the transaction, a request for foreign veterinary requirements should be obtained. Certain countries require special testing for mammals and birds within 45 days prior to shipment. Some countries are more demanding than others. For example, the Netherlands requires their special Certificate of Origin and Health for import of Psittacine birds to be signed by the U.S. federal veterinarian stating that the birds were quarantined 45 days prior to shipping and free from numerous diseases. All of this is required in addition to the U.S. International Health Certificate.

Shipments of Artiodactyls to Japan (not CITES or ESA regulated) requires a series of tests and inspections. Japan's requirements state that the animals be inspected 15 days and 24 hours prior to shipment by the U.S. federal veterinarian. Lack of attention to details can set back shipments by as much as several weeks.

Advanced preparation with airlines on international transactions is a very important first step. Do not wait until the last week prior to the desired transport date to make arrangements. For example, one has the permits, the veterinary tests, the crates and the animals are ready to ship in the next four days. However, the plane to that destination on that particular week is not pressurized. Other problems one might encounter may be with connecting flights. Some countries' connecting flights only operate on certain days and times. On rare occasions, a trans-shipment has to take place from one aircraft carrier to another. Always obtain written confirmation of acceptance by both carriers. On lengthy flights, always provide "In case of emergency" instructions with a possibility of contacting the nearest zoo in that country. Extensive delays can be a problem, since airports in some countries do not have the facilities or personnel to care for the animals.

In summary, it is nearly impossible to outline all the variables involved in shipping animals. Experience, knowledge of the subject and proper planning are the best preparations. The important factors to remember are that timing and orchestration of all events is critical. If the regulations and health requirements are obtained in advance and the requirements are discussed with all individuals involved, then most problems can be avoided.
Table 1
Animal Shipping
Requirements specific to each NYZS mammalian order
(Check CITES, ESA and New York state status on each species)

Marsupialia
Importations of live Australian fauna and CITES listed species from Australia require approval under Australia's Wildlife Protection (Regulation of Export and Imports) Act of 1982. This Act ensures that both importer and exporter have suitable expertise and facilities to care for a given species. The Bronx Zoo has received approval for various taxa; however, each import/export must receive individual clearance. The Act is administered by the Australian National Parks and Wildlife Service, head office Canberra.

Insectivora
No special regulations; however, remember that Tupai is still considered a primate by USDI.

Chiroptera
The CDC requires a permit for bat shipments for import, export and movement within the country. Bat shipments require special labels issued by CDC which are affixed to the shipment crates.

   The receiving institution must obtain the permits.

   Importation and interstate shipment of Pteropus also require USDI Injurious Wildlife Permit in addition to the above.

Primates
Only those institutions registered with the Department of Health and Human Service, Center for Disease Control (HHS), can import nonhuman primates. Temporary bans on the importation of certain taxa and/or animals of certain geographical origin can be in effect at any time.

APHIS regulations provide special guidelines on the shipping of nonhuman primates (CFR 9, 3.85 through 3.91.).

Edentata
No special regulations

Lagomorpha
No special regulations
Rodentia
The CDC requires a permit for the importation of rodents. Importations and interstate shipments of *Mastomys* require a USDI Injurious Wildlife Permit. Some states of the United States ban the keeping of certain exotic rodent species without special permission.

Carnivora
Viverids: Mongooses require a USDI Injurious Wildlife Permit.

Pinnipeds: North American, Caribbean and Pacific species come under the Marine Mammal Protection Act and require a permit for collection and export.

Domestic shipment of Pinnipeds does not require a permit; however, NMF must approve all institutions receiving Pinnipeds.

APHIS has special regulations on shipping procedures in CFR 9, 3.112 through 3.117.

Ursids: Polar bears only are considered marine mammals and fall under the Marine Mammal Protection Act. See Pinnipeds above. Note that polar bears are also listed on CITES Appendix II.

Proboscidea
Note that all elephants are now classified as USDI endangered and are listed on Appendix I of CITES. Check state regulations. Georgia, for example, requires a TB test on elephants.

Hyracoidea
No special regulations

Perissodactyla
Post-entry quarantine of at least seven days is required of all Perissodactyls by the USDA (CFR 9, 92.11d (i), except those to Canada which require special health certificates (CFR 9, 92.24).

Post-entry quarantine of at least 60 days is required of horses coming from the continent of Africa and may enter the United States only through the Port of New York and be quarantined there (CFR 9, 92.308 (2).

In order to qualify for release from quarantine, all horses from any part of the world must test negative to the following tests: dourine, glanders, equine piroplasmosis, equine infectious anemia and others as determined (CFR 9, 92.308 (3).

In addition to CITES and permits, a USDA permit for all imported elephants, hippos, rhinos and tapirs must be obtained. Under the same ruling, the animals must be treated for ticks (CFR 9, 93.3 and 93.4).
Interstate shipment is subject to tests and inspections as described in the USDA's State-Federal Health Requirement and Regulations. This manual is organized by state in alphabetical order.

Artiodactyla
Wild ruminants and swine imported from any part of the world except Canada shall be quarantined for not less than 15 days and are subject to inspection, disinfections and tests as may be required by the Deputy Administrator of Veterinary Services (CFR 9, 92.400, subpart D and 92.500, subpart E).

Animals are automatically tested for TB and brucellosis. Other tests depend on country of origin and taxon (see CFR reference above).

Animals coming from countries which are not declared free of rinderpest and/or hoof and mouth disease must be quarantined at least 120 days, part of which can be in USDA-approved facilities overseas.

Interstate shipment is subject to tests and inspections as described in the USDA's State-Federal Health Requirement and Regulations. This manual is organized by state in alphabetical order.
Table 2
Domestic Shipping Checklist

Date __________________________ Species __________________________
Destination __________________________

1. Sent transaction form

2. Check permit requirements (Federal State & CDC)*

3. Check with animal departments and AHC

4. Check airline schedule - check with receiving zoo

5. Make airline reservation - give details to other zoo

6. Create breeding loan(s) if required

7. Make up airway folder:
   a. Health certificate*
   b. Crate labels (2 per crate)
   c. Feeding instruction label (1 per crate)
   d. Animal data transfer form*
   e. USDA form (mammals only)*
   f. Print specimen reports
   g. Permits (Federal, State, CDC)
   h. Airway bill
   i. Invoice for sale
   j. Crate invoice
   k. Specimen ID request form from receiving zoo
   l. Flight info for departments
   m. Guaranteed payment (airline request)
   n. Letter of acclimation (airline request)

8. Shipping details to supervisors, curators, AHC

9. Note on calendar

10. Check with airline day of shipment

11. Check with receiving zoo postshipment

12. Information to records keeper for ARKS

* Needed for pickup
EXTRA-LABEL USE: THE FDA IN YOUR PHARMACY

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The science and art of zoological medicine requires veterinarians to call upon their knowledge of the biology of the species, its diet, behavior, anatomy, responses to confinement as well as idiosyncrasies of the individual animal combined with clinical diagnostic tools in order to make an accurate assessment of medical problems. Once a diagnosis is made the veterinarian must decide on appropriate pharmaceutical intervention to correct the problem. At this point in the process there have been no laws broken and the veterinarian has no risk of blundering into "regulatory hell" and a life of crime. But once the veterinarian selects a pharmaceutical and administers it to the animal, our diagnostic hero steps into "extra-label land".

The zoo veterinarian today must manage the medical needs of over 600 species representing more than 60 families of the animal kingdom under continued management in North America. These species range from rare and endangered toads to marine mammals and may have environmental requirements varying from arctic to tropical. The individuals may have been sourced from the wild and adapted to confinement and others born and hand-reared in the facility. The diversity of species and complexity of factors affecting medical situations addressed by the zoo veterinarian is unequalled in medicine or biological science today.

Now that the medical environment that the zoo veterinarian has to work in has been defined, we can examine the impact of current FDA regulations upon what pharmaceutical tools are available to them to do their job.

Of the over 120 pharmaceuticals used in treatment of conditions of animals in zoological collections today, only four are approved for use in non-domestic species: ketamine hydrochloride for use in primates, xylazine as a sedative for deer, yohimbine hydrochloride as a reversal of xylazine sedation in deer, and carfentanil citrate as an immobilizing drug for deer.

By use of any pharmaceutical in any other species other than just described, the veterinarian is by the strictest interpretation of the Food, Drug and Cosmetic Act, in violation of the Act and Federal Regulation 21 CFR. Fortunately, the Food and Drug Administration Center for Veterinary Medicine (FDA/CVM) has a policy that permits the veterinarian to use a drug in an extra-label fashion if there is no approved drug for that use and if a client-patient relationship has been established. That is the good news. The bad news is that this is only a policy, and this policy has been changed four times since 1984. In todays FDA regulatory environment 99% of the veterinary pharmaceuticals used in zoos are under this permissive policy of FDA/CVM. Unfortunately in the biopolitical reality of Washington, that permission can be revoked at any time, depending upon the political and public pressure that day. In some extreme cases this could result in a "policy du jour".

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What impact does today's status of the FDA/CVM's extra-label use have on your life as a veterinarian practicing zoological medicine today? In reality, because species in zoological collections do not enter the food chain, and extra-label use of pharmaceuticals in these species do not constitute a human health hazard the implications of extra-label use and regulatory exposure are greatly diminished. Accurate and complete clinical records on any animal in which extra-label drugs are used will do much to reduce the regulatory risk and liability. Although nothing will completely insulate one against a "press and pressure group" attack following a drug related public relations disaster (i.e. Shedd Aquarium), good clinical records documenting the decision-making process to use a drug in an extra-label fashion will greatly assist in "damage control" following an incident.

At this time there is legislation within the 103rd Congress to make extra-label use the veterinarians' right rather than having the veterinarian continue to rely upon the goodwill of the current FDA/CVM policy. Enactment of such legislation as part of the Food, Drug and Cosmetic Act will demand that veterinarians maintain the highest professional standards and record keeping regarding extra-label use of unapproved pharmaceuticals in any species.

This legislation will also be a two-edged sword. While it will provide veterinarians greater legal authority to use any pharmaceutical they can legally obtain in animals under their care, codification of extra-label use as an amendment to the Food, Drug and Cosmetic Act may discourage pharmaceutical research and development required for new molecules or formulations needed specifically for veterinary medicine. Economic incentive must somehow be maintained to encourage development of new pharmaceutical tools for zoological medicine. Only time will tell how this could affect future availability of new pharmaceuticals.

Veterinarians in zoological medicine are forced into daily regulatory conflict by the existing laws. It is vital that zoo veterinarians continue to be aware and properly manage the regulatory exposure of their professional activity to minimize what can be embarrassing or even disastrous publicity should a drug related incident occur. Furthermore, they should encourage the enactment of legislation to make extra-label use part of their right as medical professionals.
VETERINARY PRACTICE ACTS...ARE THERE ANY SURPRISES IN YOUR PRACTICE ACT?

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Veterinarians in every state are regulated in the practice of their profession by practice acts. These laws are in place to ensure the public that anyone practicing veterinary medicine meets certain standards. Though practice acts vary in their details, and sometimes in very important ways, all of them do the following: 1) Prohibit the practice of veterinary medicine and surgery without a license; 2) Define the practice of veterinary medicine and surgery; 3) Place in an examining board responsibility for approving schools and courses of study, determining the qualifications of applicants, administering examinations for a license, and issuing licenses; 4) Define an accredited school of veterinary medicine and surgery; 5) State the qualifications for receiving a license and the fees to be charged; 6) State the grounds for refusing to issue a license or to suspend or revoke a license, and set forth the procedure to be followed in so doing; 7) Provide that administrative decisions are subject to judicial review; 8) Provide for reciprocity with other states having equal standards (although all acts do not so provide); 9) Require that licenses either be recorded locally or be registered with a state agency (although some states may not require this); 10) Provide for the renewal of licenses; 11) Provide penalties for violation of the practice act or of any regulations adopted under it. 1 There are also certain categories of persons that are exempted from the application of state practice acts. Federal or state veterinarians in the course of their duties, students and staff members of veterinary colleges, and owners treating their own animals are among these exempt categories. Most practice acts also make some concessions to the livestock industry and state that certain activities done by livestock owners such as dehorning and castration are not included in the practice of veterinary medicine.

One very important way practice acts may effect zoo veterinarians is in the regulation of non-veterinarians used as consultants. It is very common for zoo veterinarians to consult with physicians or dentists as well as other veterinarians in order to treat the variety of animals in zoo collections. While practice acts vary in the specific language used, the intent is clear, veterinarians do not treat humans and physicians do not treat animals. 2

Upon reviewing the Illinois Practice Act the question of non-veterinary consultants was put to the State Veterinary Board. They stated Illinois Veterinarians were empowered to "engage or collaborate with other parties necessary to accomplish the task at hand as long as performance is under the supervision of an Illinois licensed veterinarian". 3 Illinois however has a veterinary board that acts only in an advisory capacity to the Depart of Professional Regulation a state government agency that regulates practice, carries on investigations and administrative hearings and ultimately determines penalties. The question of non-veterinary professionals was put to the Illinois Department of Professional Regulation with a much different response. The Department stated "It would appear that under the current reading of the Act, a non-veterinary medical expert may not assist in the diagnosis, prognosis, or treatment of animals, even under the supervision of a licensed veterinarian". 4
This interpretation might not only affect zoo veterinarians but veterinarians that call on other professionals such as nutritionists to help arrive at a diagnosis. The Illinois State Veterinary Medical Association drafted with the help of the author, support of the American Association of Zoo Veterinarians and the American College of Zoological Medicine an exemption to the practice act. The following category of persons is exempted from the act under the pending Illinois Veterinary Practice Act of 1994, "Members of other professions when called for consultation and assistance by a veterinarian licensed in the State of Illinois and who acts under the direction and control of such veterinarian".

It would be prudent for all veterinarians to be familiar with the practice acts in their state. This is no less true of zoo veterinarians whose unique type of practice may put them in conflict with those who regulate veterinary medicine.

LITERATURE CITED

3. Illinois Department of Professional Regulation. pers comm.
A MOLECULAR EPIDEMIOLOGIC STUDY OF HEMOGREGARINE INFECTIONS IN CAPTIVE REPTILES

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The most common group of intracellular sporozoan hemoparasites found in reptiles are the hemogregarines. Within the family Hemogregarinidae, there are three genera commonly seen in reptiles: Hemogregarina, Karyolyssus, and Hepatozoon. All three genera have heteroxenous lifecycles involving schizogony within the reptile and sporogony within an invertebrate vector. Generic classification is based on the pattern of development within the vector and usually cannot be done from stages seen in blood films. Thus far most hemogregarines described in snakes have been shown to be in the genus Hepatozoon. Hemogregarine species infecting reptiles often produce little or no pathologic change in their natural hosts. Within unnatural host species however, Hepatozoon spp. infections have been shown to cause significant disease. Lesions associated with hemogregarine infections include: multifocal hepatitis, splenitis, pancreatitis, and pneumonia. The purpose of this study was to evaluate a Polymerase Chain Reaction (PCR)-based strategy capable of differentiating morphologically similar species of hemogregarines at the molecular level.

The 1991 Lincoln Park Zoo (Chicago) snake collection, several privately owned snakes, and a Burmese tortoise from the Minnesota Zoo were surveyed for hemogregarines. All snakes were bled by either cardiac puncture or venipuncture and Wright’s stained blood smears were microscopically examined. Several colubrid, boid and vipersid snakes were found to be parasitemic. Generic classification of selected hemogregarine isolates was accomplished by xenodiagnosis in laboratory reared Aedes aegypti mosquitoes; the sporogonic patterns of a hemogregarine from an infected western cottonmouth (Agkistrodon piscivorus leucostoma) demonstrated it to be Hepatozoon mocassini. All isolates of hemogregarines were molecularly characterized at the ribosomal DNA (rDNA) locus by PCR amplification and restriction enzyme analysis. Amplification of the rDNA from all five hemogregarine isolates resulted in the robust production of 590 base pair (bp) DNA fragments. Restriction enzyme digestion of these 590 bp fragments revealed characteristic restriction endonuclease patterns for parasites of each host species. The finding of distinctive hemogregarines in each host species indicates that active transmission across host species was not common within these collections.

LITERATURE CITED

AN OUTBREAK OF MALIGNANT CATARRHAL FEVER IN *Rangifer tarandus*

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Ronald E. Werardin, DVM, PhD
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Peregrine L. Wolff, DVM
Minnesota Zoological Garden, Apple Valley, MN, USA

This paper describes three clinical cases of sheep-associated malignant catarrhal fever (SA-MCF) in reindeer (*Rangifer tarandus*), outlines serological and molecular biological tests available for the diagnosis of this disease, and proposes a reservoir and possible method of transmission of the virus in this outbreak.

Case 1, a 10 year old, neutered, male reindeer presented on 30 June 1992, with an acute onset of seizures. On physical exam the animal was in sternal recumbency, depressed and slightly prodromal. It had normal heart and lung sounds but depressed gastrointestinal sounds with normal feces. Its sclera were injected and no oral lesions were seen. It appeared painful to abdominal palpation. Abdominocentesis was performed but all taps were dry. Blood was drawn for complete blood count (CBC) and serum chemistries. Due to reoccurrence of the seizures, the animal was euthanized with 350 mg (3-4 mg/kg) Beuthanasia i.v. (Beuthanasia-D Special, Schering-Plough Animal Health, Kenilworth, NJ 07033, USA).

Case 2, an eight year old female reindeer presented on 9 July 1992. The animal was recumbent and reluctant to rise. Physical exam revealed mild mucopurulent ocular and nasal discharge, with slightly edematous conjunctiva and no oral lesions. Tenderness was evident in the caudal abdomen and around the vulva, which was slightly swollen. Gastrointestinal sounds were depressed but within normal range. Feces were mucous covered but pelleted. Blood was drawn for CBC and serum chemistries. The animal was found dead the following morning.

Case 3, a two year old female reindeer presented 20 July 1992 as slightly depressed. Physical exam revealed lethargy and anorexia with dark brown, liquid diarrhea. When recumbent it exhibited bilateral twitching of the rear legs. Blood was drawn for CBC and serum chemistries. In the afternoon, the animal was recumbent and euthanasia, with 280 mg (3-4 mg/kg) Beuthanasia i.v., was elected.

Necropsies were performed at the Minnesota Veterinary Diagnostic Laboratory. Tissues were collected and fixed in 10% neutral, buffered formalin, cut, embedded in paraffin, and stained with hematoxylin and eosin for light microscopy. In all three cases gross lesions were minimal and histologic lesions consisted of perivascular lymphoid cuffing. The perivascular lesions involved all components of the vascular wall and were characterized by fibrinoid necrosis of the tunica intima and the tunica media.
Hematologic changes are presented in Table 1. Two of the cases had a leukocytosis, but white blood cell morphology in all three cases was unchanged. Cattle with MCF show a leukopenia with shrunken and pyknotic small lymphocytes. The findings of this study suggest that Cervidae may not show the same hematologic changes seen in Bovidae.

MCF serology results for the reindeer and the sheep (Ovine spp.) and goats (Caprine spp.) are presented in Table 2 and Table 3. The indirect immunofluorescence assay (IFA) titers in the two reindeer were indicative of MCF and supported the histopathologic diagnosis. The majority of the goats had positive IFA titers which supported the conclusion that these animals were the carriers of the virus in this outbreak.

The goats and sheep were never in direct contact with the reindeer. It is unknown why the virus was transmitted in 1992 rather than past years. It was hypothesized that fomite transmission via boots, feedpans, and cleaning instruments may have contributed to the infection of the reindeer.

In conclusion, three reindeer at the Minnesota Zoo died of MCF within three weeks of each other. All presented with different clinical signs and none survived more than 24 hr. Diagnosis of MCF was supported by histopathology and serology. Goats and sheep were implicated as the carriers and transmission probably occurred via fomites.

Table 1. Hematological results and serum chemistries for reindeer with MCF.

<table>
<thead>
<tr>
<th>Hematology</th>
<th>Values</th>
<th>Normals ± 9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case 1</td>
<td>Case 2</td>
</tr>
<tr>
<td>WBC (10^3/μl)</td>
<td>11.7</td>
<td>4.6</td>
</tr>
<tr>
<td>Hemoglobin (gm/dl)</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>49.5</td>
<td>36</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>26.3</td>
<td>33.2</td>
</tr>
<tr>
<td>Seg neutrophils (10^3/μl)</td>
<td>10.53</td>
<td>2.9</td>
</tr>
<tr>
<td>Band neutrophils (10^3/μl)</td>
<td>0.23</td>
<td>0.32</td>
</tr>
<tr>
<td>Lymphocytes (10^3/μl)</td>
<td>0.58</td>
<td>1.06</td>
</tr>
<tr>
<td>Monocytes (10^3/μl)</td>
<td>0.35</td>
<td>0.18</td>
</tr>
<tr>
<td>Basophils (10^3/μl)</td>
<td>0.0</td>
<td>0.05</td>
</tr>
<tr>
<td>Eosinophils (10^3/μl)</td>
<td>0.0</td>
<td>0.05</td>
</tr>
<tr>
<td>Thrombocytes</td>
<td>ADQ</td>
<td>ADQ</td>
</tr>
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</table>
(Table 1 continued)

Serum chemistries

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>181</td>
<td>101</td>
<td>87</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>24</td>
<td>32</td>
<td>101</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.7</td>
<td>1.2</td>
<td>6.7</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>7.9</td>
<td>6.7</td>
<td>7.1</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>6.4</td>
<td>6.2</td>
<td>7.9</td>
</tr>
<tr>
<td>Sodium (meq/l)</td>
<td>140</td>
<td>140</td>
<td>133</td>
</tr>
<tr>
<td>Potassium (meq/l)</td>
<td>3.3</td>
<td>3.3</td>
<td>6.0</td>
</tr>
<tr>
<td>Chloride (meq/l)</td>
<td>103</td>
<td>103</td>
<td>99</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>ND</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Total Protein (gm/dl)</td>
<td>6.7</td>
<td>5.4</td>
<td>7.0</td>
</tr>
<tr>
<td>Albumin (gm/dl)</td>
<td>ND</td>
<td>2.3</td>
<td>3.0</td>
</tr>
<tr>
<td>AST (iu/l)</td>
<td>101</td>
<td>64</td>
<td>70</td>
</tr>
<tr>
<td>ALT (iu/l)</td>
<td>12</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.8</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Alk phos (iu/dl)</td>
<td>390</td>
<td>167</td>
<td>132</td>
</tr>
<tr>
<td>CPK (iu/l)</td>
<td>ND</td>
<td>45</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ISIS data
WBC = white blood cells
MCHC = mean corpuscular hemoglobin concentration
AST = aspartate transferase
Alk phos = alkaline phosphatase
ND = not done
ALT = alanine transferase
CPK = creatine phosphokinase

Table 2. MCF diagnostic test results for the reindeer.

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFA</td>
<td>+ 1:100</td>
<td>ND</td>
<td>+ 1:100</td>
</tr>
<tr>
<td>SN</td>
<td>-</td>
<td>ND</td>
<td>+ 1:4</td>
</tr>
<tr>
<td>PCR</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
</tr>
</tbody>
</table>

IFA = indirect immunofluorescence assay
SN = serum neutralization
PCR = polymerase chain reaction
ND = not done
* serum inadvertently lost during shipping
Table 3. MCF serological test results of sheep and goats.

<table>
<thead>
<tr>
<th>Goat#</th>
<th>July</th>
<th>September</th>
<th>July</th>
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<tbody>
<tr>
<td></td>
<td>IFA</td>
<td>SN</td>
<td>IFA</td>
</tr>
<tr>
<td>7240</td>
<td>-</td>
<td>-</td>
<td>1:100</td>
</tr>
<tr>
<td>7242</td>
<td>-</td>
<td>-</td>
<td>1:100</td>
</tr>
<tr>
<td>7266</td>
<td>-</td>
<td>-</td>
<td>1:100</td>
</tr>
<tr>
<td>7267</td>
<td>-</td>
<td>-</td>
<td>1:100</td>
</tr>
<tr>
<td>7308</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7309</td>
<td>-</td>
<td>-</td>
<td>1:20</td>
</tr>
<tr>
<td>7310</td>
<td>-</td>
<td>-</td>
<td>1:20</td>
</tr>
<tr>
<td>7315</td>
<td>-</td>
<td>-</td>
<td>1:20</td>
</tr>
<tr>
<td>7316</td>
<td>-</td>
<td>-</td>
<td>1:20</td>
</tr>
<tr>
<td>7333</td>
<td>1:20</td>
<td>1:8</td>
<td>ND</td>
</tr>
<tr>
<td>7388</td>
<td>1:100</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>7389</td>
<td>1:20</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>6848</td>
<td>-</td>
<td>-</td>
<td>1:100</td>
</tr>
<tr>
<td>6872</td>
<td>-</td>
<td>-</td>
<td>1:100</td>
</tr>
<tr>
<td>6853</td>
<td>1:20</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>6854</td>
<td>1:100</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>6909</td>
<td>1:100</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>6957</td>
<td>1:100</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>6537</td>
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<td>6834</td>
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<tr>
<td>6845</td>
<td>1:100</td>
<td>-</td>
<td>ND</td>
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<tr>
<td>6847</td>
<td>1:100</td>
<td>-</td>
<td>ND</td>
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</tbody>
</table>
(Table 3)

<table>
<thead>
<tr>
<th>Sheep#</th>
<th>IF A</th>
<th>SN</th>
<th>PCR</th>
<th>ND</th>
<th>ND</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>7314</td>
<td>-</td>
<td></td>
<td>1:20</td>
<td>-</td>
<td>-</td>
<td>7117</td>
</tr>
<tr>
<td>5672</td>
<td>-</td>
<td></td>
<td>1:4</td>
<td>ND</td>
<td>ND</td>
<td>N/A</td>
</tr>
<tr>
<td>5153</td>
<td>-</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>N/A</td>
</tr>
<tr>
<td>7116</td>
<td>-</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>N/A</td>
</tr>
</tbody>
</table>

IFA = indirect immunofluorescence assay  
SN = serum neutralization  
PCR = polymerase chain reaction  
ND = note done  
N/A = not applicable (i.e. adult animals, or themselves dams)  
*IFA and SN titers are reported at highest positive concentration
The purpose of this study was to determine the characteristics of three carfentanil citrate antagonists in the domestic goat (Capra capra). After pre-anesthetizing with xylazine hydrochloride (0.05 mg/kg) i.m., nine domestic goats were immobilized with carfentanil citrate (0.03 mg/kg) i.m. three times at ≥ 7 day intervals. Prior to, during, and following a 30 min period of anesthesia, physiological parameters (temperature, heart rate, respiratory rate, blood pressure, arterial oxygen saturation, and blood gases) and behavioral parameters were obtained. Each individual's immobilization was reversed with one of three antagonists, naloxone (2 mg/kg i.m.), naltrexone (1 mg/kg i.m.), or nalmefene (0.5 mg/kg i.m.). There were no statistically significant differences in the physiological measurements in the immobilized animals following the administration of any of the three antagonists. There was no significant difference in time (\( \bar{x} = 10 \) min) from the administration of the antagonist to standing between the antagonists. Eleven of the 27 immobilizations renarcotized, five naloxone (\( \bar{x} \) time to renarcotization was 8.11 hr), and six nalmefene (\( \bar{x} \) time to renarcotization was 10.20 hr). There were no renarcotizations following the administration of naltrexone.

The major significant result was that of the eleven renarcotizations, none of them occurred when naltrexone was used.

The use of a pre-anesthetic is an appropriate consideration in captive animal opioid immobilizations. All three opioid antagonists will reverse the carfentanil immobilization of domestic goats in about the same amount of time. Use of naltrexone will result in fewer renarcotizations. Naltrexone was the best antagonist, at the doses used in this study, in the carfentanil-immobilized domestic goat and presumably in non-domestic artiodactyla.
Table 1. Significant levels for each effect in a crossover repeated measures analysis of variance for each variable measured on nine goats in a study of the three antagonists of carfentanil.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Tx</th>
<th>Tx x Time</th>
<th>Time</th>
<th>Phase</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>0.3815</td>
<td>0.6078</td>
<td>0.0001*</td>
<td>0.0110*</td>
<td>0.2054</td>
</tr>
<tr>
<td>Pulse (bpm)</td>
<td>0.6678</td>
<td>0.4594</td>
<td>0.0001*</td>
<td>0.1669</td>
<td>0.5634</td>
</tr>
<tr>
<td>Respiratory rate (rpm)</td>
<td>0.6636</td>
<td>0.9734</td>
<td>0.0001*</td>
<td>0.0371*</td>
<td>0.1052</td>
</tr>
<tr>
<td>SaO₂ (%)</td>
<td>0.5389</td>
<td>0.4178</td>
<td>0.3005</td>
<td>0.4016</td>
<td>0.8522</td>
</tr>
<tr>
<td>pH</td>
<td>0.9366</td>
<td>0.0913</td>
<td>0.0001*</td>
<td>0.0384*</td>
<td>0.9723</td>
</tr>
<tr>
<td>pCO₂ (mmHg)</td>
<td>0.8323</td>
<td>0.2351</td>
<td>0.0143*</td>
<td>0.2235</td>
<td>0.4349</td>
</tr>
<tr>
<td>pO₂ (mmHg)</td>
<td>0.7013</td>
<td>0.1418</td>
<td>0.0001*</td>
<td>0.1539</td>
<td>0.0395*</td>
</tr>
<tr>
<td>BE-ECF (mmol/L)</td>
<td>0.9331</td>
<td>0.6989</td>
<td>0.0001*</td>
<td>0.0258*</td>
<td>0.0124*</td>
</tr>
<tr>
<td>BE-Blood (mmol/L)</td>
<td>0.9282</td>
<td>0.6012</td>
<td>0.0001*</td>
<td>0.0226*</td>
<td>0.0045*</td>
</tr>
<tr>
<td>SBC (mmol/L)</td>
<td>0.9341</td>
<td>0.5413</td>
<td>0.0001*</td>
<td>0.0336*</td>
<td>0.0051*</td>
</tr>
<tr>
<td>HCO₃ (mmol/L)</td>
<td>0.9403</td>
<td>0.8976</td>
<td>0.0001*</td>
<td>0.0378*</td>
<td>0.0429*</td>
</tr>
<tr>
<td>TCO₃ (mmol/L)</td>
<td>0.7633</td>
<td>0.8013</td>
<td>0.0008*</td>
<td>0.1096</td>
<td>0.0252*</td>
</tr>
<tr>
<td>O₂ Sat (%)</td>
<td>0.3823</td>
<td>0.2636</td>
<td>0.0001*</td>
<td>0.3136</td>
<td>0.0940</td>
</tr>
<tr>
<td>O₂ CT ml/dl</td>
<td>0.7567</td>
<td>0.4971</td>
<td>0.0271*</td>
<td>0.8080</td>
<td>0.7208</td>
</tr>
<tr>
<td>BP rate (bpm)</td>
<td>0.9631</td>
<td>0.7174</td>
<td>0.0001*</td>
<td>0.1302</td>
<td>0.6188</td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>0.0955</td>
<td>0.6234</td>
<td>0.0028*</td>
<td>0.6560</td>
<td>0.5541</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>0.0093*</td>
<td>0.8846</td>
<td>0.0025*</td>
<td>0.8392</td>
<td>0.3254</td>
</tr>
<tr>
<td>Average (mmHg)</td>
<td>0.0246*</td>
<td>0.7113</td>
<td>0.0024*</td>
<td>0.8423</td>
<td>0.4320</td>
</tr>
</tbody>
</table>

* Significant difference.

ECF = Extra cellular fluid

SBC = Standard bicarbonate content

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Table 2. Time intervals (minutes).

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Minutes between administration of Xylazine HCl(^1) and Carfentanil citrate(^2)</th>
<th>Minutes between administration of antagonist and when the goat stood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naloxone(^3)</td>
<td>10.0</td>
<td>30.6</td>
</tr>
<tr>
<td>Naltrexone(^4)</td>
<td>10.4</td>
<td>29.8</td>
</tr>
<tr>
<td>Nalmefene(^5)</td>
<td>9.9</td>
<td>26.8</td>
</tr>
</tbody>
</table>

\(^1\) Rompun, Miles Inc., Shawnee, KS 66201, USA.
\(^2\) Wildnil, Wildlife Pharmaceuticals, Ft. Collins, CO 80524, USA
\(^3\) Narconil, Wildlife Pharmaceuticals, Ft. Collins, CO 80524, USA
\(^4\) Dupont-Merck & Co., Inc., Wilmington, DE 19880, USA
\(^5\) Anaquest, Inc., Murray Hill, NJ 07974, USA
Table 3. Average values for physiological data collected from nine goats times three (n = 27) immobilized with xylazine/ carfentanil over time and reversed at T30 with one of three antagonists (naloxone, naltrexone, or nalmeefene).

<table>
<thead>
<tr>
<th>Measurement</th>
<th>T-35</th>
<th>T5</th>
<th>T15</th>
<th>T25</th>
<th>T5 +15</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>38.8</td>
<td>38.6</td>
<td>38.3</td>
<td>38.0</td>
<td>37.6</td>
<td>38.5 - 40.5</td>
</tr>
<tr>
<td>Pulse (bpm)</td>
<td>59</td>
<td>52</td>
<td>50</td>
<td>50</td>
<td>61</td>
<td>70 - 80</td>
</tr>
<tr>
<td>Respiratory rate (rpm)</td>
<td>16</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>12</td>
<td>9 - 15</td>
</tr>
<tr>
<td>SaO₂ (%) (n=23)</td>
<td>94</td>
<td>96</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.394</td>
<td></td>
<td></td>
<td>7.446</td>
<td>7.401</td>
<td></td>
</tr>
<tr>
<td>pCO₂ (mmHg)</td>
<td>41.42</td>
<td></td>
<td></td>
<td>39.46</td>
<td>42.1</td>
<td></td>
</tr>
<tr>
<td>pO₂ (mmHg)</td>
<td>72.45</td>
<td></td>
<td></td>
<td>88.78</td>
<td>(4.9)</td>
<td></td>
</tr>
<tr>
<td>BE-ECF (mmol/L)</td>
<td>0.30</td>
<td></td>
<td>3.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBC (mmol/L)</td>
<td>1.19</td>
<td></td>
<td>3.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCO₃ (mmol/L)</td>
<td>25.28</td>
<td></td>
<td>27.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCO₂ (mmol/L)</td>
<td>26.12</td>
<td></td>
<td>27.12</td>
<td>20 - 25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂Sat (%)</td>
<td>89.37</td>
<td></td>
<td>28.31</td>
<td>21 - 26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂CT (l/dl)</td>
<td>16.43</td>
<td></td>
<td>96.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure (n=26)</td>
<td></td>
<td>17.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>98</td>
<td></td>
<td>82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>68</td>
<td></td>
<td>55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mmHg)</td>
<td>83</td>
<td></td>
<td>68</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T-35 = Time 35 minutes before expected recumbency (after xylazine preanesthetic).  
T0 = Time of recumbency.  
T5 = Five minutes after recumbency.  
T5 +15 = Time standing plus 15 minutes.  
ECF = Extra cellular fluid  
SBC = Standard Bicarbonate Content
SUBPERIOSTEAL PROLIFERATIONS IN THREE SPECIES OF FRUIT BAT (Pteropus giganteus, P. poliocephalus AND Rousettus aegypticus)

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Nodules were noted on the long bones of several Indian fruit bats (Pteropus giganteus), at Metropolitan Toronto Zoo, examined for weakness and anorexia in 1977. The diet was altered and no further cases were seen for 13 years. In 1990 lesions were again identified on Indian fruit bats; in addition, Egyptian fruit bats (Rousettus aegypticus) and grey-headed flying foxes (P. poliocephalus) were affected. Nodules were hard to identify due to roosting posture. Lesions were well advanced before they resulted in loss of condition with a reduction in activity and associated decline in feed intake.

A study was initiated to classify the lesions, and thus attempt to identify the etiology of the condition. Increased bone deposition is associated with a number of diseases, which may be considered in the following main groups: congenital, toxic, metabolic and nutritional, traumatic, infectious or neoplastic. Over an eight month period bats in the collection were evaluated by bimonthly physical examination, lesion monitoring, blood sampling, and whole body radiographs. A retrospective study of clinical and necropsy records was undertaken. Inquiries were made at other institutions that had acquired Chiroptera from the Toronto collection, regarding the appearance of similar bone proliferations. Tissues taken at postmortem examination were investigated by histology, bacterial culture, and for fluoride content.

The youngest bats to be affected were two years old. Of the total population of bats greater than six months old in the collection, 58% of Indian fruit bats (n = 43), 16% of Egyptian fruit bats (n = 45) and 100% of grey-headed flying foxes (n = 8) have developed bone nodules. A mild variation in distribution of lesions was noted in the different species, though the pectoral limbs were the most frequently affected sites. Radiography indicated the nodules were periosteal and continuous with the cortex, and consisted of low density bone with a rounded fluffy to palisading appearance. Serum phosphorus, calcium, and vitamin D levels were normal, but alkaline phosphatase levels were elevated, relative to reference levels for P. giganteus, in all the bats regardless of lesion status.
No bacteria or mycoplasmas were isolated from bone samples. Histologic examination of the bone indicated a reactive rather than neoplastic proliferation of the subperiosteum. Bone fluoride levels were high relative to the normal human levels, but interpretation of this finding in isolation cannot be made.

Unaffected Chiroptera transferred to other collections did not develop bone lesions. The lack of lesions in transferred bats, the multi-species incidence of the disease, the distribution of the proliferations, the inability to isolate an agent, and the histological appearance of the lesions are most compatible with changes due to a metabolic or nutritional imbalance. Further work is in progress to analyze the diet and tissues in order to identify the cause of the bone lesions in the bats.
ASSISTED REPRODUCTION IN ELD'S DEER: A MODEL FOR GENETIC MANAGEMENT OF ENDANGERED UNGULATES

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Smithsonian Institution, National Zoological Park, Department of Animal Health and Department of Conservation, 1500 Remount Road, Front Royal, Virginia 22630, USA

The North American, captive Eld's deer population consists of 140 individuals distributed among several herds separated by as many as 5,000 km. This species and these populations typify the challenges associated with genetically managing captive, wild hoofstock. Eld's deer reproduce well in captivity, but their excitable temperament increases the risk of stress and injury during long-distance transport. They also tend to express self-destructive behaviors which discourages their routine transport among institutions to maximize genetic vigor. The results are that: (1) the carrying capacity for the species has been reached in North American zoos; and (2) Eld's deer have become inbred within individual breeding facilities.

Zoo managers now face a dilemma. How can genetic diversity in the Eld's deer (and other rare cervid species) be maintained without increasing animal numbers? Germ plasm cryopreservation, combined with artificial insemination (AI), has potential for overcoming these problems while providing insurance against further losses in genetic diversity as a result of additional inbreeding, disease or other unforeseen catastrophes. The Eld's deer is a prime candidate for demonstrating how 'assisted reproductive biotechnology' can be integrated with sound animal husbandry to manage and preserve genetic diversity within a fragmented captive population.

Several North American zoos have active research programs aimed at applying assisted reproductive techniques (including germ plasm cryopreservation) to conserving rare taxa. For more than a decade, the 'frozen zoos' concept also has been touted as holding great promise for preserving endangered species and genetic diversity. Although AI, in vitro fertilization and/or embryo transfer have been heralded as tools for enhancing captive breeding, these techniques have not yet proven consistently useful for producing offspring from any endangered mammal. Nevertheless, there is a broad consensus that, for wild taxa, AI could be particularly valuable for (1) ensuring reproduction between genetically valuable but behaviorally incompatible pairs, (2) eliminating the risks of animal transport and (3) providing an avenue for infusing genes between wild stocks and captive populations, many of which are genetically stagnant. These biotechniques have not yet become routine largely due to our historical failure to develop strong biological databases first before assisted reproduction is attempted. For AI to be successful, detailed prerequisite information must include (1) understanding the female's reproductive cycle to allow identifying or manipulating estrus/ovulation, (2) identifying safe and reliable methods for collecting and storing spermatozoa and (3) developing approaches for the proper deposition of sperm at the optimal time and site in the female. We now have fulfilled these criteria for the Eld's deer, with the reproductive database being as complete as for any other zoo-maintained endangered ungulate in North America. This success has been largely based upon our...
parallel efforts to improve management and manipulation strategies for this species in captivity by optimizing facilities design, animal nutrition, hand-rearing protocols, behavioral conditioning and husbandry.5

The first offspring were produced in Eld’s deer using AI and frozen-thawed spermatozoa in 1992.4 Sperm donors were selected prospectively on the basis of genotypic value to the North American Eld’s deer population. Three males were selected, and semen was collected by electroejaculation and stored frozen. Intravaginal progesterone-releasing devices (CIDR devices) were used to synchronize estrus and ovulation in 20 hinds. CIDR devices were removed after 14 d, and each hind was anesthetized 70 h later for transabdominal, intrauterine AI by laparoscopy. Female reproductive activity was monitored by assessing steroid hormonal metabolites in voided urine. Nine hinds (45%) delivered 10 offspring representing the largest number of pregnancies yet produced in a single AI trial for any endangered mammal.4 The inbreeding coefficients in all of these young were < 0.25 (0.000, n = 6; 0.009, n = 1; 0.031, n = 1; 0.062, n = 1; 0.250, n = 1) providing a clear example of how assisted reproduction can be combined with sound management and husbandry to produce genetically-valuable offspring.

Young now have been produced from 32 mammalian species using AI with frozen-thawed spermatozoa.7 Seven of the 12 ungulates in which AI has been successful have been cervids (white-tailed deer, fallow deer, red deer, wapiti, reindeer, axis deer and Eld’s deer). Thus, this taxon appears particularly amenable to the application of AI technology. However, we still lack evidence that assisted reproduction can be implemented on a ‘real-life’ and practical basis for bettering regional collections and, more importantly, for contributing to species conservation.

If the zoo community commits to captive breeding a specific taxon/species with the aim of developing genetically-viable reservoir populations, then an immediate consideration must be perfecting management schemes permitting appropriate genetic interchange using natural or ‘assisted’ means. Clearly, it is nonsensical for taxon advisory groups (TAGs), species survival plans (SSPs) and studbook keepers to treat isolated (and often inbred) groups as contiguous members of a regional or global population if there is no built-in mechanism for exchanging genetic material. Without this fundamental capability, one must question the wisdom of maintaining highly stress-susceptible ungulates in captivity, particularly if the goal is species ‘conservation’.

Historically, zoos have focused upon formulating basic reproductive databases and developing the actual assisted reproductive techniques. In some cases, technology appears to be outpacing advancements in basic animal husbandry/management. For example, applying AI to Eld’s deer requires animal manipulations for CIDR device insertion/withdrawal and for laparoscopic insemination. However, most Eld’s deer in North American zoos are managed under conditions that do not permit animals to be safely and routinely manipulated. The utility of AI is only as effective as our ability to apply the technology at the husbandry level. Therefore, as these technologies continue to develop, there is need for a host of concurrent, managerial efforts including designing and constructing the physical facilities allowing AI to be practically applied.
To achieve this goal, biologists, veterinarians, curators and collection managers must join forces to develop and implement integrative strategies that take full advantage of the potential usefulness of assisted reproduction. For excitable ungulate species, semi-intensive management holds promise for improving our ability to genetically manage small captive populations. Such approaches may include hand-rearing, behavioral conditioning and facilities design that incorporates such features as shift corridors, holding pens and drop-chutes to permit routine animal isolation for veterinary procedures, management and research. These strategies are likely to initially increase costs. However, long-term benefits include a decreased need for anesthesia, reduced incidence of self-destructive behaviors and an improved ability to manage the genetics consistent with goals established by the TAG and SSPs.

We contend that the Eld's deer serves as the prototype model of a highly excitable, endangered species that has benefitted from combined intensive management and assisted reproduction. For this specific species, an approach that combines traditional husbandry-management with biotechnology (including germ plasm cryopreservation) may be the only realistic hope of maintaining sufficient genetic diversity without burdening limited captive breeding space with excessive individual animals. The Eld's deer provides one of the first examples in which prospective sire and dam selection, germ plasm banking, AI and urinary hormone monitoring have been used together successfully for the consistent production of multiple offspring. In this context, dialogue is in progress with the North American Cervid TAG to develop cross-institutional strategies for applying these strategies to managing this regional population. Combined with new management initiatives, we anticipate that this approach could have broad applications to preserving a host of rare ungulate species.

ACKNOWLEDGMENTS

This research was supported by grants from the Scholarly Studies Program of the Smithsonian Institution, the NOAAHS center, Friends of the National Zoo and a National Institutes of Health Career Development Award (SLM).

LITERATURE CITED

EMBRYO TECHNOLOGY IN DOMESTIC CATTLE AND ITS APPLICATION TO ENDANGERED SPECIES CONSERVATION

Leslie A. Johnston, PhD* and Naida M. Loskutoff, PhD*
Omaha’s Henry Doorly Zoo, Omaha, Nebraska 68107, USA

In 1982, Brackett et al. reported the birth of the first domestic bull calf resulting from the in-vitro fertilization of mature ova collected by laparoscopy. By 1986, techniques were further developed for producing calves from immature oocytes recovered from abattoir ovaries after in-vitro maturation and fertilization. Initially, methods for culturing bovine zygotes were insufficient for supporting in-vitro development to uterine stages suitable for embryo transfer; therefore, fertilized ova were cultured for several days in the ligated oviducts of cattle, sheep or rabbits. Since then, a number of independent investigators have reported successful in-vitro culture systems for the development of bovine zygotes up to the hatched blastocyst stage. Although a variety of culture conditions and medium constituents have been reported to influence in-vitro development, somatic cell co-culture has been found to be an effective means of conferring developmental competency to cultured embryos. The first calves produced from an entirely in-vitro culture system resulted from embryos that were co-cultured with follicular granulosa cells. Also using granulosa cell co-culture, Fukuda et al. (1990) first reported the birth of calves resulting from in-vitro-produced embryos that were frozen and thawed before transfer. Although bovine granulosa or oviductal epithelial cells are most commonly used for bovine embryo co-culture, the beneficial effects of somatic cells do not appear to be limited to species, sex or even tissue type, although there can be differences in the relative degrees of embryotrophic effects. As with embryos collected from superovulated cattle, in-vitro-derived bovine embryos can be subjected to further manipulations such as embryo biopsy for sexing or embryo multiplication for the production of genetically identical calves. Loskutoff et al. (1993) reported that the pregnancy rates of recipient cattle carrying twin calves produced by embryo splitting was similar using in-vivo- or in-vitro-derived embryos and as many as four genetically identical calves have been produced from in-vitro-derived embryos by blastomere separation. The methods developed in domestic cattle for producing viable embryos in vitro have also been effective in a variety of species including sheep, goats, water buffalo and deer.

Only a few reported studies have explored the potential of gamete rescue and the application of IVM/IVF to endangered species. These studies have been limited due to the rarity of the species and the requirement that most of the raw data must be collected opportunistically from older and medically compromised individuals. As a consequence, these studies have met with only limited success in comparison to studies using domestic cattle. To-date, immature oocytes have been recovered from four endangered ruminant species representing a broad diversity of taxa.

The first rescue of immature oocytes from an endangered ruminant was attempted five years ago with a banteng (Bos javanicus) in poor health. Ovaries were recovered following euthanasia and shipped air express to the processing laboratory. Twenty immature cumulus-enclosed oocytes were recovered and processed for IVM and heterologous IVF. This initial study demonstrated for the first time that nondomestic ruminant oocytes were capable of
IVM using maturation conditions established for domestic cattle. Although embryos were not produced, fertilization did occur as indicated by the presence of 2 pronuclei and remnant sperm tails in the cytoplasm.

Gaur (*Bos gaurus*) ovaries were opportunistically obtained from 3 adult cows and maintained at ambient temperature for 7-8 h before processing for IVM/IVF. Ninety-five oocytes were recovered from the three sets of ovaries, of which 65 were suitable for culture. These immature gaur ovarian oocytes were capable of in-vitro maturation and fertilization with thawed homologous sperm and resulting embryos advanced to blastocysts in culture and established a pregnancy in vivo.7

Although more distantly related to domestic bovids, immature oocytes were rescued from ovaries of a klipspringer (*Oreotragus oreotragus*) which died from pregnancy ketosis.10 Eighteen cumulus-enclosed immature oocytes were placed into culture and matured 24 h. Although a percentage of oocytes had degenerated by this time, fertilization of 12 remaining oocytes with homologous fresh semen resulted in one 4-cell embryo. This single embryo advanced to 16-cells before being cryopreserved using a bovine protocol.

More recently, a 4 year old giraffe (*Giraffa camelopardus*) died of unknown causes. Although this female was >10 months pregnant, 60 cumulus-enclosed oocytes were recovered and placed into culture. After 24 h, 75% of the oocytes matured and after fertilization with homologous frozen sperm, 42% (n=19) cleaved to the 2-cell stage. Three of these embryos developed to the early blastocyst stage (60-70 cells) by day 7.8

Based on these limited studies, oocyte rescue has the potential to become a viable approach for rescuing genetic material from endangered ruminants which die naturally or are euthanized for medical reasons. Unlike previous studies with endangered felid oocyte recovery, it appears possible to recover quality immature oocytes from donors which have been classified into a variety of categories including terminally ill and pregnant individuals. Although more research is required to optimize maturation conditions for each taxa, these studies demonstrate that rescued oocytes are capable of maturing and fertilizing in vitro and have the potential of developing in vivo.

**LITERATURE CITED**


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Understanding basic reproductive processes is crucial for determining why some animals reproduce and others do not. Some nondomestic felid species reproduce poorly in captivity, and there is little physiological information available to help identify what causes reproductive failure in certain species, populations or individuals. One of our goals is to develop databases on the hormonal interrelationships regulating reproductive activity. However, monitoring hormonal patterns using traditional blood sampling methods is not practical for these species. Instead, noninvasive techniques for assessing reproductive function need to be developed. Metabolism of estradiol (E2) has been described for the domestic cat, and it was found that >90% of this steroid was excreted in feces which explains the failure of urinary monitoring techniques to track reproductive patterns in felids. Our objectives were to determine excretion rates and metabolism of progesterone (P4) in the domestic cat, and then to develop and validate a fecal progestogen radioimmunoassay (RIA) for monitoring ovarian function in the domestic cat and representative nondomestic felid species.

A radiolabel infusion study was conducted in the domestic cat to assess P4 metabolism and excretion. Three anestrous females were injected i.m. with 3 μCi 14C-P4 (75 mCi/mmol; New England Nuclear) and 100 μg unlabeled P4 in 2.0 ml 0.9% NaCl. Following isotope injection, syringes were rinsed with ethanol and the residual radioactivity counted and subtracted from the pre-injection total. Cages were checked at 2 h intervals and all urine and feces collected for 5 d post-injection. Feces and urine contained 96.7 ± 0.5% and 2.6 ± 1.2%, respectively, of the radioactivity as a percentage of total radioactivity administered. The majority of radioactivity in the urine was detected in the first sample collected between 8 and 13 h post-injection. Peak radioactivity in feces also occurred in the first sample collected between 11 and 50 h (28 ± 11 h). Based on differential extraction with diethyl ether (1:10 v/v), 78% of P4 metabolites were excreted as conjugated (aqueous phase) rather than free (organic phase) steroid forms. Using a gradient of 30-100%
acetonitrile:dH₂O over 80 min (1 ml fractions, 1 ml/min flow rate), HPLC analyses detected
3 radioactive peaks, 2 polar (presumably conjugate) peaks at fractions 5-7 and 8-10 (48 and
42% of the total radioactivity, respectively) and a less polar peak at fractions 38-39 (10%).
None of these peaks co-eluted with the ³H-P₄ reference tracer (fractions 35-36). Conjugated
metabolites were not enzyme-hydrolyzable, and, based on HPLC analyses, the percent
hydrolyzable by sulphatase (8%) and β-glucuronidase (7%) appeared to represent residual
conjugates entering the ether phase.

A rapid method for extracting fecal P₄ metabolites (herein referred to as progestogens) from
cat feces was developed based on the method of Wasser et al.³ Dried, pulverized fecal
material (0.1-0.2 g) was boiled in 5 ml of 88% ethanol:dH₂O for 20 min and centrifuged at
500 g for 10 min. The supernatant was recovered and the pellet resuspended in 5 ml of 88%
ethanol, vortexed for 1 min and recentrifuged. The two ethanol phase supernatants were
combined, dried completely and then redissolved in 1 ml methanol. This technique resulted
in a recovery of 87.2 ± 1.4% (n = 6) for metabolized ¹⁴C-P₄, and >90% for exogenous ¹⁴C-
P₄ added to fecal samples before extraction. The P₄ RIA developed in this laboratory
utilized a monoclonal P₄ antibody (produced against 4-P-11-ol-3, 20-dione hemisuccinate:BSA) provided by Dr. Jan Roser (University of California, Davis), an ¹²⁵I-
labeled P₄ tracer (Pantex, Santa Monica, CA) and P₄ standards. The assay was incubated
at 4°C for 24 h in a total volume of 500 µl. Standards (100 µl) and/or sample (diluted 1:800
to 1:8,000 with RIA buffer; 0.01 M PO₄, 0.5% BSA, 2 mM EDTA, 0.9% NaCl, 0.01% thimerosal,
pH 7.4) were incubated with antibody (1:100,000 in 100 µl) and P₄ tracer (20,000
c.p.m. in 100 µl) for 4 h. Antibody-bound complexes were precipitated with sheep anti-
mouse gamma globulin (1:80 in 200 µl; 20 h incubation) followed by addition of 1 ml rinse
buffer (0.01 M PO₄, 0.9% NaCl, 0.01% thimerosal, 5% polyethylene glycol, pH 7.4) and
centrifugation for 30 min at 1500 g. The antibody bound 30-40% of the iodinated P₄ tracer
with 3% nonspecific binding. Assay sensitivity, based on 90% of maximum binding, was 3
pg/ml, and the intra- and inter-assay coefficients of variation were <10%. This assay was
validated for fecal extracts from the domestic cat (Felis catus), leopard cat (Felis bengalensis),
cheetah (Acinonyx jubatus) and clouded leopard (Neofelis nebulosa) by demonstrating: 1)
parallelism between dilutions of pooled fecal extracts and the standard curve; and 2)
significant recovery of exogenous P₄ (3.75-120 pg) added to fecal extracts (domestic cat, y
= 0.95x + 1.11; r = 0.99; leopard cat, y = 1.08x - 1.13, r = 0.99; cheetah, y = 1.16x - 0.42;
r = 0.99; clouded leopard, y = 1.08x - 1.94; r = 0.99).

Co-chromatographic HPLC profiles of extracted fecal samples from the domestic cat
revealed P₄ immunoreactivity coincided with 1 presumably conjugated (fractions 8-10) and
1 unconjugated (fractions 38-39) metabolized progestogen peak. Isotope infusions were not
performed in nondomestic species, but progestogen immunoreactivity in leopard cat and
c clouded leopard fecal eluates was similarly associated with a conjugated (fractions 7-8;
>90% of the total immunoreactivity) and unconjugated (fractions 38-39) peak, suggesting
that P₄ metabolism may be similar among these species. Interestingly, RIA of fecal eluates
from the cheetah revealed 3 immunoreactive peaks constituting 42% (fractions 7-8), 51%
(fractions 43-36) and 7% (fractions 38-39) of the total radioactivity, with the second peak
co-eluting with the ³H-P₄ tracer. Thus, with the exception of the cheetah, fecal metabolites
other than unconjugated P₄ appear to predominate in felids.

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Longitudinal profiles of fecal progestogen excretion were examined in samples collected 3-5 times weekly for 2 wk before artificial insemination (AI) or natural mating through parturition to 2 wk after birth. Progestogen profiles were generated for 3 pregnancies resulting from AI (leopard cat, n = 1; clouded leopard, n = 1) or natural mating (clouded leopard, n = 1), and for 5 nonpregnant luteal phases (i.e., pseudopregnancy) after AI (clouded leopard, n = 3) or natural mating (cheetah, n = 1; clouded leopard, n = 1). For AI, follicular development was stimulated using equine chorionic gonadotropin and ovulation was induced using human chorionic gonadotropin administered 5 and 2 d before laparoscopic AI, respectively. In the leopard cat, fecal progestogen concentrations were low before AI (5.2 ± 0.9 µg/g dry feces), increased within 5 d after AI and peaked at ~1500 µg/g on Day 25 of pregnancy. Progestogen concentrations remained elevated throughout pregnancy (overall mean, 874 ± 66 µg/g); however levels gradually declined from ~750 µg/g on Day 40 to ~400 µg/g one day before parturition. Baseline concentrations were detected within 2 wk post-partum. Overall mean baseline progestogen concentrations in the clouded leopard (3.8 ± 1.7 µg/g) were similar (P > 0.05) to those observed in the leopard cat, whereas average basal concentrations in the cheetah (0.8 ± 0.2 µg/g) were lower (P < 0.05). Mean progestogen concentrations during the nonpregnant luteal phase (cheetah, 98.2 ± 10.3 µg/g; clouded leopard, 68.3 ± 8.1 µg/g) or pregnancy (clouded leopard, 41.1 ± 5.6 µg/g) were substantially lower (P < 0.05) than those observed in the leopard cat, suggesting that species differences exist in the quantity of progestogens excreted. Duration of pregnancy was 63 d in the leopard cat, and 88 and 89 d for the clouded leopard. The nonpregnant luteal phase duration was 50 d for the cheetah, and 45 ± 2.5 d (range, 39-51 d) for the clouded leopard, or about half that of a normal pregnancy.

In summary, P₄ in the domestic cat is metabolized and excreted primarily into feces. We have identified at least 3 major radioactive metabolite peaks using HPLC, although it is possible that several metabolites could be associated with each of these peaks. We also have developed and validated a P₄ RIA using an antibody that crossreacts with 2 of the 3 radioactive metabolite peaks present in the domestic cat. Although radiolabel infusion studies were not conducted in the nondomestic species, comparisons with the domestic cat suggest that the leopard cat and clouded leopard excrete similar immunoreactive metabolites, whereas the cheetah excretes an additional metabolite that may be unconjugated P₄. These preliminary data provide good evidence that fecal progestogen analyses are useful for monitoring changes in corpus luteum function during pregnancy and nonpregnancy in several felid species. We conclude that longitudinal monitoring of fecal progestogen excretion, in conjunction with fecal E₂, should provide a valuable tool for assessing ovarian function in felids. Given that this technology can be applied across taxa, then we predict that fecal steroid analyses will be valuable for: 1) characterizing ovarian activity over time; 2) determining the prevalence of induced versus spontaneous ovulation; and 3) providing valuable feedback on how to improve assisted reproduction strategies, making them a practical management tool. Because the duration of a nonpregnant luteal phase appears to be about half that of pregnancy, it may also be possible to distinguish between these two reproductive states during the second half of a suspected pregnancy.
ACKNOWLEDGMENTS

We thank Dorothy Bowers and Anneke Moresco for assistance with assay validations and sample analyses. We also are very appreciative of the collaborative efforts of the staff at The International Wildlife Conservation Park/Bronx Zoo, Nashville Zoo, Phoenix Zoo and the National Zoological Park and Conservation and Research Center. This project was supported, in part, by the AAZPA Conservation Endowment Fund/Ralston Purina Big Cat Survival Fund, the Center for New Opportunities for Animal Health Sciences (NOAHS), the Friends of the National Zoo (FONZ), and the Scholarly Studies Program of the Smithsonian Institution.

LITERATURE CITED

Assisted reproduction is being developed as a management strategy for maximizing genetic diversity in captive and wild populations of endangered species. However, the success of these techniques including artificial insemination, in vitro fertilization (IVF) and embryo transfer may be limited by several factors such as: 1) poor ejaculate quality and high proportions of structurally-abnormal sperm; 2) failure to recover large numbers of embryos from females; and 3) an inadequate number of available animals. To overcome these limitations, it may be necessary to apply advanced methods of gamete biotechnology that have been developed in humans and domestic livestock species. For example, Felidae is an endangered taxon in which there are several species, including the cheetah (*Acinonyx jubatus*), that produce poor sperm quality and exhibit compromised zona pellucida (ZP) penetration and IVF success. New technologies for assisting IVF may be beneficial for enhancing reproductive efficiency in this species.

Procedures for assisting IVF have evolved rapidly, primarily due to research conducted in the human fertility field where male factor infertility frequently is the cause of failed conception. Four primary procedures for assisting IVF are: 1) zona pellucida piercing (ZnPd); 2) partial zona dissection (PZD); 3) subzonal sperm insertion (SUZI); 4) sperm injection (SI). ZnPd involves chemical or mechanical manipulation of the oocyte to induce very small channels through the ZP. These channels provide paths that offer little resistance to sperm penetration, but sperm motility still is essential for successful oocyte penetration. PZD is a more extreme form of ZnPd where a glass needle is used to shear off an entire section of the ZP. Unfortunately, the incidence of polyspermy (the entrance of >1 sperm into the oocyte) tends to be high in both procedures. For SUZI, one or more sperm are aspirated into a very fine-tipped micro-pipette and then injected into the perivitelline space (PVS) adjacent to the oolemma of the oocyte. SUZI obviates the requirement for sperm penetration but fusion with the oolemma still must occur. Finally, SI eliminates the necessity for most of these events associated with sperm function. A single sperm is aspirated into a micro-pipette, injected through the zona and deposited directly within the ooplasm. SI has been successfully applied to an endangered species, the gorilla (*Gorilla gorilla*). Although polyspermy is not a risk associated with this procedure, oocyte lysis and parthenogenetic activation are major concerns, and perhaps the greatest risk is the complete lack of any natural selection for the fertilizing spermatozoon. All four of these assisted IVF procedures have proven successful for enhancing IVF in humans and investigations concerning their utility in other species currently are being initiated.

The utility of ZnPd for enhancing sperm penetration has been demonstrated recently in the cat (*Felis catus*). Sperm were collected from normospermic (>60% normal sperm/ejaculate) and teratospermic (<40% normal sperm/ejaculate) domestic cats that serve as research models for nondomestic felids. Conspecific oocytes mechanically pierced (6 holes/zona) were co-incubated with sperm (2 x 10^5 motile/ml) for 6 hours, then fixed in 10%
buffered formalin and evaluated for the number of sperm bound to the zona and penetrating the outer half of the zona, the inner half of the zona and into the PVS. For both normospermic and teratospermic groups, inner zona penetration was greater \((p<0.05)\) in ZnPd than control oocytes (normospermic, 52.3% vs. 34.9%; teratospermic, 32.0% vs. 17.2%). Similarly, ZP piercing increased \((p<0.05)\) the proportion of oocytes with sperm in the PVS (normospermic, 11.7% vs. 0.8%; teratospermic, 7.0% vs. 0.8%). A ZnPd study utilizing a heterologous ZP penetration assay also was conducted recently in an endangered felid species, the cheetah. ZnPd and nonmanipulated domestic cat oocytes were co-incubated with sperm from six adult cheetahs maintained at Fossil Rim Wildlife Center in Glen Rose, TX. Ejaculates contained an average of 11.7% structurally-normal sperm. Similar to the domestic cat results, a greater proportion \((p<0.01)\) of ZnPd oocytes contained sperm within the inner ZP (39.2%) compared to nonmanipulated oocytes (12.8%). ZP piercing also resulted in a higher PVS penetration rate in the ZnPd oocytes (2.7%) compared to controls (0%). These results indicate that this assisted IVF procedure enhances sperm penetration in cheetahs as evidenced by a 3-fold increase in ZP penetration following zona piercing. These data also suggest that this gamete micromanipulation procedure may facilitate IVF efficiency in teratospermic felids. Although the ZnPd, PZD, SUZI and SI procedures offer potential benefits for assisting reproduction in cases of severe male-factor infertility within small populations of endangered species, it is likely that a limiting resource will be the female's gametic contribution. Ideally, superovulation followed by natural breeding, embryo collection and embryo transfer into several recipients would provide an effective strategy for optimizing the female's genetic contribution to a population. In nondomestic species, however, this regimen does not reliably result in the retrieval of large numbers of embryos. When only one or few embryos are obtained from a valuable female, there are several biotechniques available that may increase the final yield of offspring: 1) embryo dissection; 2) blastomere disaggregation; and 3) nuclear transplantation. These embryo micromanipulation procedures were developed primarily for use in domestic livestock, but also could be considered for assisting reproduction of nondomestic animals.

Embryo bisection is the most common procedure for enhancing the number of offspring resulting from a limited number of embryos. This technique requires a relatively low level of micromanipulative expertise and can be highly successful when healthy morulae or blastocysts are available. Transfer of demi-embryos following embryo bisection has resulted in live offspring in numerous domestic species including the mouse,\(^{12}\) rabbit,\(^{13}\) sheep,\(^{14}\) pig,\(^{15}\) cow,\(^{16}\) and horse.\(^{17}\) Furthermore, it has been possible to produce offspring from quarter embryos in cows,\(^{18}\) suggesting that a single morula could yield four offspring. The feasibility of using this biotechnology in nondomestic species has been demonstrated by the birth of an eland (Tragelaphus oryx) calf that resulted from the transfer of two demi-embryos to a recipient eland female.\(^{19}\)

Blastomere disaggregation is similar to embryo splitting but can be applied to embryos at a very early stage of development. Each blastomere of an embryo remains totipotent until the embryo reaches a specific developmental stage (frequently the 8-cell stage). Therefore, a single blastomere isolated from a 2-, 4- or 8-cell embryo has the capacity to develop into a viable fetus. Blastomere disaggregation successfully has been applied in the mouse,\(^{20}\)
sheep, pig, goat and cow. Although, it is possible to produce up to eight offspring from a single 8-cell embryo, the optimal yield frequently is not achieved because blastomere disaggregation is a difficult, complex procedure during which cells frequently are damaged.

Finally, nuclear transplantation offers a third method for increasing the number of offspring derived from a single embryo. For this procedure, the nuclear material is removed from a single-cell embryo and replaced with the nucleus from a blastomere of a multi-cell embryo at a later stage of development. This "older" nuclear material is re-programmed by the recipient embryo's cytoplasm so that it functions as the nucleus of a one-cell and not a later stage embryo. After this new embryo cleaves, it too can serve as a nuclear donor. Theoretically, the cycle could continue indefinitely to produce an infinite number of offspring all derived from the nuclear material of a single embryo. This procedure generally is referred to as cloning and requires a high level of technical skill but has proven successful in the mouse, sheep, cow and pig.

In time, embryo biotechnology may prove important for increasing the production of endangered species offspring. However, the benefit of utilizing these three embryo micromanipulation methods (bisection, blastomere disaggregation, nuclear transfer) for enhancing the genetic contribution from a female of an endangered population or species is questionable largely because each method, if successful, produces genetically identical offspring. Therefore, the end result could ultimately impact negatively on the genetic diversity of a small population.

Finally, when the number of females is the limiting factor in an endangered population, the ability to transfer embryos to recipient females of a non-endangered species is desirable. Embryo inner cell mass (ICM) transplantation could facilitate the success of interspecies pregnancies. This procedure involves blastocysts which are embryos that have differentiated into ICM (fetal) and trophoblast (placental) cell lineages. The ICM is isolated from the blastocyst of the desired species and transferred into a trophoblastic vesicle (a blastocyst with the ICM removed) derived from a non-endangered, but closely related, species. The resulting chimeric embryo then is transferred into a female of the non-endangered species. Theoretically, the conspecific trophoblast will serve as a barrier at the fetal-maternal interface protecting the xenospecific fetus from rejection by the maternal immune system. In short, the offspring of an endangered species could develop to term in utero safely surrounded by the placental tissue of the non-endangered, recipient species. Although technically difficult, ICM transplantation between species has been successful resulting in a sheep giving birth to a goal.

In summary, as the earth's resources continue to diminish, those species that escape extinction likely will exist only in small, isolated populations. Over time, these populations will experience the effects of inbreeding that commonly result in reduced reproductive efficiency. Intensive animal husbandry practices, including assisted reproduction may become necessary to sustain animal populations both in situ and ex situ. Therefore, it is critical that we develop the biotechnology necessary for overcoming reproductive deficiencies...
in a variety of species. Although gamete biotechnology and assisted reproduction will never replace natural breeding, these techniques may serve as valuable tools in our efforts to save species from extinction.

LITERATURE CITED

STRUCTURE AND FUNCTION OF THE AAZPA CONTRACEPTION COMMITTEE

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The AAZPA Contraceptive Task Force was formed in July, 1989, with two primary objectives: 1) to survey the efficacy and safety of currently used contraceptive techniques, and 2) to initiate and coordinate research into alternative forms of contraception. Since that time, the temporary task force has become a permanent committee, and education, both of the public and the zoo community, has become an additional function. Members of the committee, which include curators, veterinarians and research scientists, also serve as advisors to Species Survival Plans (SSPs) and Taxon Advisory Groups (TAGs).

Surveys of carnivores, primates, and hoofstock have been conducted by Ingrid Porton and summarized on a computer database by Betsy Hornbeck, both of the St. Louis Zoo, for more efficient analysis and access. These surveys will be updated yearly, so that information remains current. The database currently contains over 1,500 records for more than 1,000 individuals of 110 different species.

The results of these surveys alert us to problems with efficacy and safety. For example, pregnancies in New World monkeys (Cebids) while melengestrol acetate (MGA) implants are in place have been reported. As a result, the Committee has initiated a study of a higher dose regimen in these species. With regard to safety, Dr. Linda Munson has used information from the carnivore survey to assist in her analysis of the deleterious effects of MGA in large felids. The survey database will also be used to evaluate dosages of MGA, especially in large felids. The database also contains information on forms of contraception other than MGA, such as birth control pills, Norplant, and Depo-Provera. By compiling reports from various institutions, the effectiveness of these formulations can be evaluated.

Survey summaries confirmed that the MGA implant is the most widely used contraceptive used in North American zoos. MGA, a synthetic progestin supplied by the Upjohn Company, was incorporated into silastic implants and distributed to zoos for almost 15 years by Dr. U.S. Seal, then of the VA Medical Research Center in Minneapolis. After Dr. Seal's retirement from the VA, the program was transferred to Dr. Ed Plotka of the Marshfield Medical Research Foundation in Wisconsin. Upjohn has graciously agreed to continue to supply the drug without charge. However, with the rapidly increasing need for contraception in captive wildlife, Dr. Plotka now makes and sends more than 800 implants annually. Zoos which receive implants all are asked to contribute $25 per implant to the Conservation Endowment Fund of the AAZPA. Recently, AAZPA awarded annual grants to Dr. Plotka from CEF to help defray the costs of the program.

Especially considering the vast array of species which have been treated with this contraceptive, MGA has proven remarkably effective and safe, as well as reversible even after many years of treatment. The notable exception has been the felids, in which uterine and mammary hyperplasia and symptoms of diabetes mellitus, in particular, have been associated with treatment with MGA or the related progestin megestrol acetate. Similar
problems are likely in other carnivores, especially canids. In fact, progestins in general, not only MGA, have been reported to cause these conditions in domestic dogs and cats. Thus, substituting other progestins would not be a solution.

For these reasons, as well as to provide a greater selection of contraceptive choices for other taxa, the Contraception Committee has initiated various trials of alternative techniques, and attempts to stay informed about other contraceptive projects to facilitate exchange of information and to prevent duplication of effort. Projects initiated by members of the Committee include MGA in feed, Depo-Provera to seasonal breeders, vas plugs, and bisdiamine oral contraceptive.

The Bronx Zoo veterinary staff found that administration of MGA in feed to herds of hoofstock was generally successful, although antler growth was altered in barasinga. Failures seemed to be attributable to subordinate animals not ingesting a full dose. The advantages, however, include ease of administration, rapid reversal, and synchronized births.

A systematic study of the effectiveness and safety of Depo-Provera in seasonally breeding black lemurs is being conducted by the St. Louis Zoo, with collaboration by the Henson Robinson and Metro Toronto Zoos. Parameters measured include dosages, cortisol levels, color change, and weight gain (distinguishing percent gain of fat, water or muscle).

Vas plugs are being evaluated in various primates, hoofstock and carnivores with the cooperation of 15 zoos, under the direction of Dr. L. Zaneveld, who is conducting clinical trials of the devices for humans. The technique has proven effective in blocking sperm transport, but reversibility trials are not yet complete.

Bisdiamine, an oral male contraceptive recently tested in gray wolves, has proven effective at blocking spermatogenesis. Reversibility will be examined during the next breeding season. Meanwhile, possible side-effects are being evaluated. Felids and sea lions are being considered next for treatment.

In a slightly different vein, the Committee expects to become more involved in testing compounds to reduce aggression in multi-male groups, expanding on work at Fossil Rim. These studies will be directed at alleviating space constraints associated with maintaining surplus animals. Coincidentally, many contraceptives also suppress testosterone, which may result in reduction of aggression as well.

At the midyear meeting, the Committee produced contraceptive recommendations for the major mammalian taxa, including suggestions for juveniles, pregnancy and lactation. These recommendations are being published in the AAZV Newsletter and have been distributed to SSP and TAG chairs.
LITERATURE CITED

ADVERSE EFFECTS OF CONTRACEPTIVES IN CARNIVORES, PRIMATES AND UNGULATES

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Contraceptive use in zoos has evolved with the increasing commitment of zoological parks to conservation programs. The immediate need to prevent reproduction of surplus animals and to postpone pregnancy in some individuals, as part of single species conservation plans, required using available contraceptives in species for whom these contraceptives had not been safety tested. Consequently, our understanding of the effects of these contraceptives on zoo species are only now gradually emerging from the large clinical trial we are conducting.

It should be remembered that no medical procedure or pharmaceutical is completely without risks. Our goal should be to use existing knowledge to choose contraceptives that have minimal detrimental effects on the health of the animals. This report summarizes current available information on the adverse effects of contraceptives on zoo species. Adverse effects of contraceptives in free-ranging wildlife are included in the review by Kirkpatrick and Turner.²

Steroid contraceptives

The most widely used contraceptives in all zoo species are the progestins, melengestrol acetate (MGA), megestrol acetate (MA), and medroxyprogesterone (MPA). Combined estrogen-progestin contraceptives (birth control pills) also have been used in primates. All steroid hormones exert their contraceptive effects by disrupting the normal cyclical hormonal levels of the hypothalamic-pituitary-gonadal axis. Tissue sensitivity to steroids depends on the presence of specific receptors, and receptor numbers in turn are modulated by exogenous and endogenous steroids. The response to receptor binding also depends on the species and tissue. All progestins are presumed to exert their action by binding to the progesterone receptors in tissues. Progesterone receptor numbers usually are up-regulated by estrogen and down-regulated by progesterone. For zoo species, the regulation of progesterone receptor numbers and responses to receptor binding are not known, but are assumed to be analogous to those of similar domestic and laboratory species.

The response of the endometrium and mammary gland to sex steroids varies among taxa and has only been studied in domestic and laboratory animals and women. For most species, estrogens promote hyperplasia and hypertrophy of the uterus, whereas progesterone antagonizes the estrogen effects, promotes secretory differentiation of the endometrium, and causes uterine atony. In canids and felids, however, progesterone also promotes endometrial proliferation. Mammary gland growth in all species requires estrogen, progesterone, and the pituitary hormones, prolactin and growth hormone.
Adverse effects of progestin contraceptives in carnivores

Our ongoing reproductive pathology surveillance of MGA-contracepted zoo felids at the time of this writing includes 137 felids representing 22 species (MGA-contracepted = 64; non-contracepted = 73). Our data to date indicate that MGA is highly associated with more severe forms of endometrial hyperplasia and development of endometrial hyperplasia at earlier ages than non-contracepted felids. All ten felids with uterine cancer from this survey had been contracepted with progestins, as had 22 of 25 felids with mammary gland cancer. Our data also suggest that more advanced endometrial hyperplasia and cancer are associated with lower doses of and prolonged exposure to MGA (5.5 or more years). These findings confirm the predicted pathologic effects of progestins on felid reproductive organs and indicate that progestins should not be used as permanent contraceptives for felids. Minimal endometrial lesions were noted in felids contracepted with MGA for less than 3 years, and these lesions most likely would resolve with withdrawal of MGA and resumption of estrous cycles. Thus MGA may cause acceptable levels of changes that pose a minimal health risk, when used as a temporary contraceptive.

Diabetes mellitus also has been reported in MGA and MA contracepted felids, and has been presumed to be due to the potential of progestins to promote insulin resistance and aggravate latent diabetes. However, a case-control study has not been conducted to confirm this correlation.

Cystic endometrial hyperplasia, pyometra, and mammary cancer have been noted in progestin-contracepted canids and other carnivores (eg. binturong, civet, and fox), but extensive epidemiologic studies have not been conducted to confirm the association of these lesions with progestin exposure. However, in canids particularly, progestins would be expected to be a major risk factor for these diseases. Delineation of the role of progestins in disease development in other carnivores awaits further studies.

Adverse effects of mibolerone in canids and felids

The androgenic steroid, mibolerone, has been used as an effective contraceptive in wolves, jaguars, leopards, and lions. Anorexia, masculinization of lions, and increased aggression among wolves were notable side effects in this study.

Adverse effects of progestins in ungulates

Insufficient information on the effects of contraceptive doses of MGA in zoo ungulates is available, because use has been limited to a few zoos. In domestic cattle, prolonged exposure to progestins can result in accumulation of endometrial secretions (mucometra or hydrometra) and secondary endometrial atrophy. Two cases of mucometra/hydrometra have in fact been reported in zoo ungulates ingesting MGA. This condition may be reversible if atony and atrophy are not prolonged and if secondary infection does not occur. Male barasingha ingesting MGA have been reported to have abnormal horn growth (B.Raphael, personal communication).
Adverse effects of progestins in primates

Progestin-only contraceptives, such as levonorgestrel and medroxyprogesterone, have been extensively tested in laboratory primates and are currently approved for use in women. Adverse effects have been minor, such as amenorrhea and weight gain. However, some investigators are concerned that progestins may increase the risk of breast cancer when used in young women. The effects of long-term exposure of primates to MGA are not known, but would be predicted to be similar to other progestins.

Reproductive pathology surveillance of zoo primates on either progestin-only or combined progestin-estrogen contraceptives has only recently been initiated, so data is limited. Notable adverse effects of progestin-only contraceptives in lemurs have been weight gain and color changes (I. Porton, personal communication). MGA implant removal by primates (self-removal or by cagemates) has been common and has resulted in superficial wounds. One case of retained placenta and endometritis was noted in an MGA-contracepted tamarin that was (or became) pregnant while exposed to MGA. The fate of the fetus was unknown. One orangutan with an MGA implant had profound endometrial atrophy and an intraluminal endometrial "cast", but had no clinical signs associated with these findings. These are presumed to be rare events, and progestin-only contraceptives are expected to be acceptable contraceptives for zoo primates.

Vas deferens plugs

Surgically-placed silicone vas deferens plugs have been tested extensively as reversible contraceptives in humans with no adverse reactions. Knowledge of pathologic effects in other species are very limited, because clinical trials with formed-in-place soft silicone plugs have just been initiated at zoos. However, no serious adverse reactions are anticipated. In preliminary tests on impala and horses, small granulomas and mild inflammatory reactions were noted near the site of insertion and minimal tissue changes associated with healing were present. None of these lesions impinged on the lumen of the vas, nor were any pathologic changes within the vas containing the plugs. These preliminary findings suggest that this method will be safe for temporary or permanent contraception of males.

Surgical contraception

The most prevalent methods of permanent contraception are surgical ovariohysterectomy or vasectomy. Male gonadectomy (castration) is usually not chosen because social hierarchies can be disrupted and secondary sexual characteristics will be lost. Female gonadectomy without removal of the uterus also is not optimal for some species, because of the potential for developing infections in the post-pubertal, atonic uterus. The risks involved in any of these surgical procedures are minimal because current anesthetics are safe, and the procedures are relatively simple. The risks would be the same as for any surgical minor procedure.
Other contraceptives

Clinical trials with the anti-spermatogenic contraceptive bisdiamine and immunocontraception with zona pellucida vaccines in zoo species are in initial stages, and pathology surveillance for adverse effects has just been enacted. Therefore at the time of this writing, no information is available on their systemic or gonadal effects in zoo species. Because drug metabolism differs among species, extrapolation of bisdiamine safety data to zoo species may be inappropriate. The same is true for species variation in response to zona pellucida (ZP) vaccines. In laboratory dogs, mice, and primates, the ovarian effects of ZP immunization depended on the species, origin and purity of the immunogen, dose, and type of adjuvant. In some cases, permanent destruction of the ovary occurred. Information gained from these studies should be used to chose the best system for temporary or permanent immunocontraception in zoo species. The variation in ovarian response noted in these laboratory animal studies also confirm the importance of conducting carefully controlled clinical trials that include histopathological analysis of gonads to determine the species-specific effects of ZP vaccines.

AAZPA contraceptive committee adverse reaction reporting system

To address the critical need for more information on the safety of contraceptives in zoo species, the AAZPA Contraceptive Committee has established a system for reporting adverse reactions. Any reproductive problems or unexplained diseases in contracepted animals should be reported to this center by completing the enclosed form. Each contribution to this database increases our collective understanding of the risks of using certain contraceptive methods. Information from this databank will be reported annually at the AAZPA meeting.

Ongoing safety assessment trials

The optimal method to assess the safety of contraceptive methods in new species is to conduct controlled prospective clinical trials, rather than retrospective epidemiological analyses. Toward this aim, we have proposed that safety trials be conducted for the following contraceptives:

1. Zona pellucida vaccines from native or recombinant proteins with various adjuvants.
2. Vas deferens plugs
3. High dose melengestrol acetate in a 24-month trial
4. Bisdiamines and indenopyridine
5. Medroxyprogesterone in primates and seasonal carnivores
6. LHRH vaccines

These trials need surplus animal volunteers that are targeted for permanent contraception. If you are interested in contributing your animals to these trials, contact Dr. Cheri Asa, St. Louis Zoological Park, Forest Park, St. Louis, MO 63110 (314-781-0900 X 488) or Dr. Linda Munson, Dept Pathobiology, CVM, University of Tennessee, P.O. Box 1071, Knoxville, TN 37901 (615-974-8235).
REFERENCES

REPORT OF ADVERSE REACTIONS IN CONTRACEPTED ANIMALS

REPORTING INSTITUTION: ____________________________________________

REPORTING VETERINARIAN/CURATOR: ________________________________

ANIMAL SPECIES: __________________________________________________

ANIMAL ID: STUDBOOK #__________ ISIS #___________________________

Contraceptive type and dose Animal weight Inclusive dates of contraceptive use

__________________________  _____________  _________________________

__________________________  _____________  _________________________

__________________________  _____________  _________________________

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
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IMMUNOCONTRACEPTION IN ZOO ANIMALS: VACCINATING AGAINST PREGNANCY

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Three years of research with immunocontraception of captive female exotic species with a porcine zona pellucidae (PZP) vaccine have been completed. As of June 1993, 17 zoos in 6 countries are participating in these studies. A total of 173 animals representing 35 different species have been immunized, including 24 species of ungulates, 8 carnivores and 3 species of bears (see below). As of June 1993 results are known for 11 of these species. Contraceptive efficacy ranged from a low of 77% for tur to a high of 100% for tahr, sika deer and Przewalski's horses. Based on the results of this research, as well as 6 years of PZP immunocontraceptive research with feral horses, 4 years with white-tailed deer and 15 years with nonhuman primates, the data indicate that (1) PZP immunocontraception is highly effective across a wide range of species, (2) there are differences in contraceptive effectiveness according to the type of adjuvant used, (3) no significant health problems result from PZP immunocontraception over 3 years in captive exotic species or in white-tailed deer or feral horses after 4 and 6 years respectively, (4) the vaccine is safe to give to pregnant animals, (5) the vaccine can be administered remotely, by dart, (6) a single annual booster inoculation is sufficient to extend contraception for a second year and (7) the contraceptive effect of PZP immunization is reversible after short-term use (up to 2-3 years).

Issues of concern include (1) the number of inoculations necessary for contraceptive antibody titers (2) the amount of PZP antigen necessary for contraception, (3) appropriate adjuvants, (4) the most effective delivery systems, (5) and long-term effects upon ovarian function, after 5-7 years of consecutive treatment, and research during the past year has focused on these potential problems. Species with well-defined seasonal breeding lasting 1-3 months can be treated successfully with a single inoculation and will produce contraceptive antibody titers for up to 200 days following administration. Species with longer breeding seasons can be treated with two inoculations given over 4-5 weeks. Preliminary evidence indicates that the "standard" dose of PZP antigen (65 µg of antigen or about 5,000 zonae)
can be reduced to between 50%-90% and still generate contraceptive antibodies. From an economic viewpoint, this means that the cost of contraception can be reduced from the current $25/dose to somewhere between $2.50-$12.50/dose.

The concern over adjuvants arose primarily because of the possibility that Freund's Complete Adjuvant (FCA), which has previously been shown to be highly effective in generating high antibody titers, may lead to tuberculosis-positive test reactions in animals thus treated. Also, a small percentage of these animals produced small abscesses. A recent study of alternative adjuvants indicates that Freund's Incomplete Adjuvant (FIA), Carbopol® (Goodrich Co., Cleveland, OH), FIA = Quil-A (Superfos Specialty Chemicals, Vedback, Denmark) and DEAE-dextran (Sigma Chemical Co., St. Louis, MO) all produce contraceptive antibody titers when used with PZP vaccine. Muramyl dipeptide, and Ribi Adjuvant System did not produce contraceptive antibody titers.

Remote delivery is possible, but not all delivery systems are adequate because of the high viscosity of the antigen/adjuvant emulsion. The system which has provided the best results thus far is the Pneudart® 1.0 cc barbless dart (Pneudart, Inc., Williamsport, PA) fired from a Pneudart capture rifle, blowpipe or CO₂-powered pistol.

Long-term effects of the PZP vaccine upon ovarian function are not well understood and are the most important focus of the studies that are currently underway. While there is no evidence for debilitating side effects from PZP immunocontraception through 6 years of treatment, most available data indicate that developing ovarian follicles may also become the focus of action for anti-PZP antibodies, as well as ovulated ova, and that ovarian pools of oocytes may be depleted by prolonged use of the vaccine. Two species, rabbits and dogs, appear to have ovarian function disrupted even after short-term use; but there is no evidence of this in other species thus far. Reversibility of the contraceptive action of PZP after short-term use (1-2 years) has been documented in domestic mares. The majority of feral horses treated for 4 consecutive years demonstrated ovulatory cycles based on urinary estrone conjugates and behavior. At the Bronx Zoo a muntjac contracepted in 1991 produced a fawn in 1993, and at the Toronto Metropolitan Zoo, 3 of 6 tur contracepted in 1990 produced kids in 1993. At the Zoologischer Garten Koln, 3 banteng, which were contracepted in 1991 and which subsequently stopped demonstrating estrous cycles, were all cycling again in 1993. A protocol for the study of long-term effects of PZP contraception upon ovarian function and reproductive tract histopathology has now been developed and will be implemented in future studies. There can be no guarantee that unanticipated long-term effects will not appear, but annual updates of data will be provided to all participating zoos as this research progresses. We do not recommend that valuable animals in SSP breeding programs be treated with the PZP vaccine until the data base for long-term effects is broader.

Species treated as of June 1993 are given below. Those marked with asterisks have been treated successfully; for all others, data were not yet available at the time this abstract was prepared. These species included Przewalski's horse,* banteng,* sambar deer,* axis deer,* sika deer,* muntjac deer,* Himalayan tahr,* North American wapiti,* white-tailed deer,* West Caucasian tur,* African lion, tiger, black buck, Indian wolf, North American bison,
pygmy hippo, addax, ibex, onager, river hippo, impala, kudu, giraffe, waterbuck, fallow deer, aoudad, mouflon sheep, cougar, jaguar, bobcat, leopard, European brown bear, Kodiak bear, Asiatic black bear, Asian small-clawed otter. Contraception failed to be achieved in the river hippo but may have been the result of a lack of adjuvant or poor injection. Although contraceptive effectiveness has not yet been assessed, very high antibody titers have been achieved in the Indian wolf, African lion and tiger.
THE ROLE OF ZOO VETERINARIANS IN CAPTIVE CONSERVATION PROGRAMS

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Introduction

As recently as 1982, author Robert Bendiner noted that "Until the last decade, a resident veterinarian was the rarest animal to be found at the zoo." Fortunately, that situation has changed and increasing numbers of trained zoo veterinarians have played a growing role in the conservation of threatened and endangered species. Although still vital functions of veterinary medicine in the zoo, the treatment of individuals and attention to catastrophic epidemics (e.g., rhinotracheitis in nondomestic felids) has been supplemented with a renewed emphasis on herd health concepts applied on a national basis. The growth of Species Survival Plans (SSPs) and other Regional Species Conservation Plans (RSCPs) offers zoo veterinarians a remarkable opportunity to participate in the broader issues of disease and population management.

As these changes take place within the zoo community, the continued destruction of wild habitats results in dramatic declines in many wild populations. The effect of these changes is to bring the management of many small populations, whether they be captive or wild, closer together. For some species, e.g., the black-footed ferret (*Mustela nigripes*), the California condor (*Gymnogyps californianus*), the Arabian oryx (*Oryx leucoryx*), captive breeding has resulted in their survival. For others, such as the addax (*Addax nasomaculatus*) and the golden lion tamarin (*Leontopithecus rosalia*), captive groups now make up a significant portion of the total population. However, the ultimate reality of species conservation lies beyond the borders of any institution. The finite resources of zoological parks in a seemingly infinite world of endangerment prevent zoos from becoming "arks" for all (it has been estimated that zoological parks could manage 2000 vertebrate species). In reality, they may offer "lifeboats" to selected species that represent the tip of the sinking iceberg of diversity. Given those limited resources, it is critical that zoo veterinarians maintain their focus on the larger conservation picture that includes education and research, captive breeding and field conservation. Regional Species Conservation Plans offer a vitally important avenue for such opportunities.

Regional Conservation Management Plans

For captive conservation plans to work on a numerical, epidemiological, and genetic basis, it is necessary to manage numbers larger than the individuals of a species housed at any single institution. The level of cooperation necessary to achieve such an effort requires a heretofore unachieved level of coordination. In North America, the organization challenge resulted in the American Association of Zoological Parks and Aquariums' (AAZPA) establishment of Species Survival Plans (SSPs). These plans, now over 65 in number, manage North American populations as a region. They are the forerunners of similar plans.
established by zoological associations in Europe, Australia/New Zealand, India, Africa and South America - each area represented by an alphabet soup of appropriate initials. The term Regional Species Conservation Plans (RSCPs) is a generic term for them all.

The next step in this effort is the international coordination of the regional plans. Currently, they are supervised by the Captive Breeding Specialist Group (CBSG), a 300+ member committee of the World Conservation Union (formerly, the IUCN). Part of the Species Survival Commission of the WCU, the CBSG focuses its efforts on species in which captive conservation efforts are deemed promising. The "mechanics," eg, the identification and location of animals, is aided by another international organization, the International Species Inventory System (ISIS).

Although this report will focus on Species Survival Plans of the AAZPA, the principles are equally applicable to most RSCPs. SSPs are established under the auspices of the Wildlife Conservation and Management Committee (WCMC) of the AAZPA and derive their authority from that organization. Each SSP Committee is chaired by a Species Coordinator and members are elected from institutions holding the species in question. The Chair may appoint Advisors to the Committee as needed. Often, expertise in nutrition, reproduction, and medicine is solicited, and as Veterinary Advisors to these groups, herein lies one of the best opportunities for zoo veterinarians to contribute to the population management of a species.

With this opportunity comes all the problems associated with the medical management of small populations. Whether captive or wild, they offer unique challenges for veterinarians. O'Brien et al. have noted that the deleterious effects of inbreeding include the potential for altered resistance to disease. Dr. Linda Munson has succinctly noted that disease could prove to be a greater modifier of small populations in the future than the past because of greater habitat and genetic restrictions. The high morbidity of corona-viral infections in cheetahs (Acinonyx jubatus) may illustrate such a situation. It is a disease that may be aggravated by the species genetic homogeneity and like many other diseases, complicates the movement of animals between institutions for breeding purposes. In addressing these problems, the Cheetah SSP Committee has been a leader in applying an interdisciplinary approach: its research advisory group includes a nutritionist, a geneticist, clinical veterinarian, veterinary pathologist, reproductive physiologist and a behavioralist.

In the role of SSP Advisor, veterinarians have unique opportunities to serve as a central "clearing house" for medical information pertaining for a species. Obviously, causes for mortality and determining their epidemiology are vital statistics. The medical status of the black rhinoceros (Diceros bicornis) in the early 1980s illustrates the point. Isolated cases of hemolytic anemia had been noted, but review of the captive black rhinoceros populations and correlation of the data identified a syndrome that was the leading cause of death in captivity. Further review of the data linked isolated cases of fungal pneumonia, encephalomalacia and oral/skin ulcers into more readily recognized syndromes that could be addressed by focussed research efforts. Such correlations would have been difficult, if not impossible, to make without a central review of medical information.
Beyond the identification of disease, Veterinary Advisors are central to the determination of its etiology and prevention. Veterinary Advisors should review preventive medicine measures, e.g., vaccination, nutrition, management protocols, for a species and develop a preventive medicine program. In studying causes for disease, the recommendation of uniform testing standards, identifying clinical research needs, and establishing research projects are all vital. Often these lead to tissue collection protocols, and ideally, creation of central storage facilities for biological products of the species; all activities that can be coordinated by a Veterinary Advisor.

Preventive medicine is also the emphasis as some captive populations are being prepared for return to the wild. Preventive measures must be designed to prevent the spread of disease concomitant with the translocation (the same is true when wild animals are brought into captivity, or wild animals are translocated between habitats). As golden lion tamarins return to Brazil the problems of infectious disease and genetic defects must be addressed. In others, the issues may be more mundane, but no less important, e.g., it is critical that Arabian oryx returning to the Middle East be free of tuberculosis. Again, the information generated by Veterinary Advisors can be a critical reference regarding the prevalence of disease in captivity.

The need for Veterinary Advisors continues to grow, indeed one source suggests that 240 advisors will be needed by the year 2000 (presently there are approximately 100 full-time zoo veterinarians).3 Noting the increasing role of Veterinary Advisors to the SSPs, the Animal Health Committee of the AAZPA, in cooperation with the Infectious Disease Committee of the American Association of Zoo Veterinarians and a working group of the Disease and Reintroduction Conference held in 1992 in Oakland, California, has taken steps to describe the roles and responsibilities of Veterinary Advisors. A copy of that document is appended to this report. It is hoped that these guidelines will serve not only as guidance to those already serving in advisory roles, but also encourage SSP Chairs to appoint a Veterinary Advisor to each Committee. Additionally, an advisory group to the Animal Health Committee is being formed is being organized to identify and address ongoing issues that face Veterinary Advisors in SSPs and other regional conservation plans.

Summary

As the wild grows smaller and zoological institutions expand their horizons, the line between the wild and captivity grows more indistinct. The status of the black rhinoceros in Kenya illustrates the point. Black rhinoceros populations in large, open and poorly protected parks have been poached to near extinction, and the stable, surviving populations are housed in smaller, fenced and protected parks and ranches containing from 15-100 animals.5 Perhaps a quote discussing elephant populations best summarizes the situation for many species, "The distribution of human and elephant populations has changed from one characterized by human islands in a sea of elephants to increasingly small islands of elephants in a sea of people."4 In reality, whether it be the fencing around the rhinoceroses, or the sea of people around the elephants, the number of small populations and the significance of their management continues to grow. Thus the line between the historical approaches to "zoo"
and "wildlife"medicine is also blurring, as disease, nutrition, genetic and reproductive studies in zoological institutions and in the field become increasingly interchangeable. Active, thoughtful participation in Species Survival Plans and other RSCPs is one way zoo veterinarians can play a vital role in this larger mosaic of species preservation.

LITERATURE CITED

GUIDELINES FOR VETERINARY ADVISORS
TO REGIONAL CONSERVATION PLANS

As the role of Regional Conservation Management Plans (RCMPs) for species continues to grow, it is vital that veterinary input be standardized for the health of these protected species. At the present time, many of the North American Species Survival Plans (SSP) Coordinators have appointed Veterinary Advisors (see attached list); ideally each RCMP will identify a source of veterinary input and advice (in this report, SSP will be used, but the suggestions also apply to TAGs and FIGs). However, it is also advisable that the role of veterinary advisor be established and clarified via reasonable guidelines. The benefit of such direction would be two-fold: 1) it offers the SSP Coordinator a reasonable expectation of the role of a Veterinary Advisor, and 2) it would supply the Veterinary Advisor with an outline of basic standards that should be met, regardless of the species involved. It is hoped that AAZPA's Animal Health Committee could submit such proposed guidelines to its Wildlife Conservation and Management Committee (WCMC) for their consideration and inclusion in SSP protocols.

Commitment and Responsibilities of Veterinary Advisors
to Regional Conservation Management Plans

1. Institutional commitment:
   Each Regional Species Conservation Plan (RCMP) should designate a Veterinary Advisor for each species or taxa being intensively managed. The Veterinary Advisor should serve as a full member of the RCMP Committee and any reintroduction committees. The Veterinary Advisor must have a letter of commitment from his or her institutional employer supporting the nomination and approving the commitment to the RCMP.

2. Responsibilities of the Veterinary Advisor:

   A. Identify major medical problems in the species/taxa and pursue methods of diagnostic evaluation, treatment and prevention. Data collection, and diagnostic and treatment methods for particular disease problems should be coordinated through regional zoo and wildlife infectious disease committees.

   B. Identify scientific specialists in the areas affecting the health and well-being of the species and where possible, aid in the coordination of their efforts.
C. Customize the quarantine, movement, reporting, preventive medicine, accounting and necropsy protocols so they best suit the species in question. These data should be made readily available to the SSP Committee and holding institutions.

D. Facilitate centralized sera and tissue banking.

E. Propose testing requirements and recommendations for the prevention of disease transmission during interzoo movements as well as the reintroduction of species.

F. Provide regular reports (at least annual) to the RCMP Committee as well as the regional veterinary coordinator. These should include:
   - summary of deaths and their causes
   - description of significant illnesses (morbidity report)
   - results and availability of significant diagnostic tests
   - other significant medical activities

G. The Veterinary Advisor should review proposed research protocols and methods that pertain to his/her species and advise the RCMP Committee on the value and health risks posed by such proposals.

H. Advise the RCMP Committee on animal welfare as it pertains to movement, reintroduction, and/or breeding.

I. The Veterinary Advisor should cooperate closely with a nutritional specialist to encourage the use of a scientifically based system of dietary husbandry for captive animals.
THE IDENTIFICATION AND EVALUATION OF DISEASE WITHIN SPECIES WITH DESIGNATED SPECIES SURVIVAL PLANS

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Increased habitat pressure has lead to more extensive human management of wild and captive populations of animals. To various degrees, man controls the environmental factors to which these animals are exposed. The most effective and prudent role of a veterinarian working within a Species Survival Program (SSP) is the identification and evaluation of these factors relative to disease. This process should result in protocols to eliminate or reduce situations adversely affecting that species. In the broadest terms this would be the study of morbidity which can be defined as 1) a diseased state, ill health or 2) the result of exposing an organism or group of organisms to the causes of disease. Morbidity, as it relates to SSP's, can further be broken down into two categories: A) Syndromes which are the aggregate of signs, symptoms, or other manifestations considered to constitute the characteristics of a morbid entity and the term is usually used when the cause of the condition is unknown; and B) Disease which is a condition which alters or interferes with the normal state of an organism and is usually characterized by the abnormal functioning of one or more of the host's systems, parts, or organs. It may result from an inherent metabolic abnormality including congenital and hereditary defects and degenerative processes or from such factors as stress noxious stimuli, toxic agents, injury, or infection. A given disease is often manifested by a characteristic set of signs and symptoms although a host organism can be asymptomatic while having microscopic, serologic or immunologic evidence of disease.

Identification

The SSP veterinarian is responsible for the seemingly insurmountable task of identifying all of the possible causes of morbidity within a certain species. The sources of information are necropsy results, literature searches, surveys, serological screening, and prospective studies. Necropsy records accumulated through the years from zoos and other institutions that manage populations of that species need to be assembled and reviewed. The veterinarian should be concerned with both the wild and captive population and be aware of diseases that occur in either/or populations. The historical aspects of the species in captivity, can be obtained from the studbook keeper and the ARKS program. The detail and quality of the post-mortems will vary widely but in general, has improved in recent years. The importance of the completeness of a post-mortem and subsequent records will soon become apparent in that the animal may have several significant disease processes occurring as well as the cause of death. The literature search should include the morbidity of the wild and captive populations of concern as well as species that may transmit problems to the species in question when exposed in the wild or in captivity. Literature searches and necropsy results should reveal the most important and severe causes of morbidity.

However these sources may not expose the more chronic and less severe causes of morbidity in the population that, in the long term, may have significant impact on the viability of the species. These syndromes and diseases may be uncovered with management and viability
surveys sent to institutions housing the species asking about difficulties and concerns. The veterinarian may have to conduct a survey of serological tests of the wild and captive population to elicit the incidence of carrier states with no clinical signs, eg, herpes viruses in cranes and primates. Management changes and behavioral data should be examined for possible causes of poor population viability, such as the high infant mortality in many species.

On a prospective basis the veterinarian should customize quarantine, movement, preventative medicine, and necropsy protocols to promote the identification and surveillance of morbidity. Of essential importance is sufficient, meaningful documentation which includes permanent animal identification, to compile medical records and pedigree.

Evaluation

The causes of morbidity that are of the most concern are those that pose a significant threat to the long-term survival of the captive and/or wild population. One of the most potentially devastating situations is the exposure of the species of concern to similar species with diseases which are foreign and may be more pathogenic in their new host. The largest concern to reintroduction plans is that diseases artificially introduced in captivity will be transported back to the wild. The advent of zoogeographic regions in zoos as opposed to houses holding all similar species will reduce but not eliminate this potential. Not all causes of morbidity by definition are perilous. Prioritization must be attempted since financial and personal resources are usually limited. Recently, at the Captive Breeding Specialist Group conference at Oakland, the Captive Disease Monitoring Working Group modified the Mace/Lande criteria for assessing extinction threats for species.\textsuperscript{1} The purpose of this modification was to assess the threat of an infectious disease. The criteria must be applied for every infectious agent of the species under investigation. A further modification of the program is presented to include the wider definition of morbidity. The system is flexible enough to allow professional judgement of the veterinary advisor. The system needs periodic review, because of the emergence of new disease, changes in prevalence, new therapeutic and vaccine developments as well as the quantum leaps in diagnostic technology.\textsuperscript{1}
Morbidity Risk Assessment
I. Assessment Criteria

| Mace/Lande category of susceptible species | Endangered/critical
|                                          | Vulnerable
|                                          | Safe
| Incidence of Morbidity                   | High
|                                          | Low
|                                          | Affects multiple species
|                                          | Vertically transmitted
|                                          | Has asymptomatic carrier
| Prevalence of Morbidity in Population     | High
|                                          | Low
| Outcome of Morbidity                      | Usually fatal
|                                          | Causes permanent debility
|                                          | Rarely fatal
|                                          | Causes temporary debility
| Availability of Prevention                | Effective vaccine/therapeutic agents available; management/nutritional changes possible.
|                                          | No effective vaccine, treatment available, no nutritional/management changes possible.
| Diagnostic Tests                          | Reliable
|                                          | None/unreliable
| Public Health/Agricultural Concern        | High
|                                          | Low

If a species has been assigned to an SSP, it already has been designated as endangered or critical. Therefore the first category of the Mace/Lande criteria does not have to be included in the categories of morbidity risk assessment. For the purposes of this modification of the Mace/Lande criteria for the evaluation of morbidity, incidence is defined as the number of new cases arising after exposure while prevalence is the number of cases, old or new, existing within a population. In terms of infectious disease, incidence is synonymous with infectivity or virulence.
Morbidity Risk Assessment
II Categories of Risk

High Risk Morbidity:
A) is usually fatal or causes permanent debility, or
B) is rarely fatal or causes temporary debility but is of high incidence and prevalence, or
C) is rarely fatal or causes temporary debility but is vertically transmitted, or
D) is rarely fatal or causes temporary debility but has asymptomatic carriers and no diagnostic tests, or
E) affects multiple species and is of high incidence with no effective vaccine, treatment, or diagnostic test, or
F) is of public health or agricultural concern and has a high incidence.

Low Risk Morbidity:
A) is of low incidence and prevalence and causes temporary debility or is rarely fatal and has reliable vaccines therapeutics and diagnostic tests available, or
B) is of high public health or agricultural concern but low incidence or prevalence, or
C) is of low public health or agricultural concern.

Roadblocks to making an accurate and valuable evaluation of the morbidity are numerous:

1) the lack of sensitive and specific diagnostic tests,
2) the lack of standardization between laboratories and test interpretation,
3) the reluctance of zoological institutions to disclose existing problems or survey for possible problems,
4) the general lack of knowledge and epidemiology of many of the morbidity problems we face within an SSP,
5) the inability to readily transport biological specimens across national boundaries for diagnostic purposes,
6) the difficulty accessing recent information.

Once morbidity has been prioritized, resources can be channeled to those problem areas of high concern. Members of the wildlife and zoo field, affiliated universities, and the infectious disease committee of the AAZV should be approached to help clarify problems.

Risk evaluation of interzoo movement for SSP management benefits could be accessed utilizing a modified decision tree designed by the Risk Assessment Working group at the CBSG meeting for reintroductions.
Decision Analysis Tree for Risk Assessment for Interzoo SSP Movement

Is morbidity status of source and designation communities known?

NO

Conduct morbidity survey

YES

Is there a risk of a type 1 or 2 event?

YES

Is there a significant risk of type 1 event?

YES

Is the species' probability of extinction (or SSP failure) high if move is not made?

NO

STOP THE MOVE

YES

Develop a morbidity containment strategy

NO

PROCEED

Is there a significant risk of a type 2 event?

YES

Is there a significant risk of a detrimental type 2 event?

NO

PROCEED

PROCEED

NO

STOP THE MOVE

Type 1 event - morbidity occurs in the source animals but not in the destination community

Type 2 event - morbidity is common to both communities and there is concern of deleterious consequences from the disturbance of the dynamic relationship between the hosts and morbidity

Type 3 event - morbidity in the destination population only
The role of the SSP veterinarian is not only to identify and evaluate morbidity from a historical perspective, but document, monitor, and disseminate new emerging problems and solutions.

REFERENCES

DISEASE MONITORING IN CONSERVATION PROGRAMS BY PATHOLOGY SURVEYS

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Design of species or taxon conservation programs requires knowledge of the problems impeding preservation of the population. Disease is only one factor affecting population dynamics, but the impact of disease cannot be assessed without knowledge of the prevalent and serious diseases in the population. Furthermore, action plans to reduce the impact of disease on species survival cannot be initiated without this information. Not all SSP/TAG species will have disease problems requiring specific actions, but target species cannot be identified without preliminary surveillance. Then for the target species, diseases prevalence statistics can be used to assess if animal transfers should be restricted or if research resources should be invested.

When veterinary advisors are first assigned to American Association of Parks and Aquariums (AAZPA) Species Survival Plans (SSPs) or Taxon Advisory Groups (TAGs), they usually are disheartened by the shortage of published information on their assigned species. When Murray's book and Medline fail, they are left with the overwhelming task of acquiring this information from primary sources (clinical and pathology records). Substantial variation in the quantity or quality of these records can result in over or under-estimation of the prevalence of a disease. Furthermore, diseases that have been published may assume an importance that is out of proportion to the relevance of that disease to population survival. This also is true if serology or parasitology surveys are used as a measure of disease, because these data do not necessarily signify disease as much as exposure to an agent or a commensal relationship. Only through pathologic evaluation can the pathogenicity of an agent and true disease prevalences be determined.

Prospective pathology surveys are the optimal method of precise disease monitoring for SSP/TAG conservation programs because all animals that die can be systematically evaluated by the same method. Design and enactment of appropriate protocols for comprehensive pathologic evaluations will result in systematic recording of most clinical and subclinical diseases in the population within a few years. Information from retrospective analysis of clinical and pathology records then can be included with the prevalence statistics from the prospective study in a complete disease database for the SSP/TAG. Regardless of the impact of this survey on the subsequent SSP Action Plan, no disease surveillance in any species will be in vain, because any new knowledge will contribute to the enormous information shortage that currently exists for non-domestic animal disease.

SSP/TAG pathology surveys use epidemiologic methods

A disease database is built on information from individual animals, but disease concerns of a species are based on the prevalence statistics of epidemiology. The SSP/TAG veterinary advisor must focus on the predominant, fatal, or transmissible diseases in the population and
forego concern for individual animals unless their genetic contribution is indispensable. This represents a fundamental departure from our veterinary training.

For the pathologist, this focus on epidemiology indicates that determining the cause of death of each individual animal becomes secondary to screening all tissues for pathologic changes. A minor lesion in an animal may be significant to the population if this lesion represents one manifestation of a prevalent disease. For example, mild veno-occlusive disease (VOD) may be an incidental finding in an individual cheetah, but a significant addition to the high prevalence statistic for VOD in the captive population. For that reason, all gross or histopathologic lesions should be recorded by a pathologist regardless of the severity or relevance to a clinical condition and/or death of an animal.

Guidelines for optimizing data collection in SSP/TAG pathology surveys

A prospective pathology survey provides vital information for conservation programs and can contribute significantly to the science of zoological medicine. However, these goals cannot be met if certain guidelines are not followed. The rationale for these guidelines is that consistent collection of compatible data results in information that can be collated and shared through common information networks. Compliance with these guidelines exacts a minimum of additional time, while yielding a maximum of information for zoological medicine.

The guidelines are as follows:

1. Develop a customized necropsy protocol using the CBSG/AAZV and Canid TAG prototypes: one for captive animals and one for free-ranging animals.

2. Provide copies of the protocols to veterinarians at all facilities housing the species/taxon and to key field personnel who may have access to free-ranging animals.

3. Necropsy all animals that die and have all organs examined histopathologically.

4. Designate a pathologist who is experienced with the species or taxon to evaluate the tissues.

5. Report all data using the selected zoological pathology "dictionary".

6. Enter your data into a network-accessible database.

7. Provide reports of your findings to contributing institutions and to the AAZPA.
Necropsy protocols

For any research project, planned data collection using standard methods results in more useful information. In order to promote consistent biomaterials collection during disease monitoring, a "generic" necropsy protocol was designed for captive wildlife at the 1992 CBSG/AAZPA/AAZV meeting on "International Conference on Implications of Infectious Diseases for Captive Propagation and Reintroduction Programs of Threatened Species". This protocol is designed for use by clinical veterinarians, veterinary pathologists, or highly trained staff and presumes a certain level of anatomical knowledge. In contrast, a prototype protocol for free-ranging animals was devised for the 1993 Canid TAG Manual that included anatomical illustrations for performance of a necropsy by untrained field personnel. Protocols for both captive and free-ranging animals should be customized for your species or taxon to include unique anatomic features, disease problems, or research protocols deemed important for SSP goals. Avoiding complexity in these protocols will increase your compliance rate, as will inclusion of special media or fixatives, packaging materials, and addresses.

Provide these protocols and materials to key international personnel at zoos and wildlife preserves who can contribute tissue or information to a global disease network for your species. Acquisition of necessary permits for importation of tissues to the species pathologist will facilitate international compliance. Providing feedback for their efforts in the form of necropsy reports and disease summaries to the contributing facilities and parks will give them the incentive to continue the disease monitoring protocols. Attempts also should be made to interface with IUCN/SSC and other regional conservation disease-monitoring programs that affect your species of interest.

Communicate to all facilities or parks supporting your species or taxon that necropsy of all animals is essential, regardless of the state of decomposition or the need to determine the cause of death. If only selected individuals are necropsied, then prevalence statistics will be biased, and important subclinical diseases will be overlooked that could be contributing to infertility or morbidity in your species.

The role of the pathologist

Designating a specific SSP/TAG pathologist to analyze all tissues from your species will result in increased recognition of disease patterns in the population and simplify communication of important data. Although expertise with the particular species or taxon is preferred, this is not always possible with the current shortage of zoo/wildlife pathologists. Therefore, any experienced, well-trained comparative pathologist with an interest in the species and willingness to consult with other pathologists would be appropriate.

SSP/TAG pathologists should examine all tissues histopathologically during the first stages of the pathology survey until the important diseases are identified in the species in question. Besides conducting the prospective pathology survey, the SSP/TAG pathologist should review all retrospective pathology records and interpret the "pathspeak" for the SSP/TAG clinical veterinary advisor. Through their experience in interpreting tissue responses to injury, the
pathologist can determine if a disease may have a significant impact on a species or if that
disease likely is inconsequential. Comparative pathologists also can focus research efforts
by combining their observations of the SSP species disease problems with their
understanding of disease pathogenesis in other species.

SSP/TAG pathologists should agree to code all observed lesions regardless of clinical
importance and to use the coding methods and dictionary chosen by consensus for
MedARKS. This zoological pathology-based dictionary in under development by a
consortium of zoo and wildlife pathologists and will be available through ISIS/MedARKS
within two years.

Networking

Our knowledge of human and domestic animal disease is founded on decades of meticulous
pathologic studies correlated with clinical parameters. This work represents the consolidated
efforts of thousands of physicians and pathologists who shared their information through a
common medical language. In zoological medicine and pathology, we are still in our infancy,
but the learning process can be accelerated with contemporary information management
tools if the initiative is present among veterinarians to cooperate on this effort. Information
networks require compatibility to work, which is why standardized necropsy protocols and
pathology terminologies should be integral components of SSP/TAG disease monitoring
programs. Use of the MedARKS Pathology Module by all SSP/TAG pathologists and
veterinarians also would open new avenues of communication that will benefit all
participants by increasing our knowledge of wild animal diseases.

SSP/TAG pathology surveys can benefit global conservation efforts by networking with
disease monitoring systems globally. Currently, efforts are underway to obtain a consensus
amongst zoo and wildlife pathologists and veterinarians throughout the globe to develop a
single registry for wild animal disease. This universal wildlife disease database would permit
entry and extraction of epidemiological data from a network of zoos, wildlife parks, and
federal wildlife information banks. A wildlife disease reporting system has already been
initiated through the collaboration of the IUCN/SSC Veterinary Group and the pre-existing
IOE domestic animal surveillance program (M. Woodford, personal communication). The
cooperation of AAZPA SSPs and TAGs with this program would significantly enhance the
knowledge base of disease information in threatened species.

What zoos can do to assist SSP pathology surveys

1. Promote comprehensive necropsies of all animals and cooperation with SSP
   protocols.

2. Use a pathology data system that interfaces with global disease monitoring networks.

3. Initiate archiving of pathology specimens (wet tissues, blocks, kodachromes) and
develop serum banks.
4. Support research on important diseases affecting critical SSP species

5. Support fellowship programs in zoo and wildlife pathology to address the need for veterinary pathologists with expertise in non-domestic species.

Prototype SSP pathology surveys

Evidence that pathology surveys work is best presented by successful examples. Prototype programs have been designed for the maned wolf, golden lion tamarin (R.J. Montali) and for the cheetah (L. Munson). These programs were originally motivated by recognition that diseases were a significant component of captive management. This concern led to the design of comprehensive necropsy protocols by the SSP pathologist and the rapid accumulation of information on the prevalence of diseases in these species. These pathology surveys have been extended to include free-ranging species to determine if the disease identified in captive animals are unique to those populations or are inherent to the species. For example, cystinuria in maned wolves and diaphragmatic hernias in golden lion tamarins were identified in both captive and free-ranging animals, whereas veno-occlusive disease has been recognized only in captive cheetahs. This type of information will be essential for resolving critical disease problems in other SSP species in the future.

LITERATURE CITED

### WORKSHEET

<table>
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<td>Yes</td>
</tr>
<tr>
<td>Copy of Report Sent To RSCP Pathologist?</td>
<td>Yes</td>
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### HISTORY
(Include clinical signs, treatments, antemortem test results, diet, circumstances of death and quarantine status): ***PLEASE ATTACH COPY OF MEDICAL RECORD***
<p>| | |</p>
<table>
<thead>
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<tr>
<td><strong>General Condition:</strong> (Nutritional condition, physical condition)</td>
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<tr>
<td><strong>Musculoskeletal System:</strong> (Bone, joints, muscles)</td>
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<tr>
<td><strong>Body Cavities:</strong> (Fat stores, abnormal fluids)</td>
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<td><strong>Respiratory System:</strong> (Nasal cavity, larynx, trachea, lungs, regional lymph nodes, air sacs)</td>
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<td><strong>Cardiovascular System:</strong> (Heart, pericardium, great vessels)</td>
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<td><strong>Urinary System:</strong> (Kidneys, ureters, urinary bladder, urethra)</td>
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<td>Reproductive System: (Testis/ovary, uterus, oviduct, vagina, cloaca, penis, prepuce, accessory glands, mammary glands, placenta)</td>
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<tr>
<td>Endocrine System: (Adrenals, thyroid, parathyroids, pituitary)</td>
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<tr>
<td>Nervous And Sensory Systems: (Brain, spinal cord, peripheral nerves, eyes, ears)</td>
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**GROSS DIAGNOSIS:** (List each lesion separately. Include organ, lesion type, distribution, severity, etc.)

**LABORATORY STUDIES:** (List bacterial and viral cultures submitted and attach results, if available)
TISSUE COLLECTION LIST

TAKE DUPLICATE SETS OF TISSUES FOR THE REGIONAL SPECIES CONSERVATION PROGRAM PATHOLOGIST. Preserve the listed tissues in 10% buffered formalin at a ratio of 1 part tissue to 10 parts formalin. Tissues should be no thicker than 1 cm. INCLUDE SECTIONS OF ALL LESIONS AND samples from all tissues listed. For EMBRYOS OR NEONATES, also include the information in the NEONATAL PROTOCOL. Specific procedures for TISSUE SAMPLING are described on the last page of the protocol.

TISSUES TO SAMPLE: MAMMALS

<table>
<thead>
<tr>
<th>Heart</th>
<th>Liver</th>
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</thead>
<tbody>
<tr>
<td>Lungs</td>
<td>Stomach</td>
<td>Kidneys</td>
</tr>
<tr>
<td>Trachea/esophagus</td>
<td>Pancreas</td>
<td>Urinary bladder</td>
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<tr>
<td>Thymus</td>
<td>Small intestines</td>
<td>Uterus/Ovary</td>
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<tr>
<td>Thyroid/parathyroids</td>
<td>Large intestines</td>
<td>Testis/epididymis/prostate</td>
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<tr>
<td>Lymph nodes</td>
<td>Skeletal muscle</td>
<td>Brain</td>
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<tr>
<td>Spleen</td>
<td>Bone/bone marrow</td>
<td>Skin</td>
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TISSUES TO SAMPLE: BIRDS

<table>
<thead>
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<tbody>
<tr>
<td>Lungs</td>
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<td>Kidneys</td>
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<tr>
<td>Trachea</td>
<td>Proventriculus</td>
<td>Bursa of Fabricius</td>
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<tr>
<td>Air sacs</td>
<td>Ventriculus</td>
<td>Skin with feather</td>
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<tr>
<td>Thymus</td>
<td>Small intestines</td>
<td>Testis/ovary</td>
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<tr>
<td>Thyroid/parathyroids</td>
<td>Large intestines</td>
<td>Oviduct</td>
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<tr>
<td>Spleen</td>
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<td>Skeletal muscle</td>
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<td>Brain</td>
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TISSUES TO SAMPLE: REPTILES & AMPHIBIANS

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<tr>
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<td>Testis/ovary</td>
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<tr>
<td>Thyroid/parathyroids</td>
<td>Large intestines</td>
<td>Oviduct</td>
</tr>
<tr>
<td>Spleen</td>
<td>Skeletal muscle</td>
<td>Brain</td>
</tr>
<tr>
<td>Bone/bone marrow</td>
<td>Skin</td>
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ESSENTIAL FROZEN TISSUE: Please store 10 grams (if possible) of liver, brain, kidney in plastic bags and antemortem serum and plasma at -70 C.

SHIPPING TISSUES:
After at least 72 hrs in fixative, ship tissues in a leak-proof container with enough formalin to keep tissues moist. Tissues can be shipped by mail or other carrier to the Regional Species Conservation Program pathologist. FOR INTERNATIONAL SHIPMENTS, INCLUDE ALL NECESSARY PERMITS.

NEONATAL NECROPSY PROTOCOL

Follow the adult protocol. Also, include the following:

1. Fix umbilical stump and surrounding tissues
2. Examine of malformations (cleft palate, deformed limbs)

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3. Assess hydration (tissue moistness) and evidence of nursing/eating (food or milk in stomach).

4. Determine if breathing occurred (do the lungs float in formalin?)

For embryos: Open the coelomic cavity and fix the entire embryo. Include egg shell and membranes if available.
RECOMMENDED TISSUE SAMPLING PROCEDURES: MAMMALS

Adrenal glands: Entire gland with transverse incision

Brain/Pituitary gland: Sliced longitudinally along the midline.

Gastrointestinal tract: 3 cm long section of esophagus, stomach (cardia, antrum, pylorus), duodenum, jejunum, ileum, cecum, and colon. Open carefully along the long axis. Cross-section of tongue.

Heart: Section including atrium, ventricle and valves from right and left heart.

Kidneys: Section from both kidneys (cortex, medulla, and pelvis)

Liver: Sections from 3 lobes including capsule and gall bladder

Long bone: Submit 1/2 of a femur including growth plate.

Lungs: Sections from several lobes including a major bronchus

Lymph nodes: Cervical, anterior mediastinal, bronchial, mesenteric, and lumbar with a transverse cut.

Pancreas: Representative sections from two areas

Reproductive tract: Entire uterus and ovaries with longitudinal cut into lumen. Entire testis with transverse cut, entire prostate with transverse cut

Skeletal muscle: Cross section of thigh muscles

Skin: Full thickness of dorsal skin

Spleen: Cross sections including capsule

Thymus: Representative section

Thyroid/parathyroids: Leave glands intact

Urinary bladder/ureter/urethra: Cross section of bladder and 2 cm sections of tubular structures
FIELD NECROPSY PROTOCOL

The field necropsy usually is less detailed or necessity than those performed under ideal conditions in zoos. Rapid tissue harvesting is essential to minimize tissue autolysis. FIX THE REQUESTED TISSUES EVEN IF AUTOLYZED, because evidence of infectious disease may still be evident histopathologically. Tissues should be collected and fixed in 10% buffered formalin. They then can be stored indefinitely until sent to a pathologist. Histopathological analysis should be performed by veterinary pathologists familiar with the diseases of canids. Dr. Linda Munson, the AAZPA Canid TAG pathology advisor, is willing to provide this service free of charge. If sent to another laboratory, the results should be reported to Dr. Munson for entry into the Canid Disease Databank.

TO SHIP TISSUES (FORMALIN FIXED ONLY), PLEASE CONTACT DR. MUNSON FIRST SO THAT PROPER IMPORT PERMITS CAN BE ARRANGED:

Dr. Linda Munson  
Dept. Pathobiology  
College of Veterinary Medicine  
University of Tennessee  
2407 River Drive  
Knoxville, Tennessee 37996  
USA  
(615) 974-8235  
Fax (615) 974-5616

No one should handle a canid carcass unless they have been vaccinated against rabies. Always wear gloves when performing a necropsy!

SEND SAMPLES OF ALL DISCRETE LESIONS. ALSO SEND THE FOLLOWING TISSUES (IN ORDER OF PRIORITY):

Brain  
Lungs  
Small intestines  
Kidney  
Liver  
Lymph nodes  
Spleen  
Reproductive tract  
Large intestines  
Adrenal glands  
Urinary bladder  
Pancreas  
Heart  
Stomach

316  
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Please protect yourself from infectious agents by wearing gloves (double set) and a mask. Be cautious with sharp instruments and avoid spraying tissue fluids.

Please fix tissues in 10 percent buffered formalin at a ratio of 10 parts formalin to 1 part tissue. Tissues should not be thicker than one-half inch or they will not fix. Please place in a leak-proof container. Ship tissues by standard mail or UPS to:

Dr. Linda Munson  
Dept. Pathobiology  
College of Veterinary Medicine  
University of Tennessee  
2407 River Drive  
Knoxville, Tennessee 37996  
USA  
(615) 974-8235  
FAX (615) 974-5616

Tissues requested are highlighted in black and designated by arrows:

1. Lung

2. Heart

3. Liver
4. Spleen

5. Pancreas

6. Adrenal gland

7. Kidneys

8. Urinary Bladder
9. Reproductive Tract

Entire tract for females

Ovaries

Uterus

Female

Male

10. Brain

Fix one-half brain

Freeze one-half brain

11. Lymph Nodes along the aorta or near where the trachea attaches to the lungs

12. Gastrointestinal Tract

(Same as for pancreas)
PREVENTIVE MEDICINE CONSIDERATIONS FOR INTERZOO ANIMAL MOVES

C. Douglas Page, DVM*
Jacksonville Zoological Park, 8605 Zoo Road, Jacksonville, Florida 32218, USA

Introduction

Presently there are 63 Species Survival Plans (SSP), the majority of which use veterinary advisors to assist in establishing protocols for those aspects of husbandry related to medical management. Examples of these include protocols for vaccination, anesthesia, necropsy, and other areas of preventive medicine. As more of these programs are established, veterinarians will be called upon to participate as advisors. Veterinarians must prompt this involvement by stepping forward and offering their expertise.

Paramount to the successful transfer of a healthy specimen to another zoo is the recognition of disease processes that may be occurring in the species. This requires the practice of sound diagnostic medicine integrated with other disciplines such as pathology, parasitology and nutrition. Once a disease process has been identified, an effective antemortem diagnostic tool needs to be established and incorporated into the health screen. This may present the greatest hurdle for those involved in assessing the health of an animal prior to its transfer.

Establishing a minimal health screen

As a minimum, the pre-shipment health screen should include the signalment (age, sex, origin, and genealogy) and anamnesis. The anamnesis should provide information on past disease problems, tests performed to survey for specific diseases, and husbandry practices. Additionally, a complete physical exam, including an intestinal and external parasite screen, should be performed. A complete blood count, evaluation of serum chemistries with serum banked, screening for hematozoans, and fecal cultures for enteric pathogens may also be necessary depending on the species involved.

For example, in the recommendations for the interzoo movement of elephants,4 banked serum is forwarded to the receiving institution prior to the transfer of the animal. This enables the receiving zoo to perform additional tests if necessary, and to conduct future epidemiology studies should disease occur following the transfer. Additionally, three negative fecal cultures are required prior to shipment of an elephant due to the occurrence of salmonellosis in these species.

Vaccination protocols and parasite prevention, or control programs, have been established for some of the SSP species. The appropriate husbandry manual should be consulted so the most effective method of prophylaxis or treatment can be instituted. Prior to shipment, communication with the receiving institution regarding tests or procedures requested may expand the list of the pre-shipment health screen.
Specific health screening

Additional tests will be required for those species where specific disease processes have been identified, and methods of surveillance are available. By consulting the literature and/or the husbandry manuals, the appropriate testing procedures can be employed. The following are examples of specific disease concerns for various species.

Atoxoplasmosis has been identified as a cause of fledgling mortality in the Bali mynah, *Leucospar rothschildi*. Research is being conducted to evaluate means of treatment and control of this organism. The potentially devastating effects of this parasite are realized for the captive population, and for those specimens utilized in the reintroduction program.

The maned wolf, *Chrysocyon brachyurus*, is plagued with cystinuria. Diagnosis can be achieved with a complete urinalysis, cyanide nitroprusside test, radiography and ultrasound. Specific treatment with the disulfide exchanging drug, thiola, and urinary alkalinizing agents is promising. Protocols have also been established for vaccination, parasite prevention and necropsy of this species.

The ophidian paramyxovirus is a threat to the captive population of Aruba Island rattlesnakes, *Crotalus unicolor*. A recent epizootic in a zoo killed 4 of these snakes and affected 24 other species. Hemagglutination inhibition (HI) has been used to detect antibody titers to this virus. The presence of antibody suggests recent exposure, but does not confirm active infection. Ongoing research is directed towards prevention and serologic diagnosis of this disease.

Problem areas

As mentioned earlier, the actual monitoring and surveillance of disease in captive animals can present problems. Diagnostic tests may be unavailable, or the results of the test may be equivocal. Some of these problem areas are considered below.

The accurate diagnosis of tuberculosis continues to evade the zoo veterinarian. Additionally, pre-shipment surveillance of an animal for this disease may well result in more questions than answers. This is true in elephants, hoofstock, many species of primates, marsupials, and avian species, to name a few.

The intradermal skin test for TB is known to produce false positive reactions in certain species such as orangutans and Asian elephants. One of the problems we face with this diagnostic test is the lack of standardization in its application. Before we can determine the validity of a test, we must first eliminate the variables that exist. For example, for a given species, we must all use the same site for injection, and the same dose and strength of test antigen. After we have standardized the test, we must then develop a means to substantiate the results. The search for the latter has superseded the former, thus leading us to investigate ELISA tests, lymphocyte transformation, and DNA probes in an effort to identify TB positive animals.
In avian species, perhaps the most legitimate screen for TB involves a CBC, radiographs and a liver biopsy on the specimen(s) in question. Additionally, and not unlike *Mycobacterium paratuberculosis* in hoofstock, the history of the incidence of TB in the flock over the past 5 years is a good indicator of a specimen's TB status prior to its shipment. This last item exemplifies the need for accurate standardized record keeping, and a thorough anamnesis as part of a pre-shipment health screen.

For other diseases, another problem we face is interpreting the results of a true positive test, and applying the results to captive management and interzoo transfers. Specific examples involve the hoofstock with an antibody titer to malignant catarrhal fever (MCF), and the felid that tests positive for feline immunodeficiency virus (FIV). What do these results mean, and how do we use them to effectively manage a species? Does the positive test correlate with clinical disease, or the potential thereof? The extrapolation of disease surveillance data from one species to a closely related species can be an effective tool, however, we must be cautious in our interpretations of the test results for that species. Certain tests may be too sensitive for our needs, and may be as detrimental to a captive breeding program as is the lack of an effective test for the surveillance of a specific disease.

**Conclusions**

The successful transfer of a healthy specimen from one zoo to another requires communication and cooperation between the two institutions. Disease processes must be identified, and tests for surveillance of the disease must be established and utilized. An interdisciplinary approach to problem solving is required if we are to continue to improve upon existing, and develop new, aspects of preventive medicine for the many species within zoos. A commitment to actively participate in SSP programs, and to collaborate on research efforts with colleagues is critical to the advancement of the veterinarian's role in conservation programs.

**LITERATURE CITED**

Reintroduction of captive animals to the wild is currently receiving considerable attention. Several species reared in captivity have been or are currently being reintroduced to the wild including the golden lion tamarin (Leontopithecus rosalia), black-footed ferret (Mustela nigripes), red wolf (Canis rufus), Arabian oryx (Oryx leucoryx), scimitar-horned oryx (Oryx dammah), Pere David's deer (Elaphurus davidianus), whooping crane (Grus americana), Bali mynah (Leucopsar rothschildi), California condor (Gymnogyps californianus), Andean condor (Vultur gryphus), thick billed parrot (Rhynchopsitta pachyrhyncha), Virgin Island boa (Epictrates monocellatus) and many others. Preventive medicine has become a vital part of reintroduction programs. 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 and prevent the introduction of diseases into the area's indigenous animal or human population. This discussion will outline preventive medicine issues that should be developed and tailored to the specific species considered for reintroduction. The author has served as veterinary advisor for the Bali mynah (Leucopsar rothschildi) Species Survival Plan (SSP) for the past 3 years and many examples utilized in this discussion come from this experience.

In the initial planning stages of a reintroduction project, a disease and medical problem preventive medicine program should be developed. Protocols should be continually re-evaluated and updated as more information becomes available. The Bali mynah reintroduction program has been ongoing since 1985. Minimal medical screening was performed prior to the Bali mynah releases in 1988 and 1990. Shortly after the first release, all the birds died or could not be located. These disappointing results were attributed to releasing the birds during the dry season, releasing the birds too far from any existing roost sites, and releasing too few birds (three). The second release was much more successful, because the birds were released during the wet season, the locality of the release was very near the only remaining regular Bali mynah roosting site, and a greater number of birds (thirteen) were released (Pers. Comm., Seibels). At the time of this writing a third release is being planned on a small island off the coast of Bali. It is unknown whether the birds will fly to Bali and join the current wild flock or stay on the island. There have been no Bali mynahs sighted on this island to date. In 1990, the wild Bali mynah population was estimated in the range of 12 to 18 birds. Most recent estimates are in the range of 55 to 61 (Pers. Comm., Seibels).

The first step in developing a preventive medicine program for a reintroduction project is to define the diseases in captivity of the species to be reintroduced. Veterinary assistance is critical in defining medical problems in captivity. Many SSPs have recently added veterinary medical advisors to assist the propagation group in the overall management of SSP animals. The veterinary advisor can act to coordinate other specialists to assist in medical evaluation and management. The cheetah SSP is an excellent model to use when
developing a team of health and husbandry specialists to evaluate a captive population of a particular species. The Florida panther project is likewise an excellent model for disease evaluation of a wild population of a particular species. A pathologist should be consulted in the development of a necropsy protocol and assisting in the evaluation of health problems of a particular species. Dr. Kenneth Latimer, a pathologist at the University of Georgia, assisted in the preparation of the US Bali mynah necropsy protocol, and performs the histopathology on all Bali mynah deaths at no charge. This provides more uniform necropsies and has been the most useful diagnostic tool in defining diseases in the US Bali mynah population. A necropsy protocol was developed for Indonesian zoos and the Pre-release Training Center (PTC) in Bali and was translated into the local Indonesian language (Bahasa). Vials of 10% formalin were given to each Indonesian zoo housing Bali mynahs along with the protocol. Permits have been obtained to bring formalinized tissues to the US for histopathology. At the time of this writing, it is too early to assess the effectiveness of this effort.

The Bali mynah SSP has also received considerable assistance from Dr. Ellis Greiner, a parasitologist from the University of Florida. Atoxoplasmosis is now known to be widespread throughout the US and Indonesian captive Bali mynah populations. It is currently thought to be the most serious infectious disease in captive Bali mynahs. Dr. Greiner's expertise has been utilized to define the extent of the atoxoplasmosis problem and evaluate the effectiveness of therapy. There are other infectious diseases which impact the captive and wild Bali mynah populations, but these are less common than atoxoplasmosis and have not been well documented. These include chlamydiosis, newcastles disease virus (endemic to Indonesia), toxoplasmosis and salmonellosis. Some examples of infectious diseases and medical problems that have been of concern to some other reintroduction programs include eastern equine encephalitis, Mycobacterium avium, disseminated visceral coccidiosis, inclusion body disease, and mycotoxicosis in the whooping crane; callitrichid hepatitis virus, Pterygodermatites nyciticebi, intestinal campylobacteriosis, and a familial defect of the diaphragm in golden lion tamarins; bovine tuberculosis in Arabian oryx; malignant catarrhal fever in Pere David's deer; canine distemper in the black-footed ferret; to name a few. Recommendations for infectious disease screening in the various animal groups are listed in Table 1.

Surveys can be developed to define past and present medical problems. They should be written so that the data can be easily utilized and computerized.

The whooping crane recovery program recently held a health management workshop in Madison, Wisconsin in March of 1992 to develop standardized management protocols and to define and prioritize disease research needs. Participants included researchers and clinical veterinarians involved with the whooping crane. A protocol manual entitled "Whooping Crane Health Management Workshop" was developed from the meeting and is an excellent model to be utilized when considering reintroduction for other avian species.

Assessing the impact of a particular medical problem on the wild population is essential. However, this can be very difficult when the release program is in an underdeveloped country and deals with an endangered species. With only 50 Bali mynahs remaining in the
It is extremely important to carry out regular disease monitoring of a population after a release. A disease surveillance protocol should be tailored for the reintroduced species. If possible, selected animals should be physically restrained or immobilized at regular intervals and appropriate samples taken for analysis. Appropriate physical restraint and/or anesthetic protocols should be developed. Facilities should be available for field storage of biomedical samples. Field necropsies are often not feasible due to inability to locate the carcass, rapid autolysis of the carcass, and lack of medical expertise in the field. If telemetry is utilized in the reintroduced animals, carcasses may be recovered in a more timely manner. A good example of this is the documentation of a panleukopenia epizootic in wild bobcats (*Felis rufus*) in Southern Florida, where 11 of 18 radio-collared animals died in a 3 month period. Furthermore, over a 10 year period, 16 radio-collared Florida panther (*Felis concolor coryi*) carcasses were recovered and received a thorough postmortem revealing the cause of death of each animal. Without radiotelemetric technology the carcasses would not have been recovered. In future Bali mynah releases, telemetry will be utilized to monitor the released birds. Field biologists should be trained on gross necropsy techniques and sample collection. Permanent records (preferably computerized) should be developed for all aspects of the reintroduction project.

Evaluating the diseases of other species in the release area may also be of benefit to the overall knowledge of infectious disease exposure to the released species. Researchers involved with the black-footed ferret reintroduction project have surveyed for diseases in the release area concentrating on the primary predator species, coyotes (*Canis latrans*) and badgers (*Taxidea taxus*), because of their presumed importance to the epizootiology of canine distemper. The black-footed ferret's primary prey species, the white-tailed prairie dog (*Cynomys leucurus*), were also studied because they are the major host of sylvatic plague (*Yersinia pestis*). Eastern Equine Encephalitis (EEE) virus is of major concern to the whooping crane, which is being reintroduced into Florida. The sandhill crane (*Grus canadensis*) is abundant in Florida and is susceptible to similar diseases to the whooping crane, thus it is being used as the animal model for the whooping cranes. The black-winged starling (*Sturnus melanopterus*) is an abundant species closely related to the Bali mynah with an overlapping range, and thus may be a good model for diseases that occur in the wild Bali mynah population. The feasibility of utilizing this species is currently under investigation.

Domestic animals in the release area are another potential source of infectious disease to the reintroduced species. A small village currently resides in the Bali Barat National Park very close to the Pre-release Training Center. Back yard chickens are abundant in this village and a serious threat to the Bali mynahs at the PTC and the wild population of Bali mynahs. Efforts are being made to relocate this village, but this is obviously a very politically sensitive issue. The threat of the released species transferring diseases to domestic stock is often a concern and may be politically detrimental to a release project.
The potential diagnostic laboratories to be used for screening animals in a reintroduction project for infectious diseases and general health should be visited and evaluated by the veterinary advisor. Simply sending samples to a laboratory which claims to test for a particular disease may prove to be a waste of finances, because the results may be misleading, difficult to interpret, or unreliable. The lack of veterinary medical expertise on Bali and throughout Indonesia has made the development of a disease screening protocol for the Bali mynah very difficult. There are very few veterinary or medical laboratories capable of performing the necessary screening tests. The author visited all the zoos in Indonesia housing Bali mynahs in an effort to define the diseases in captivity, train veterinarians and other staff members on basic principles of avian medicine, and develop relationships with Indonesian researchers and laboratories. Involving the native people in reintroduction projects enhance local support and awareness and is essential for a successful program.

When accurate diagnostic testing is unavailable, treatment of certain diseases may be an alternative. For example, a 45 day doxycycline therapy can be utilized to treat chlamydiosis and potentially substitute for screening tests. Immunizations have been used in selected reintroduction programs, usually against pathogens that the species is susceptible to and that are endemic or epidemic in the release area. A few examples include vaccination of the whooping crane against EEE virus, the black-footed ferret against canine distemper virus, and the Florida panther (vaccinated the wild population) against rabies virus, upper respiratory virus, and feline distemper virus.

Another important aspect of developing a reintroduction preventive medicine program (especially in underdeveloped countries) is being familiar with the captive breeding facilities. The animal health history of the supplying institution or individual owner's premises should be studied, together with the incidence and prevalence of local and regional enzootic animal diseases. This has become quite complex when dealing with Bali mynahs. Most Bali mynahs released back to the wild thus far have been born in Indonesia at the Surabaya Zoo in Java. A separate breeding facility, partially funded by the AAZPA, at the Surabaya Zoo was built in 1987. There is no public access to this facility. The birds are housed as individual pairs and egg production has been excellent. Unfortunately, there has been a very high chick and fledgling mortality rate. Atoxoplasmosis is suspected to be the cause of the high mortality, because Isospora spp. oocysts (consistent with atoxoplasmosis) were found in the feces of a very high percentage of Bali mynahs at this facility. The preventive medical care and husbandry at the zoo are in need of improvement. In the past, the birds to be released have gone directly to the Pre-release Training Center (PTC) from the Surabaya Zoo without being quarantined. The PTC is designed to provide natural vegetation and diet with minimal human contact. A quarantine facility is currently being constructed on Bali in the Bali Barat National Park. This facility will be utilized to quarantine all birds prior to entry into the PTC. The design of a quarantine facility and procedures should be effective from a medical standpoint, but also designed to minimize injury to the animal (i.e. broken flight or tail feathers in a bird may significantly delay the timing of the release). Obviously, it is recommended to build quarantine facilities prior to the start of the reintroduction project. The length and conditions of the quarantine period will depend on the species to be released and the specific local disease risks which the quarantine is designed to eliminate.
(i.e. if *Mycobacterium tuberculosis* or *M. bovis* are deemed a hazard then appropriate testing protocols and isolation should be developed, if bluetongue virus is of concern then the quarantine facility should be mosquito proof). ¹⁴

Eight birds from the Surabaya Zoo are currently being housed at the PTC and have been there for well over a year. Physical examinations and diagnostic samples were collected from these birds by the author. The birds were in excellent health except they were all shedding *Isospora* spp. oocysts consistent with atoxoplasmosis in their feces. A researcher has received funding to place radiotelemetry devices on these birds and monitor them post-release. He is scheduled to start his project in April of 1993, thus it is important that the release take place in a timely manner. A pre-release treatment and diagnostic protocol has been developed for these 8 birds (Table 2). Confining integrated social groups of animals to be reintroduced in a large enclosure in the area near or within the release site for several weeks to months before a release is a very important part of any release program. ¹⁴ These birds will be taken to an island off the coast of Bali and placed in a large pre-release enclosure for an acclimation period and then released. Following this release, another group of birds from the Surabaya Zoo will be sent to Bali. A separate quarantine protocol has been developed for these birds (Table 2).

A source of birds for both captive breeding and potential release is the Amnesty Campaign. This program, administered by the Indonesian government, allows citizens who are illegally holding wild-caught Bali mynahs to register them without fear of retribution. The Bali mynah is a status symbol in Bali and throughout Indonesia and is kept in captivity for its beauty and unusual vocalizations. All of these birds are wild caught and very valuable from a genetic standpoint. Genetically unimportant birds from the US have been shipped to Indonesia to be exchanged for the privately-owned wild caught birds. Pre-shipment screening protocols have been developed for US zoos (Table 5). The birds come from a variety of US zoos and are held at the Los Angeles Zoo prior to shipment to Indonesia. Approximately 80 wild caught Bali mynahs currently kept as pets have been identified for exchange. Most of these birds will be used for captive breeding and their offspring will be released. The birds will be distributed to various Indonesian Zoos and to the PTC. The author examined several of these birds at the owner's homes in Denpasar, Bali. A high percentage of them had *Isospora* spp. oocysts in their feces consistent with atoxoplasmosis. Ataxoplasmosis organisms were also found in the monocytes from a plain blood smear from 1 young bird. Unfortunately, most of these birds are housed under suboptimal conditions and have been exposed to a variety of pathogens. Many of them were probably in the bird market at one time during their stay in captivity. These markets are stressful and overcrowded, with a variety of avian and mammalian wildlife. Because of the additional disease risks, a separate quarantine protocol has been developed for these birds (Table 2). Quarantine and preventive medicine guidelines have been distributed to all the zoos in Indonesia housing Bali mynahs. It is very important that all protocols are presented in the native language.
The animals to be released must be identified in some manner (i.e. ear tags, tattoos, bands, or transponders) so that individual recognition and recording are possible. Transponders have been placed in all released Bali mynahs. A Bali mynah recovered from poachers was identified to be a released bird by reading its transponder.

Communication is not only one of the most important parts of developing preventive medicine protocols for a reintroduction program, but for the success of the entire program. The California and Andean condor projects have had extensive veterinary input from multiple individuals and have been successful primarily because of good communication between veterinarians, field biologists, curators, etc. The medical aspects of the Bali mynah reintroduction project are still evolving. It is becoming apparent that communication at all levels is crucial to the success of the medical portion of the program.

The initial drafts for preventive medicine protocols for the Bali mynah release program were ideal protocols which did not take into account limitations in funding, facility space, lack of diagnostic laboratories, educational background of personnel involved with the project, and politics. Hopefully using examples of the successes and the failures of the Bali mynah reintroduction project have given some insight into the development of a preventive medicine program for a release project. Future reintroduction programs should develop medical management strategies prior to releasing captive animals to the wild.

LITERATURE CITED

Table 1. General recommendations for infectious disease screening in the various animal groups.

Ruminants
- Tuberculosis, brucellosis, bovine viral diarrhea, epizootic hemorrhagic disease, and bluetongue virus, rinderpest, salmonellosis.

Equids
- African horse sickness, Babesia equi and caballi, dourine, glanders, equine infectious anemia, equine herpesviruses and equine viral arteritis, salmonellosis.

Suids
- Swine fever, African swine fever, vesicular diseases, pseudorabies, Erysipelas, salmonellosis.

Canids, Mustelids, Procyonids, Viverrids
- Rabies, canine distemper, parvoviruses, salmonellosis.

Felids
- Rabies, parvovirus, Feline immunodeficiency virus (FIV), Feline leukemia (FeLV), salmonellosis.

Primates
- Salmonella, Shigella, Campylobacter, Mycobacterium spp., variety of viral and parasitic organisms depending on the primate species.

Avian
- Newcastles disease virus, avian influenza, herpesviruses, Salmonella, Chlamydia psittaci, psittacine beak and feather disease.

Table 2. Bali starling (Leucopsar rothschildi) Quarantine at Pre-release Training Center (PTC)

1) Birds currently in PTC
   a) Body weight and physical exam (preferably by a veterinarian, otherwise Bas or Mark)
   b) The bird must be eating well, have no noticeable abnormalities, normal feces and urates
   c) Screen for Newcastles Disease Virus
   d) Screen for salmonella (3 samples at least 1 week apart preferred, minimum of 2 samples, can use fresh feces, culture center of fecal sample to avoid contamination).
   e) Treat with Ivermectin and Droncit, use dosages on treatment sheet.
   f) Treat birds for atoxoplasmosis as previously described.
   g) Place birds out on Island 20 days into atoxoplasmosis treatment.
   h) Collect fresh fecal samples in Potassium dichromate as previously described.
   i) Send samples by express mail service or identify person to hand carry into US. If prior to July 1, the current permit is sufficient, otherwise it will need to be renewed.
2) White Wash Campaign birds

a) If possible screen for newcastles disease virus prior to acceptance into release program.

b) These birds must be quarantined in new quarantine building.

c) Upon arrival, obtain a weight, perform a physical exam (Mark or Bas), if obviously unthrifty the bird should not be placed at the PTC, send to Taman Safari for more strict quarantine.

d) Give ivermectin and droncit using doses on treatment sheet at the beginning of quarantine.

e) Acclimate for a few days, then start on atoxoplasmosis treatment protocol. Start feeding food item to be used for medication upon arrival (ie grapes, mealworms).

f) Enclosures should be cleaned daily utilizing an appropriate disinfectant (ie chlorox diluted 1:40 with water), the disinfectant should be rinsed with fresh water.

g) If animals are being kept in the PTC, the quarantine animals should be cleaned and fed after the PTC. Gloves should be worn (or at least handwashing with an antiseptic soap between each exhibit), a pair of rubber boots should be designated for quarantine, a foot bath with a disinfectant should be present in entry into quarantine (do not walk in quarantine with bare feet).

h) Samples should be collected for newcastles disease virus, *Chlamydia psittaci*, and 3 salmonella fecal cultures during the first 30 days of quarantine.

i) After the 30 day treatment for atoxoplasmosis, rest 1 week (during this time give a second dose of ivermectin and droncit).

j) If *Chlamydia psittaci* diagnostic tests are unavailable, the birds should be treated with a 45 day course of doxycycline at 50 mg/kg (approximately 5 mg) once daily in a preferred food item. Nystatin (100,000 units/ml) should be utilized at this time to prevent secondary yeast overgrowth at a dose of 30,000 units (or 0.3 ml) once daily.

k) During the *Chlamydia psittaci* treatment, 3 negative fecal direct and flotations should be performed (these samples should be collected 1 week apart), also samples should be collected for atoxoplasmosis screening using the previous protocol.

l) Birds should be brought into quarantine at approximately the same time period. If a new bird comes into quarantine, all other birds in quarantine will start over again. All birds should be released from quarantine at approximately the same time.

m) A prerelease physical exam and weight should be performed (preferably by Dr. Norton during his trip in late August).

n) Birds will then be placed either in the PTC for breeding and/or designated for eventual release.

o) Birds must be eating well, have no noticeable abnormalities, normal feces and urates prior to release from quarantine.
3) Surabaya Zoo (KBS) birds

a) Select only thrifty birds, preferably birds examined previously by Dr. Norton.

b) If possible perform weight and physical examination prior to leaving the Surabaya Zoo. Ivermectin and droncit can be given at this time (it would be nice if Bas or Mark could be present for the exam).

c) Test birds for newcastles disease virus preferably prior to leaving the Surabaya Zoo; also 3 negative salmonella cultures (at least 1 culture).

d) It is preferable that these birds go into quarantine rather than the PTC, this will depend on the time frame-no exposure should occur between current PTC birds, WW birds, and Surabaya birds. If housed at the same time, the keeper must be trained on appropriate disinfection, hand washing, foot bath use, cleaning feed bowls, tools and net cleaning (separate tools and nets should be used for PTC and Quarantine), food preparation, etc.

e) Screening for salmonella (3 cultures from fresh feces), newcastles disease virus (not necessary if done at Surabaya).

f) Repeat ivermectin and droncit upon arrival (must be a minimum of 10 days from last dose), this must be repeated as with WW birds if not performed at Surabaya.

g) Treat for atoxoplasmosis as previously described.

h) Collect feces 1 week after 2 doses of ivermectin and droncit, must have 3 negative fecal samples, these should be collected no less than 1 week apart.

i) Screen for Chlamydia psittaci or treat as described for WW birds.

j) Collect feces for atoxoplasmosis screening as previously described (possibly Dr. Norton to transport back to US).

k) If quarantined in PTC, it will need to be completely stripped, this must take place before the atoxoplasmosis treatment is complete, otherwise the birds will become reinfected.

l) Birds must be eating normally, have no noticeable abnormalities, normal feces and urates prior to release from quarantine or the PTC.
Table 3. Preshipment workup for Bali Mynahs going to Indonesia in August

1) Physical examination and body weight
2) Fecal direct and flotation, and appropriate deworming
3) Cloacal or fresh fecal culture for *Salmonella*
4) Complete blood count
5) Serum biochemical profile (SGOT, LDH most important)
6) Chlamydial latex agglutination
   
   c/o Dr. James Grimes
   Texas Veterinary Medical Diagnostic Laboratory
   #1 Sippel Road
   College Station, Texas 77841-3040
7) Whole body radiographs
8) Follow atoxoplasmosis diagnostic protocol
9) Treat any positive birds for atoxoplasmosis
10) Please submit a summary of the results to:

   Dr. Terry M. Norton
   Riverbanks Zoological Park
   PO Box 1060
   Columbia, SC 29202
   Phone 803-779-8717
When we bring animals into captivity for any reason, we assume ultimate responsibility and indeed, moral obligation, for their health and well-being. The field of animal nutrition is one of many specialties which must be applied in order to successfully meet the future challenges of wildlife conservation, education, and science. Due to shortages of time, human, monetary, and animal resources, and with increasing emphasis on Species Survival Plans (SSPs), the need for detailed knowledge and management protocols, including dietary information, is imperative. Asking proper questions and disseminating proper information have become critical factors in species survival.

As with all specialty advisers, SSPs should establish minimal professional standards from Nutrition Advisers including: 1) knowledge of the literature, 2) efficient utilization of finite resources, and 3) awareness of limitations.

Roles of the Nutrition Adviser

To advise means simply to recommend or guide a course of action, and implies that the giver of the advice or opinion has knowledge and/or experience underlying those recommendations. While diet preparation, presentation, feeding ecology, and feed management are also important aspects of a comprehensive nutrition program, Nutrition Advisers should have some specific training in the underlying principles and tenets of the nutritional sciences.

Three separate, yet integrated functions of Nutrition Advisers include those of Consultant, Coach, and Counselor. As a Consultant, the Nutrition Adviser may be sought regarding diet, ingredient, or nutritional supplement composition or utilization, or be referred to for technical information. The adviser should be regarded as an expert, and should be expected to answer queries in these areas, or locate people who can. The Nutrition Adviser as a Coach should be considered an instructor, mentor, guide, or manager, and as such must be thoroughly prepared with background information as well as willing to share that information. This role might realistically be best applied with students or other collaborators. The evaluation of research plans or protocols, which are often focussed on small, finite projects, can most effectively be accomplished with the adviser overseeing a more comprehensive strategy. Thirdly, in the Counselor role, the Nutrition Adviser recommends a specific course of action based on serious deliberation and mutual exchange of ideas and opinions, often affecting the very survival of a species.

Responsibilities of the Nutrition Adviser

*Literature Review* - First and foremost, the Nutrition Adviser should be thoroughly familiar with, evaluate, and summarize available published information pertaining to diet and nutrition of the species of concern. In the field of wildlife nutrition, comprehensive
literature review entails browsing not only nutrition specialty journals, but also human and animal medical publications, as well as those dealing with ecology, wildlife and zoo management, biology, physiology, behavior, and numerous peripheral topics. This must include, and in some instances should particularly target, foreign periodicals. While conference proceedings and newsletters are important forms of communication, they should not be considered primary references. Management protocols for individual species should include a bibliography of pertinent nutrition, diet, and feeding behavior literature, properly cited, for general dissemination.

A written summary which describes feeding behaviors (including meal patterns, ingredient selectivity, palatability factors) and any information on nutrient composition of diets, describing both free-ranging and captive diets, must be a starting point. If data do not exist, this fact should be identified as a possible conservation priority.

The Nutrition Adviser should be familiar with anatomical (i.e. beak architecture, dentition, gastrointestinal tract) and physiological (basal metabolism, enzyme systems) specializations of a given species, and understand how these features relate to diet processing. Knowledge of these basic biological attributes provides a solid foundation for the development of suitable feeding and nutrition recommendations.

A review of identified nutritionally-related disorders should also be addressed in the summarization.

*Diet Recommendations/Reviews* - Husbandry manuals are only beginning to incorporate systematically documented sections covering topics of Nutrition and Hand-Rearing. Based upon the literature summary, recommendations by a nutrition specialist should include: 1) the most appropriate comparative model species (domestic, laboratory, or livestock) for evaluating nutrient adequacy, 2) target range(s) for gross chemical composition of diets, and amounts of feedstuffs, and 3) appropriate methods of diet presentation, with physiological and psychological optimality of the animal the ultimate goals. Exceptions to generalities or coherent arguments that no suitable data exist should be clearly documented. While unique aspects of any given species may be identified, the basic biology underlying the science of nutrition must be the primary focus.

Diet recommendations should not imply specific ingredient or product endorsement, but rather, focus on nutrients. Ingredients from diets of free-ranging animals can rarely be duplicated in managed feeding programs, but nutrients can. Thus we must identify the nutrient levels, feedstuffs, and relationships underlying successful diets and/or feeding programs which allow management flexibility within geographic, environmental, or economic constraints. Participation in SSP groups should entitle zoological institutions access to current, updated nutrition information and recommendations for various species -- this service may be particularly valuable to facilities without staff nutritionists.

Minimal information necessary for proper diet review includes documentation of all diet ingredients (with nutrient composition), amounts offered and/or consumed, supplementation products and regimens (including some medical treatments), and occasionally housing
conditions. Sometimes these data can be compiled through well-designed surveys and result in broad diet recommendations for meeting at least minimal nutrient requirements. More often, however, data acquisition requires one-on-one communication with involved personnel. The Nutrition Adviser should be prepared to ask detailed questions designed to gather the necessary information, and provide timely follow-up. Acknowledgement of samples, diet surveys, or information requests used in such reviews is not only professional courtesy, but a responsibility of the Nutrition Advisor.

Research Priorities - Nutrition Advisers should be able to identify relevant questions for nutritional research based, again, upon the foundation literature. If essential data don’t exist, state the facts such that interested collaborators can be encouraged. Studies of vitamin E interactions with zoo herbivores, for example, have shown a clear lack of data relating dietary types and amounts of fat to status of this nutrient. The basic biochemistry underlying these relationships needs to be examined under controlled, laboratory conditions better suited to university and/or industry programs than zoos. Investigations that can appropriately be conducted in zoological facilities should be coordinated through species Nutrition Advisors when necessary to provide adequate numbers of animals and/or treatments for statistical design. In either case, appointed Nutrition Advisors should be consulted and considered the main communications conduit. Redundant, poorly designed, or extraneous investigations with limited animals, personnel, money, and time resources should be considered irresponsible and discouraged.

Information Updates - Data on diet composition, digestive physiology, feeding ecology, nutrition research, or health parameters should be regularly disseminated by the Nutrition Adviser through national and international species coordinators, publication (scientific journals, newsletters, popular press), and regular meetings of interest groups. The current system of SSP program reports through the AAZPA is an excellent step in this direction; compliance should be a requirement for all species advisers.

Specific Future Needs

A system of computerized zoo diet records, perhaps linked in some way with the International Species Inventory System (ISIS), remains the most pressing communications issue for current focus. Conservation is a matter of global concern, and should encompass institutions worldwide. The computerized module should therefore be developed in a standardized, compatible format that will encourage acceptance and use. SSP species, particularly those also managed by an EEP (European Endangered Breeding Program) could be targeted as the "prototype" groups for development of the broader system, to facilitate acceptance of the format in Europe.

Collaborative laboratory networks should be established within the zoo, academic, and private industry communities to support zoo animal nutrition research and development. Routine analytical assays and techniques utilized by commercial laboratories are often not applicable to the specialized samples or species we consider daily. Furthermore, interpretation of data, even if obtained, can be limited by experts not familiar with extensive
comparative nutrition. Information databases needed to advance the field of wildlife nutrition, preferably in computer-accessible formats, include feedstuff chemical composition, clinical chemistry, and biochemical data.

The ISIS Physiological Normals database represents an initiation of such a compilation, but much more detail gleaned from research reports, published literature, and unpublished records is necessary. One example of data awaiting a proper outlet includes the fat-soluble vitamin database from the Wildlife Nutrition Laboratory of NYZS/The Wildlife Conservation Society. While published summaries of data do exist, the computerized files are constantly updated. Plasma values alone currently consist of data from blood samples comprising >8000 individuals of >300 species -- the largest comparative compilation of these data in the world.

We envision systems whereby these data could be readily accessible to the entire zoo/scientific community, and encourage others to contribute to and utilize such information. Clearly the field of wildlife nutrition, as with other wildlife specialties, must be one of collaboration and communication.

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SCIENTIFIC STUDY IN A SMALL ZOO

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Introduction

The history of research in US zoological institutions dates back to the formation of Penrose Laboratories at the Philadelphia Zoo in 1901. Since that time research has developed into a recognized discipline within the zoological community. In 1973 the first research symposium was held at the 49th Annual American Association of Zoological Parks and Aquariums (AAZPA) Conference in Houston, Texas. Today virtually all regional and annual AAZPA conferences have a session on research. In 1982 the first issue of Zoo Biology was published and has served as a source for publishing zoological research results. Allied professional associations such as the American Association of Zoo Veterinarians (AAZV), the American Association of Wildlife Veterinarians (AAWV), the Association of Avian Veterinarians (AAV) and the International Association for Aquatic Animal Medicine (IAAAM) have also promoted research in zoos and aquariums.

Finlay and Maple published the results of a survey on zoo and aquarium research in 1986. They found that the majority of those responding carried out research. Of those, 39% had research departments or committees. Most of the research dealt with behavior or reproduction. Only 57% of respondents actually published the results of their research.

Most modern day zoos recognize the value of research, but only the largest zoos are likely to have substantial research budgets. The Cheyenne Mountain Zoo (CMZ) is similar to many small zoos in that it has no formal research department. In recent years research has come under the auspices of the veterinarian at CMZ. The first full-time veterinarian was hired by CMZ in 1983, and at that time the first efforts were made to establish a research policy.

Animal Care and Use Committee

In 1971 the National Institutes of Health (NIH) established a policy that all institutions using warm-blooded animals in projects supported by NIH grants, awards or contracts would assure acceptable standards for the care, use and treatment of such animals. To assure proper care the institution would either be accredited by a professional laboratory animal accrediting body or create its own committee to evaluate such care. Institutions were to follow guidelines in the NIH Guide and follow applicable portions of the Animal Welfare Act.

In 1973 the 1971 NIH policy was replaced by the first Public Health Services (PHS) policy. Under this policy an institutional committee was required if the institution was not accredited. In 1979 the PHS policy was revised to read that all animal using NIH grant recipients must have a committee regardless of accreditation.
The last revised PHS policy was submitted by NIH in 1985. It elaborated on Animal Care and Use Committee (ACUC) functions and membership.\textsuperscript{6} It also requires a much fuller assurance statement that is filed with PHS.

The Scientists Center for Animal Welfare (SCAW) was organized in 1978. This organization was formed as a forum for scientists within the biomedical research community to address animal welfare concerns. SCAW has developed resource materials to aid in establishing ACUC's.\textsuperscript{4}

Animal Care and Use Committees are a recent development in zoos. A workshop on animal welfare, animal rights and the zoo and wildlife fields, was held at the joint conference of the AAZV and the AAWV in 1988. Two papers encouraged the formation of Animal Care and Use Committees at zoos.\textsuperscript{3,5}

In the fall of 1990 the first ACUC of the Cheyenne Mountain Zoo was formed. The veterinarian serves as a permanent member and committee chairperson. The committee is responsible for the evaluation of animal husbandry and health programs at the Zoo. It is also responsible for reviewing all requests for scientific study to assure compliance with institutional policies and national standards of health care and use. The main criteria used when reviewing requests for scientific study are scientific merit, conservation and education. Collaboration within CMZ or with other investigators, commitment of resources, the effect on the collection and publication of findings are additional considerations.

The formation of the ACUC has proved to be an important step in the commitment of CMZ to scientific study. See addendum 1 for the CMZ written committee policies.

Collaborative research

Collaboration with universities and other outside investigators has led to numerous advances in the zoological community. In their 1986 survey Finlay and Maple found that less than half of the zoos and aquariums responding maintained a formal affiliation with a college or university. The importance of such affiliation is gaining momentum and resulted in the Annual Directory of the Consortium of Aquariums, Universities and Zoos for 1988, which lists interests of university faculty.\textsuperscript{1}

The primary focus for scientific study at CMZ is of a collaborative nature. The Zoo receives approximately 50 requests a year for scientific study from outside investigators. These requests are reviewed in monthly meetings of the ACUC. The committee chairperson (Zoo veterinarian) communicates with the investigator of each request to let them know if the Zoo will comply with the request, not comply and why or if more information is needed.

Very few zoos or aquariums have a statistically significant population of any single species. Collaborative research among zoos and aquariums provides a much larger data base upon which to conduct statistical analyses.
For CMZ another important aspect of collaborating with outside investigators is the financial consideration. The research budget for CMZ is very limited and providing subjects, samples or observations to existing, well funded studies allows us to contribute without significant expense.

Important relationships have been established by CMZ with local and regional universities. A local college anthropology department offers a primatology course in which the students compare and contrast primate behavior in the natural and captive state. The students spend time observing primates at CMZ. The research papers which the students write for the course based on their observations are reviewed by CMZ animal department staff and have provided useful information for primate management decisions. A student from another university is doing extensive research on the effect of behavioral enrichment on behaviors of gorillas at CMZ.

Cheyenne Mountain Zoo is also involved in several captive propagation programs that relate field research and reintroduction programs. Four such species survival plans (SSPs) are the Bali mynah, golden lion tamarin, Virgin Island boa and black-footed ferret. The black-footed ferret program is a cooperative effort with Wyoming Game and Fish and the United States Fish and Wildlife Service. This breeding program is intensively managed and is under the direct supervision of the Zoo's veterinarian.

Another propagation and research program at CMZ involves the endangered Wyoming toad. This is also a cooperative effort with Wyoming Game and Fish and the United States Fish and Wildlife Service. This program has a field component to captive propagation. Captive animals are introduced to field sites in containment areas during the breeding season. After egg masses are laid, adults are brought back into the captive setting and eggs are left to develop in this protected setting. The Zoo's veterinarian sits on the Wyoming Toad Recovery Group and is the Zoo liaison to the Amphibian Taxon Advisory Group.

**In-House Scientific Study**

The Cheyenne Mountain Zoo prefers to refer to research as scientific study and recognizes its importance in supporting one of the main purposes of the Zoo which is to provide survival assistance for species in peril. The senior staff encourages in-house scientific study. The veterinarian has offered a seminar in "writing for publication" and a local college professor in the psychology department has offered a seminar in scientific method and conducting a behavioral research study.

Keepers and curatorial staff are encouraged to be an integral part of collaborative research efforts. Keepers are also enlisted to complete husbandry related surveys for outside investigators.
Discussion

Scientific study in zoos involves many of the life sciences including genetics, nutrition, ethology, psychology, reproductive physiology, evolutionary biology, pathology and ecology. Both basic and applied research are carried out, but applied research most closely addresses management issues.

Species Survival Plans, Taxon Advisory Groups (TAGS) and studbooks have resulted in the ability to collect substantial data. These programs are beginning to identify and help structure the direction of zoological research. Many of the requests received by CMZ/ACUC are initiated by or sanctioned by SSP, TAG or studbook programs. This has led to the direct use of scientific method to measure and assess phenomena that are related to the care of exotic animals in captivity.

The ability to conduct valid and worthwhile scientific study is not limited to megazoos with million dollar research budgets. Small zoos with no research department have made substantial contributions and will continue to do so. In many cases the veterinarian at a small zoo is the only staff member with even an introductory level knowledge of scientific method. As such they should take the lead as research coordinator for that institution.

LITERATURE CITED

ADDENDUM 1

CHEYENNE MOUNTAIN ZOO
ANIMAL CARE AND USE COMMITTEE POLICIES

I. Name

A. The name of this committee shall be the Cheyenne Mountain Zoo Animal Care and Use Committee (CMZ/ACUC).

II. Membership

A. Zoo Employees
   1. Four: staff veterinarian, general curator, an animal supervisor and an animal keeper

B. Zoo Board of Trustees
   1. One member

C. Community Member (not affiliated with the zoo)
   1. One member

D. Others
   1. Such as the committee deems necessary

E. Committee Chairperson
   1. Zoo staff veterinarian

F. Terms
   1. One year term for supervisor, keeper, board member, and community member. Board member and community member can be appointed for consecutive terms. The supervisor and keeper members may not serve 2 consecutive terms but can re-apply after one year off.
   2. The staff veterinarian and general curator are permanent members.

III. Meetings

A. A monthly meeting shall be held initially and then as often as deemed necessary by the committee.

B. The time and location will be determined by the committee.

IV. Reporting

A. Minutes of the CMZ/ACUC meetings, including attendance, activities and deliberations will be recorded at each meeting.
B. An annual report will be prepared by the committee chairman and presented to the zoo executive director and the Animal Committee of the zoo board of trustees.

C. Records will be kept of applications, proposals and recommendations on the care and use of animals in the collection and whether CMZ/ACUC approval was given.

V. Statement of Purpose
The CMZ is committed to the scientific study of its collection as a major part of on-going conservation and education programs. Furthermore, we are committed to the humane treatment of all animals in our collection and will assure compliance with the United States Department of Agriculture (USDA) Animal Welfare Act. In addition we will strive to care for our collection by the standards set forth by the American Association of Zoological Parks and Aquariums (AAZPA) relating to accreditation, ethics and disposition of animals in the collection. We acknowledge that a return of knowledge and understanding applicable to any species constitutes a major justification for their use in scientific study. Generally it is the philosophy of the CMZ that only non-invasive, non-terminal scientific study which is of benefit to the captive management of the species be conducted. All manipulations or interventions are to be minimized while still allowing completion of the approved objectives of the study. All requests for scientific study at the CMZ must be submitted to the ACUC and approved in accordance with their guidelines.

VI. Functions of the ACUC

A. The committee will evaluate the facilities, management, policies and procedures of animal husbandry and health programs at the zoo.

B. The committee will advise the central administration on the status of activities using animals and recommend methods for improvement.

C. The committee will represent senior staff, supervisory staff and keeper level staff who are involved in activities using animals in the collection.

D. The committee will facilitate information exchange within the institution, between institutions, and with the public in accordance with the recommendations of the Animal Committee.

E. The committee will review and evaluate all requests and proposals for scientific study to assure compliance with institutional policies and national standards of animal care and use.
F. The committee will review any funding requirements for all scientific study proposals submitted to the committee and make recommendations on funding when appropriate.

VII. Scientific Study Review Process

A. Each CMZ/ACUC member shall be provided a copy of all scientific study requests for review. Not all requests will require a vote by the committee for approval, but if a member requests a review of a proposal then a full committee review will occur. Certain requests may receive expedited review by the committee chairperson or general curator as described under VII.E.1.

B. Protocol
   1. An example of a protocol for in-house projects is attached and is adapted from recommendations in the Public Health Service Animal Welfare Policy.
   2. Requests from external sources will be evaluated in the form submitted when possible.
   3. Requests should be submitted at least 2 weeks prior to review by committee except when expedited review applies (VII.E.1).

C. Justification
   1. To be determined by the committee for each request using the following criteria:
      a. Scientific merit will be based on well defined questions, appropriateness of design and techniques, qualified investigators and need for project.
      b. Conservation as it relates to improved captive management of threatened or endangered species or support of field work, will be considered.
      c. The project will be evaluated on education of staff, public and other investigators.
      d. Collaboration within CMZ, or with other institutions will be considered.
      e. Commitment of resources including people, equipment, specimens and funds should be demonstrated.
      f. The effect on the collection with respect to the species used; number of animals used; is pain or distress involved; will institutional, state and federal regulations be followed will all be considered.
      g. Publication of findings will be an important consideration.

D. Classification of Requests
   1. Category 1: Requests which involve either no living material or use of plants, bacteria, protozoa or invertebrate animal species or no manipulation of a vertebrate animal beyond what is routine (example:
surveys, request for banked serum, tissues at necropsy or behavioral observations). These requests may be approved by expedited review by the staff veterinarian or general curator.

2. Category 2: Studies that involve collection of material or data from vertebrate species in conjunction with anticipated euthanasia for another reason following the zoo protocol (examples: frozen tissues, preserved tissues, skeletal remains etc.). These requests may be approved by expedited review by the staff veterinarian.

3. Category 3: Studies on vertebrate animals which produce little or no discomfort (examples: restraining for injections, blood collection, or examinations).

4. Category 4: Studies that involve minor stress or pain of short duration to vertebrate animal species and may require tranquilization or anesthesia (examples: blood collection, cystocentesis, implants etc.).

E. Approval of Requests
1. Expedited review - Requests in Categories 1 and 2 or requests which are made prior to a major veterinary procedure for other purposes, without time for committee review, may receive expedited approval by the staff veterinarian. The general curator may also expedite approval for requests in Category 1.

2. The committee may invite review by consultants if appropriate. Consultants have no vote on approval or disapproval unless they are members of the committee.

3. The request may be approved as written by at least 4 committee members after review.

4. The request may be approved with modifications on the basis of replacement of study subjects, reduction of numbers or refinement of protocol.

5. The request may be disapproved if not within institutional guidelines or not approved by at least 4 committee members.

6. If a member has a conflicting interest (e.g. is personally involved in the investigation) he/she cannot vote on the approval of the request.

7. The CMZ/ACUC shall notify investigators in writing of their decision to approve, withhold approval or of modifications required to secure approval. If the committee withholds approval it will include a statement of the reasons for its decision.

F. Follow-up and Monitoring of Projects
1. The committee will monitor projects at appropriate intervals determined by the committee to see if the project can continue; needs to be modified or should be terminated.

2. A project can be terminated if it is not conducted in accordance with applicable provisions of the Animal Welfare Act, or CMZ policies or the ACUC policies. The committee can suspend the activity after review at a meeting if at least 4 members vote to do so.
3. Any projects longer than one year are subject to annual review by the committee. A written proposal with any modifications and an update of the project must be submitted.

4. Requests that have been approved by the committee may be subject to further review and approval by officials of the CMZ.

VIII. Publication of Results

A. In-house projects - A copy of the manuscript resulting from the project must be submitted to the committee for review.

B. All others - Any publications must acknowledge CMZ and appropriate staff and a copy of the manuscript should be submitted for review to the committee.

IX. Funding

A. In-house
   1. The committee will review all in-house requests for funding of scientific studies and suggest allocation of funds from the Conservation and Scientific Study Fund where appropriate. These allocations must be approved by the executive director in conjunction with other requests for these funds.

B. External Requests
   1. Where external requests for funds are made for scientific study the committee will review these requests and suggest which requests should be funded by the Conservation and Scientific Study Fund. The executive director will approve these requests in conjunction with all other requests.

X. Husbandry and Health Policies

A. The living conditions for the collection will be appropriate for the species and contribute to their health and comfort. The housing, feeding and nonmedical care of the animals will be directed by a general curator, veterinarian and animal supervisors who are experienced in the proper care, handling and use of the species being maintained or studied.

B. Medical care for the collection will be available and provided for by a qualified veterinarian.

C. The committee shall review existing and new husbandry and health policies and advise on such policies when necessary. They shall pay particular attention to how these policies conform to the Animal Welfare Act, AAZPA standards and any other applicable regulations relating to animals. When
appropriate they will make written recommendations to the zoo executive director regarding any aspect of the zoo's animal program, facilities or personnel training.

XI. Lines of Authority

A. The CMZ/ACUC under the direction of the committee chairperson will be directly responsible for seeing that these policies are carried out and report to the executive director and the Animal Committee of the board of trustees.

Animal Committee

---------------------------------Executive Director---------------------------------

| Veterinarian                  |
| Animal Care and Use Committee |
| General Curator              |
Regional Species Survival Programs (SSPs) were developed by the American Association of Zoological Parks and Aquariums (AAZPA) beginning in the early 1980's.\(^1\) Taxon Advisory Groups (TAGs) and Faunal Interest Groups (FIGs) followed in the late 1980's and early 1990's. As of 1991, there were 56 SSP programs and 40 TAGs. The AAZPA has dictated that there be 200 SSPs by the year 2000.\(^1\) The inclusion of veterinarians, pathologists, nutritionists and other medical experts within these Regional Species Conservation Programs (RSCP) has paralleled their development albeit at a slightly slower rate.

With the growing concern over the impact of infectious diseases on species conservation programs, movement of animals between captive collections and species reintroductions or translocations, there has been increasing attention addressed toward the role of the veterinary advisor. Following the development of a working group report, (entitled: Monitoring, Investigation, and Surveillance of Disease in Captive wildlife; generated by a group of 40 +, zoo veterinarians at the International Conference on the Implications of Infectious Diseases on Captive Propagation and Reintroduction Programs of Threatened Species\(^2\)) guidelines outlining the commitment and responsibilities of veterinary advisors for RSCPs were proposed and accepted by Wildlife Conservation and Management Committee of the AAZPA.\(^3\) Concurrently the establishment of a Veterinary Advisors Group was proposed by the AAZPA's Director of Conservation and Science.

The Veterinary Advisors Group (VAG), will be housed as a subcommittee of the AAZPA's Animal Health Committee. The Veterinary Advisors Group will be structured to provide a number of services. Three proposed services are outlined below:

1) to act as a support and advisory body to the veterinary advisors of the SSPs, TAG's and FIG's;  
2) to act as a source of information concerning protocols for the commitment and responsibilities of veterinary advisors for coordinators of any Regional Species Conservation Programs; and  
3) to be an informational resource on veterinary issues that may impact Regional Species Conservation Programs.

The proposed composition of the VAG will be, an executive core group of 8 - 12 working members, along with a much larger general membership. The core members will most likely include: 1) the chair of the VAG; 2) the chairs of the AAZPA's Animal Health Committee, AAZV's Infectious Disease Committee and the IUCN/SSC Veterinary Specialist Group; 3) a designated representative of WCMC; 4) veterinarians that have played integral roles or
expressed keen interest in the development of protocols for the veterinary advisors; and 5) professionals from other pertinent disciplines such as pathology, epidemiology, diagnostic testing, and nutrition. The general membership will at a minimum include all designated or acting veterinary advisors.

It is important that this group develop and maintain an effective communication network. Information of concern to veterinary advisors must to be disseminated to all RSCP’s both nationally and internationally. Striving to establish a global communication network, will allow all members of the VAG to more appropriately respond to the veterinary issues affecting the conservation of threatened species.

LITERATURE CITED

CONSERVATION WORKSHOP FOR THE VETERINARY ADVISORS OF SPECIES SURVIVAL PLANS AND TAXON ADVISORY GROUPS

Peregrine L. Wolff, DVM,*
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Donald L. Janssen, DVM
San Diego Zoo, P. O. Box 551, San Diego, CA 92112, USA

The number of AAZPA approved SSP's and TAG's continues to grow. The role of the Veterinary Advisor within these programs has now been defined. In an attempt to aid the Veterinary Advisors in fulfilling their designated role in these conservation programs, the decision was made to schedule time within the program for a Veterinary Advisor Workshop. The aim of the workshop is to allow the Veterinary Advisors to develop goals for their respective programs and to share information with other veterinarians whose institutions are currently holding such species or taxa.

In order to assist all members of AAZV, a current list of the Veterinary Advisors was generated. This was developed by sending a questionnaire to the species coordinators of all listed North American SSP's and TAG's. The questionnaire asked whether the group had a Veterinary Advisor and, if so, whether the advisor was the official advisor (i.e., recognized by the SSP or TAG) or was merely an acting advisor. The same question was asked for pathologists and nutritionists.

The results of this survey are listed in Table 1, 2 and 3. The lists are broken down into SSPs and TAGs. The SSP list is divided into Gastropods, Amphibians, Reptiles, Birds and Mammals. Each species is listed alphabetically by the genus common name (i.e., Rhino, Southern Black; Rhino, Sumatran; Rhino, White) The TAGS are listed alphabetically by taxon. If the SSP/TAG does not have a Veterinary Advisor, then the column containing this information is marked NONE. If the Species Coordinator failed to return the questionnaire then the column is left blank.

These tables are a first attempt to develop a comprehensive list of the veterinary and other health advisors for the SSPs and TAGs. If you feel that information within this table may be incorrect, or if you have updated information, please do not hesitate to contact the first author.
Table 1: Species, species coordinators, veterinary advisors, pathology advisors, nutritional advisors for gastropods, amphibians, reptiles and birds.

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<td>Rail, Guam</td>
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\(^a\) = Official Veterinary Advisor  
\(^b\) = Acting Veterinary Advisor  
\(^c\) = Official Pathology Advisor  
\(^d\) = Acting Pathology Advisor  
\(^e\) = Official Nutritional Advisor  
\(^f\) = Acting Nutritional Advisor  
\(^g\) = Pending Approval  
\(^h\) = Official Parasitology Advisor  
\(^i\) = Infectious Disease Advisor  
\(^j\) = Behavior Advisor  
\(^k\) = Reproduction/Physiology Advisor  
\(^l\) = Genetics Advisor  
NONE = SSP Coordinators did not indicate any advisors in the category  

NOTE: This table represents the results of a survey sent to SSP coordinators. Blank lines indicate no response to the survey. This is not a finalized table.
Table 2: Species, species coordinators, veterinary advisors, pathology advisors, nutritional advisors for mammals.

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\(^a\) = Official Veterinary Advisor  \quad \(^b\) = Acting Veterinary Advisor  
\(^c\) = Official Pathology Advisor  \quad \(^d\) = Acting Pathology Advisor  
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\(^g\) = Pending Approval  \quad \(^h\) = Official Parasitology Advisor  
\(^i\) = Infectious Disease Advisor  \quad \(^j\) = Behavior Advisor  
\(^k\) = Reproduction/Physiology Advisor  \quad \(^l\) = Genetics Advisor  

NONE = SSP Coordinators did not indicate any advisors in the category  
* = Chair of Advisory Group  

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Table 3: Taxon, taxon coordinators, veterinary advisors, pathology advisors, and nutritional advisors.

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VETERINARY CONSIDERATIONS IN THE SHIPPING AND RECEIVING OF AVIAN SPECIES

Jacqueline M. Zdziarski, DVM, and Noha Abou-Madi, DVM, MS*
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Jeffery R. Zuba, DVM
San Diego Wild Animal Park, Zoological Society of San Diego, Escondido, CA 92109, USA

A preventative health program should be established for avian species at every zoological institution. To ensure the transfer of healthy birds this program should include the routine examination and testing of animals prior to shipment to other institutions as well as upon receiving new acquisitions into your facility. There are also various state and international regulations regarding specific diagnostic testing and permits necessary before an animal can be shipped interstate or intercontinental. Since laws change, it is advised to contact the receiving state or country before each new shipment. Arrangements should be made in advance (at least two weeks) to ensure receipt of test results, to prepare an adequate crate which meets federal and/or international requirements and to prepare appropriate facilities at the receiving institution. Contact the receiving institution veterinarian prior to testing to determine if there are any specific requirements. After testing inform the receiving institution of any problems or concerns prior to shipping. A valid health certificate, medical records, diet and husbandry information should accompany all shipments.

Strict quarantine procedures are aimed at safeguarding the existing collection from the introduction of infectious diseases. The quarantine facilities should be isolated from all collection and hospitalized birds. Equipment and animal care staff should be assigned specifically to clean and maintain the quarantine area. All other zoo personnel should be restricted from the quarantine space. Ideally boots, gloves, masks and protective clothing should be worn while working with quarantined animals due to zoonotic potential. Foot baths should be maintained at entryways to the facilities and a broad spectrum disinfectant should be utilized in daily cleaning of the quarantine area. A minimum 30 day quarantine period should be enforced with an "all-in, all-out" philosophy being ideal.

Following the stress of shipment and change in environment, a bird's resistance may be lowered. The bird may start shedding infectious agents, parasites or show clinical signs of an on-going subclinical disease process. Particular attention should be paid to diet, cage requirements, over-crowding, as well as other environmental and social factors to help minimize stress.

All foreign acquisitions legally entering this country must pass through a USDA approved federal quarantine station. It is important to identify and contact this facility to determine if any disease developed during their quarantine period. The death of any bird during federal quarantine or at the broker's facility should be investigated.
All animals arriving into a zoological quarantine facility should be accompanied by a valid health certificate, a complete medical history, diet and husbandry information. Upon arrival all birds should be inspected for injuries or disease and a body weight should be taken. Animal care staff should be trained to recognize abnormal behavior and clinical symptoms of disease and have a knowledge of the diets, husbandry, and restraint procedures for the animals under their care. Food and water intake, stool production and character, and general physiologic factors should be monitored by quarantine animal care staff. A more thorough physical exam including diagnostic testing should be completed within the first week of arrival.

Ideally, a minimum data base (MDB) should be obtained on all animals pre-and post-shipment. Due to size constraints, the veterinarian must decide the priority of tests requested. Also, specific species will require additional testing protocols. The MDB should include: a complete physical exam, body weight, sexing, determination if pinioned or tenectomized; and permanent identification. A complete blood count, hemoparasite check and chemistry profile, serum banking, cloacal and/or choanal bacterial culture and sensitivity and fungal culture, and fecal examination (including a direct smear, flotation, gram stain, and special stains for Giardia or Cryptosporidium) should be included. Baseline radiographs are recommended. If possible, samples should be collected at this time for specific research requests. Parasitized birds should be treated with an appropriate anthelmintic and three negative fecals should be obtained before the bird is released from quarantine. Appropriate vaccinations should be administered during the quarantine period. As previously mentioned all clinical birds should be separated, subjected to diagnostic testing and treated appropriately. Gross necropsies and histopathology should be performed on all dead birds.

Below follows a listing of most major groups of birds with specific disease entities to consider when shipping and receiving birds. Available tests and laboratories which provide diagnostic services are included. This is not meant to be a complete list of diseases of birds and the reader is encouraged to refer to the additional sources listed.

1. SPHENISCIFORMES & PELECANIFORMES (penguins, pelicans)

   a. Infectious Diseases
   i. Viral diseases
      -Avian Poxvirus
         **Diagnosis:** demonstration of the typical intracytoplasmic inclusion bodies in proliferating, hypertrophic epithelial cells on histopathology. Viral isolation can be performed at most state laboratories.

         -**Herpesvirus** has caused mortality in penguins.
         **Diagnosis:** identification of intranuclear inclusion bodies on histopathology.
ii. **Bacterial diseases**
- **Coliforms** due to poor water quality.
- **Clostridium botulinum**
  Diagnosis: a mouse bioassay is used to identify the toxin. Submit 2-3 ml fresh or frozen serum; state laboratory.

iii. **Fungal diseases**
- **Aspergillus spp.**
  Diagnosis: ELISA titers are performed by The Raptor Center at the University of Minnesota College of Veterinary Medicine, 1920 Fitch Ave., St. Paul, Minnesota 55708 USA, (612-624-4745). Send 0.5-1.0 ml of plasma on ice via overnight express.

b. Parasitic Diseases
- **Plasmodium**
- **Pouch lice**

c. Miscellaneous
- pododermatitis
- molting disorders
- foreign body ingestion
- penguins are predisposed to gout
- certain penguin species are susceptible to uropygial gland infections.

2. **GRUIFORMES** (cranes)

a. Infectious Diseases
i. **Viral diseases**
- **Inclusion Body Disease of Cranes (Herpesvirus)**
  Diagnosis: serology is performed by the National Wildlife Health Research Center (NWHRC), however results may not be available for 4-6 weeks. Call Sharon Goldade or Joshua Dein at 608-271-4640 to advise of shipping/arrival date. Send at least one ml. serum per bird in a labeled cryo-vial. Send frozen sample overnight express on ice packs or dry ice to NWHRC, USFWS, 6006 Schroeder Road, Madison, WI 53711-6223 USA.

ii. **Bacterial diseases**
- **Mycobacterium avium**
  Diagnosis: most human diagnostic laboratories culture feces for mycobacteria. Be sure that the diagnostic lab is using a *M. avium* specific DNA probe. In *M. avium* positive cases in cranes, tuberculin skin testing using avian PPD and OT injected interdermal on the crown has yielded positive reactions.
confirmed by biopsy in *M. avium* positive cases (personal communication, Bret Snyder, 903 10th St., SW, Rio Grande Zoological Park, Albuquerque, NM 87102, USA, 505-843-7413 X261). Interdermal testing may be a viable way to screen a flock, however all test positive birds should be cultured. The NWHRC is currently working on an Elisa for avian TB.

*Salmonella spp.*

**Diagnosis:** cloacal cultures should be taken. One negative culture is not considered significant, therefore, multiple cultures are recommended. Serotyping should be performed on all *Salmonella* positive cultures to help determine if it is actually causing disease. Your state lab in conjunction with the National Veterinary Services Laboratory (NVSL, 13th and Dayton Rd., Ames, Iowa 50010, USA, 515-239-8212) can perform the serotyping.

### iii. Fungal diseases

*-Aspergillus spp.*

b. Parasitic Diseases

- Capillaria
- Coccidia (visceral coccidiosis)
- *Cynthia coscoroba*

c. Miscellaneous

- foreign body ingestion
- leg deformities in growing chicks

#### 3. CICONIIFORMES and PHOENICOPTERIFORMES (storks, flamingos)

a. Infectious Diseases

i. *Viral diseases*

- *Poxvirus*

  **Diagnosis:** same as above.

  **Control:** the fowl pox vaccine is of questionable efficacy in flamingos. Currently, research is underway to develop a specific flamingo pox vaccine (personal communication, Terry Campbell, Sea World of Florida, 7007 Sea World Drive, Orlando, FL 32820, USA, 407-351-3600 X351). An autogenous vaccine can be made.

- *Newcastle Disease* (Paramyxovirus type 1)

  **Diagnosis:** viral isolation from lung, spleen, and affected tissues. Submit frozen tissues to the NVSL via the state lab.
ii. **Bacterial diseases**
- Coliforms
- *Mycobacterium avium*
- *Pasteurella multocida* (Avian Cholera)

iii. **Fungal diseases**
- *Aspergillus spp.*

b. **Parasitic Diseases**
- external parasites
- sarcocystis has been diagnosed on postmortem exam.

c. **Miscellaneous**
- Pododermatitis
- leg deformities
- white muscle disease is not uncommon when transporting flamingos.

4. **ANSERIFORMES** (ducks, geese, swans)

a. **Infectious Diseases**

i. **Viral diseases**

- **Duck Virus Enteritis** (Herpesvirus)
  
  **Diagnosis:** viral isolation and identification from affected tissues. Send esophagus, liver, spleen and intestine samples to a state laboratory which can forward the samples to the NVSL. Viral isolation is difficult. A serologic test is available through the NVSL. Submit 1 ml of frozen serum.
  
  **Control:** a modified live-virus vaccine is available and has been found effective in controlling DVE outbreaks. The state veterinarians permission is necessary for shipment (International Duck Research Cooperative, Inc., P. O. Box 217, Eastport, NY 11941, USA, 516-325-0600).

- **Avian Influenza** (Orthomyxovirus)
  
  **Diagnosis:** virus isolation and identification from tracheal and cloacal swabs and/or lung, spleen and affected tissue samples. A serologic test is also available. Submit tissues or 1 ml serum refrigerated on ice to the NVSL through a state laboratory.

- **Duck Viral Hepatitis** (Picornavirus)
  
  **Diagnosis:** viral isolation.

- **Newcastle Disease** (Paramyxovirus type 1)
  
  **Diagnosis:** viral isolation from lung, spleen, and affected tissues. Submit frozen tissues to the NVSL via a state lab.
- Gosling plague (Parvovirus)
  Diagnosis: this virus has never been diagnosed in the United States. If this disease is suspected, contact a state lab and NVSL.

ii. Bacterial diseases
- *Clostridium botulinum* type C (Limberneck)
  Diagnosis: a mouse bioassay is used to identify the toxin. Submit 2-3 ml serum to your state lab.
- *Pasteurella multocida* (Avian Cholera)
  Diagnosis: culture from heart blood or abdominal organs.
  Control: poultry vaccines are available (Solvay Animal Health, Inc., 1201 Northland Dr., Mendota Heights, MN 55120-1139, USA, 1-800-524-1645).
- *Pasteurella antipestifer* (Infectious Serositis)
  Diagnosis: culture, serology is available.
  Control: vaccines have been used.
- *Salmonella gallinarum* and *Salmonella typhimurium*
  are the most common species isolated from waterfowl.
  Diagnosis: culture feces.
- *E. coli*
- *Mycoplasma synoviae*
  Diagnosis: culture affected joints.

iii. Fungal diseases
- *Aspergillus spp.*

b. Parasitic Diseases
- *Cyathostoma bronchiolus*
- *Thermyzon spp.* (leeches)
- *Leucocytozoon*
- *Plasmodium*
- lice

5. GALLIFORMES (fowl, quail, pheasants)

a. Infectious Diseases
i. Viral diseases
- Newcastle Disease (Paramyxovirus type 1)
  Diagnosis: virus isolation; contact local state veterinary diagnostic laboratory or NVSL.
  Control: vaccine available for poultry (extra label use; safety and efficacy not known in exotic avian species; provide at own risk; Solvay Animal Health, Inc.).
- **Marek's Disease** (Herpesvirus)
  Diagnosis: virus isolation from blood (EDTA or heparin) or affected tissues; state diagnostic laboratory or NVSL.
  Control: poultry vaccine (Solvay Animal Health, Inc.).

- **Reticuloendotheliosis** (Retrovirus)
  Diagnosis: viral isolation; NVSL.

- **Avian Influenza** (Orthomyxovirus)
  Diagnosis: submit 0.5-1.0ml serum for serology, virus isolation from tracheal and cloacal swabs or affected tissues; state diagnostic laboratory or NVSL.
  Control: poultry vaccine (Solvay Animal Health, Inc.).

- **Avian Encephalomyelitis virus** (Picornavirus)
  Diagnosis: serology, virus isolation from the brain is very difficult.

- **Poxvirus**
  Diagnosis: demonstration of the typical intracytoplasmic inclusion bodies in proliferating, hypertrophic epithelial cells on histopathology or viral isolation.
  Control: fowl, pigeon and quail vaccine (Keenum, Inc., P. O. Box 1706, 1900 Coleman Rd., Anniston, AL 36201, USA, 205-831-6530).

- **Quail bronchitis** (Adenovirus Group 1)
  Diagnosis: demonstration of seroconversion between acute and convalescent sera or viral isolation from affected tissues (trachea and lungs are best), NVSL.

- **Marble Spleen Disease** (pheasants); **Hemorrhagic Enteritis** (turkeys) (Adenovirus Group 2)
  Diagnosis: viral isolation and identification from spleen or intestines.

- **Lymphoid Leukosis** (Retrovirus)
  Diagnosis: histology of lesions.

### ii. Bacterial diseases

- **Salmonella gallinarum**
  Diagnosis: culture feces.

- **Mycoplasma gallisepticum**
  Diagnosis: choanal cultures.

- **Mycoplasma synoviae**
  Diagnosis: culture affected joints.

- **Mycobacterium avium**
  Diagnosis: histology of affected tissues or granulomas using an acid fast stain; culture.

- **Erysipelothrix rhusiopathiae**

- **Chlamydia psittaci**

- **Clostridium colinum** (Ulcerative Enteritis)
iii. **Fungal diseases**
- Aspergillus spp.
- Candida albicans

b. **Parasitic Diseases**
- Capillaria spp.
- Syngamus trachea
- Ascaridia spp.
- Heterakis gallinarum
- Cestodes

6. **PSITTACIFORMES** (parrots)

a. **Infectious Diseases**

i. **Viral diseases**

- **Psittacine poxvirus**
  
  **Diagnosis:** demonstration of the typical eosinophilic intracytoplasmic inclusion bodies in proliferating, hypertrophic epithelial cells on histopathology. Viral isolation can be difficult.

  **Control:** killed virus vaccine, questionable availability (Maine Biological Laboratories, Waterville, Maine 04903-0255, USA, 207-873-3989).

- **Avian Polyomavirus**
  
  **Diagnosis:** a polyomaviral DNA probe test is available. Send cloacal swabs for routine screening or affected tissues collected at necropsy to Avian Research Associates Inc. Laboratory, Suite 101, 100 Techne Center Dr., Milford, Ohio 45150, USA (513-248-4700). Send samples unrefrigerated via direct mail.

- **Psittacine Beak & Feather Disease** (Circovirus)
  
  **Diagnosis:** a viral DNA test is available which detects nucleic acid in white blood cells. Submit 0.2-0.5ml whole blood in heparin micro-collection tubes, unrefrigerated via direct mail to Avian Research Associates Inc. Laboratory at above address. On birds with abnormal feather growth, affected feathers should be plucked, fixed in formalin and sent to Dr. Ken Latimer at the University of Georgia, College of Veterinary Medicine, Department of Pathology, Athens, Georgia 30602, USA (706-542-3221) or to another veterinary pathologist familiar with the lesions of psittacine beak & feather disease.

- **Papillomavirus**
  
  **Diagnosis:** biopsy of mass lesion can be submitted for histopathology, however it is difficult to determine if the lesion is caused by an infectious agent or is due to chronic irritation. Viral isolation is difficult.
- **Pacheco's Disease (Herpesvirus)**
  
  **Diagnosis:** demonstration of eosinophilic intranuclear inclusion bodies in the liver and spleen on histopathology.
  
  **Control:** killed virus vaccine, questionable availability; reports of adverse reactions to vaccine.

ii. **Bacterial diseases**

- **gram negative enteritis**
  
  - *Salmonella spp.*
  
  - *Chlamydia psittaci*

  **Diagnosis:** an Elementary Body Agglutination (EBA) test is available for routine screening of all avian species for chlamydial antibody activity. Other serologic tests are also available. Submit 0.2 ml serum in a micro-collection serum separator tube to Texas Veterinary Medical Diagnostic Laboratory (TVMDL), Drawer 3040, College Station, Texas 77841-3040, USA (409-845-3414). Culture yields a definitive diagnosis.

- *Mycobacterium avium*

  **Diagnosis:** submit fecal culture.

iii. **Fungal diseases**

- *Candida albicans*

- *Aspergillus spp.*

  **Diagnosis:** serology, see Raptor Center, University of Minnesota above.

b. **Parasitic Diseases**

- *Capillaria*

7. **PASSERIFORMES**

a. **Infectious Diseases**

i. **Viral diseases** - See appropriate listing above for diagnosis and control.

- Avian poxvirus

- Newcastle disease (Paramyxovirus type 1)

- Avian Influenza (Orthomyxovirus)

ii. **Bacterial diseases**

- *Chlamydia psittaci*

- *Mycobacterium avium*

- *Campylobacterjejuni* and *C. fetus*

- *Mycoplasma spp.*

- *Yersinia Pseudotuberculosis*
iii. Fungal diseases
   - **Candida albicans**
   - **Aspergillus spp.**

b. Parasitic Diseases
   - **Leucocytozoon**
   - **Plasmodium**
   - **Sternostoma tracheoculum**
   - Toxoplasmosis
   - Atoxoplasmosis
     **Diagnosis:** liver or splenic impression smears stained with a Wright's stain reveal zoite forms in the cytoplasm of macrophages. Oocysts are intermittently shed in droppings.
   - Nematodes
   - Coccidiosis
   - Cnemidocoptic mange

c. Miscellaneous
   - Hemochromatosis
     **Diagnosis:** liver biopsy
   - Congestive Heart Failure

8. COLUMBIFORMES (pigeons, doves)

a. Infectious Diseases
i. Viral diseases
   - Avian Pox
   - Paramyxovirus type 1 (distinct from Newcastle Disease)
     **Diagnosis:** serology, virus isolation; NVSL.
   - Herpesvirus 1
     **Diagnosis:** serology available, send 0.5 cc of serum; NVSL.

ii. Bacterial diseases
   - Salmonella spp.
   - Mycoplasma spp.
   - Chlamydia psittaci
     **Diagnosis:** serology available; TVMDL.
iii. **Fungal diseases**  
   - *Aspergillus* spp.  
   - *Candida* spp.

b. **Parasitic Diseases**  
   - Ascarids  
   - Capillaria  
   - Cestodes  
   - Trematodes  
   - Coccidia  
   - Tetraceres  
   - Toxoplasmosis  
   - Hexamitiasis  
   - Trichomoniasis  
   - *Plasmodium*

9. **CORACIIFORMES** and **PICIFORMES** (hornbills, toucans)

a. **Infectious Diseases** (no unique diseases, similar to passerine species)
   i. **Viral diseases**
   ii. **Bacterial diseases**  
      - gram negative enteritities
   iii. **Fungal diseases**  
        - *Candida* spp.  
        - *Aspergillus* spp.

b. **Parasitic Diseases**

c. **Miscellaneous**  
   - Hemochromatosis  
     **Diagnosis:** liver biopsy.  
   - Beak fractures

10. **STRUTHIONIFORMES** (ratites)

a. **Infectious Diseases**
   i. **Viral diseases**  
      - *Eastern Equine Encephalitis*  
        **Diagnosis:** serology available, send 2cc of serum to NVSL.  
      - *Western Equine Encephalitis*  
        **Diagnosis:** same as above.  
      - *Newcastle Disease* (Paramyxovirus type 1)
- Avian Influenza Virus

ii. Bacterial diseases
   - Mycoplasma spp.
   - Salmonella spp.
   - Chlamydia psittaci

iii. Fungal diseases
   - Candida albicans
   - Aspergillus spp.

b. Parasitic Diseases
   - Chanderella quiscali (cerebral nematodiasis)
   - Philophthalmus gralli (eye fluke)
   - Capillaria
   - Ascarids
   - Cestodes

c. Miscellaneous
   - Gastrointestinal foreign body
   - Trauma
   - Leg deformities in juveniles

11. FALCONIFORMES and STRIGIFORMES (falcons, raptors, owls)

a. Infectious Diseases
i. Viral diseases
   - Avian pox
   - Fatal Inclusion Body Hepatitis (Herpesvirus)
     Diagnosis: identification of viral particles by electron microscopy of affected tissues; NVSL.
   - Newcastle Disease (Paramyxovirus type 1)
   - Lymphoid Leukosis

ii. Bacterial diseases
   - Salmonella spp.
   - Pasteurella multocida
   - Listeria monocytogenes
   - Yersinia pseudotuberculosis
   - Chlamydia psittaci

iii. Fungal diseases
   - Aspergillus spp.
     Diagnosis: serology, Raptor Center, University of Minnesota, see above.
b. Parasitic Diseases
- Capillaria
- Ascarids
- Coccidia
- Trichomoniasis
- Plasmodium
- Leucocytozoon
- Haemoproteus

c. Miscellaneous
- pododermatitis

12. APODIFORMES (hummingbirds)

a. Infectious Diseases (mostly unknown)
   i. Viral diseases
   
   ii. Bacterial diseases
   - Gram negative enteritities
   - Chlamydia psittaci
     Diagnosis: serology, see Texas A&M University above.
   
   iii. Fungal diseases
   - Candida albicans
     Diagnosis: budding yeast on wet mount or culture.

b. Parasitic Diseases

c. Miscellaneous
- Torpor is a natural occurrence in hummingbirds and is used by these highly metabolic birds as a means to conserve energy, usually at night. For unknown reasons, only certain hummingbirds will go into torpor, so this state is often confused with clinical illness.

REFERENCES

PRODUCTION MEDICINE FOR CERVID FARMERS

Jerry Haigh FVRVS
Department of Herd Medicine and Theriogenology, Western College of Veterinary Medicine, Saskatoon, Saskatchewan S7N 0W0, Canada

Introduction

The species of deer that are currently farmed or ranched on the North American continent include wapiti (Cervus elaphus canadensis), red deer (Cervus elaphus scoticus), fallow deer (Dama dama), sika deer (Cervus nippon), white-tailed deer (Odocoileus virginianus), mule deer (Odocoileus hemionus), reindeer (Rangifer tarandus) and axis deer (Axis axis), also known as chital. There are also wapiti/red deer and wapiti/sika hybrids, as well as a few hybrids of Mediterranean and Mesopotamian fallow deer. Moose (Alces alces) have been domesticated at various times, but seldom with prolonged success due to their specialized husbandry needs.

Much of what has been learned about production medicine for deer species has the potential to be of considerable benefit in zoo programs. Areas with particular value include the highly developed handling systems, the nutrition, the understanding of diseases and their prevention, and perhaps most of all to those interested in endangered species propagation, the whole area of reproduction. Artificial breeding, both in terms of artificial insemination (AI) and embryo transfer (ET) have become routine matters in the red deer industry, but wapiti, chital and white-tailed deer have all been successfully inseminated. Hundreds of ETs have been successfully carried out in red deer, and workers in this field consider the success rate to be at least equal to that of the cattle industry.  

If a veterinarian is to become involved in the industry, the most fundamental requirement is that she or he must become educated about the biology of the animals and how they differ from traditional species of livestock. The herd health approach to deer farming is much more prevalent in New Zealand than anywhere else, which reflects both the maturity of the industry there and the larger number of veterinarians and farm consultants who have become familiar with its facets. A production medicine attitude cannot develop among farmers until such time as veterinarians show competence and a willingness to become involved. In some circumstances this may mean that the veterinarian may have to show a willingness to make some visits to learn the practical on farm activities.

The use of deer as agricultural animals is extremely ancient, but in Europe their popularity suffered a serious decline in the middle ages, partly due to their inability to produce tallow on a year-round basis. Tallow was essential for both soap and light. The Chinese have written records of the use of deer that are over 2,000 years old, and records of farming them that are several centuries old. In the West, domestication of deer has only been rediscovered in the last 100 years or so. Indeed, Judge J. D. Caton wrote specifically of the husbandry of wapiti, and in 1905 an extension pamphlet originating from the U.S. Biological Survey gave details of husbandry. In 1877 Caton wrote:
The wapiti is much better adapted to domestication than any of the other deer with which I have experimented. In the first place, they are much more healthy.¹

The main thrust of a deer production industry has come in the last 20 years with rapid developments in New Zealand and similar but slower developments in many other countries.

It appears from the literature that the first set of papers to specifically address production medicine for the deer farmer were published in 1987.²⁻⁸⁻⁹⁻¹⁰ Wilson identified the evolution of a veterinary input into deer farming "from humble beginnings when most veterinarians feared involvement, many actively avoided involvement, and a few keen individuals sought involvement."¹⁰

The principal goal of any health program for the livestock industry should be to optimize production and minimize disease incidence.
For the deer farmer, as well as the veterinarian, the first steps toward achieving these goals must be a thorough understanding of the industry and the animals being farmed or ranched. In a deer operation, objectives may include the production of breeding stock, antler production, development of a hunting preserve, a tourist facility or venison unit.

The frequency of routine visits on a deer farm may be dictated by the size of the operation and the amount of work that can be accomplished in a given time. It could be limited to perhaps three or four a year, which would include visits at velveting time, for semen testing of stages, pregnancy checking and for TB testing. At specified times the veterinarian may also collect blood samples for evaluation of trace mineral (especially copper) status. At any and all of these times the veterinarian can take time to evaluate nutritional management tied to local fodder availability. On large operations the veterinarian may become involved in artificial breeding and other management decisions relating to pasture management and feeding programs. Some of the areas in which a veterinarian can become involved as a farm adviser are listed in Table 1. The goals to which the deer farmer can aspire, in terms of production, are listed in Table 2.

Implementation

For a health and production approach to management to succeed, there is a need for all parties to examine the potential modifying effects of management practices on farm profitability. They are outlined in Fig 1a and developed in Fig 1b.
Rising cost of production Increased value of product(s)

Good

Profit

Loss

Bad

Quality of management

Good

Bad

Figure 1a. Relationships among fiscal and management components in a livestock production operation. (from Haigh and Hudson 1993)

Moderating Factors

Changes in the relationships depicted in Fig 1a may occur as circumstances governing them change. The moderating factors are listed in Table 3.

One of the moderating factors that is examined in more detail is losses from disease which can be broadly categorized into three types. The first are individual animal deaths, which may be unfortunate, but can be valuable as long as postmortem examination is carried out and the information gleaned acted upon. The second is a catastrophe such as might occur from any disease with high morbidity and mortality such as blue tongue in white-tailed deer, or from flooding or mass poisoning. The third category, which may be the least obvious but potentially almost as damaging as the catastrophe, is subclinical disease that may adversely affect reproduction, productivity or other parts of the farm activity. Examples of health-related causes of loss are listed in Table 4.

The old adage that "prevention is better than cure" is based upon sound scientific principles. For instance, some diseases can continue to adversely affect productivity after the clinical condition has been cured by treatment, and stunted growth due to late calving or undernourishment cannot be satisfactorily cured once growth plates have closed in the long bones.6

The overall effects of all of the above components are depicted in Fig 1b.
Yearly Calendar

It is not possible to provide a yearly calendar for farming activities of all species of deer under all management conditions in North America without becoming overly complicated. A schematic calendar of activity for the various classes and ages of animals on a wapiti or red deer farm is outlined in Fig 2.

Benefits

The economic benefits of a herd health program have been outlined by Morris and by Radostits and Blood. In the current climate of expansion, where product value is high, especially in the case of breeding stock, such a program, which must be farmer driven to be a success, may be difficult to initiate. However, even in such times production medicine can be of value in optimizing management. When prices stabilize, those farmers with well-structured programs are likely to reap the greatest rewards. A successful program includes more effective management by a farmer, introduction of specific disease prevention techniques, regular veterinary visits, optimum nutrition, optimum breeding programs and possibly some unidentified variables.
LITERATURE CITED


Table 1

Subjects areas in which the veterinarian can assist the deer farmer

<table>
<thead>
<tr>
<th>Stock acquisitions</th>
<th>Records</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species choice</td>
<td>Production records</td>
</tr>
<tr>
<td>Breed</td>
<td>Velvet production</td>
</tr>
<tr>
<td>Vendors records</td>
<td>Growth patterns</td>
</tr>
<tr>
<td>Health history</td>
<td>Reproduction</td>
</tr>
<tr>
<td>Management</td>
<td>Breeding season</td>
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<tr>
<td>Farm layout</td>
<td>Stag: hind ratio</td>
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<tr>
<td>Handling systems</td>
<td>Targets</td>
</tr>
<tr>
<td>Nutrition programs</td>
<td>Artificial breeding</td>
</tr>
<tr>
<td>Reproductive programs</td>
<td>Record analysis</td>
</tr>
<tr>
<td>Disease prevention</td>
<td>Body weights</td>
</tr>
<tr>
<td>Elective procedures</td>
<td>Antler weights</td>
</tr>
<tr>
<td>Velveting</td>
<td>Reproductive performance</td>
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<tr>
<td>Reproductive evaluation</td>
<td>Strategy analysis</td>
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<tr>
<td>Artificial breeding</td>
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<tr>
<td>Diagnosis and treatment</td>
<td></td>
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<tr>
<td>TB testing</td>
<td></td>
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<tr>
<td>Current therapy</td>
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<tr>
<td>Advances in science and technology</td>
<td></td>
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<tr>
<td>Pharmacy</td>
<td></td>
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<tr>
<td>Surgery</td>
<td></td>
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</table>
Table 2
Production goals for the deer farmer (from Haigh and Hudson 1993)\textsuperscript{4}

<table>
<thead>
<tr>
<th>Desired increases</th>
<th>Desired decreases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conception rates</td>
<td>Interval between first and last calves born</td>
</tr>
<tr>
<td>Calving rates</td>
<td>Feed costs</td>
</tr>
<tr>
<td>Weaning rates</td>
<td>Disease incidence</td>
</tr>
<tr>
<td>Weaning weights</td>
<td>Labor effort or time</td>
</tr>
<tr>
<td>15-month weights</td>
<td>Incidence of dystocia</td>
</tr>
<tr>
<td>Slaughter weights or velvet yields</td>
<td>Time to predetermined slaughter weights</td>
</tr>
<tr>
<td>Stocking rate</td>
<td></td>
</tr>
</tbody>
</table>

Table 3
Factors which can moderate simple relationships between fiscal and management components of a production medicine scheme

<table>
<thead>
<tr>
<th>Value of products</th>
<th>Cost of production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breeding stock</td>
<td>Feed</td>
</tr>
<tr>
<td>Venison</td>
<td>Medications</td>
</tr>
<tr>
<td>Velvet antler</td>
<td>Veterinary attention</td>
</tr>
<tr>
<td>Coproducts</td>
<td>Transport, etc.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Health related causes of loss</th>
<th>Uncontrollable factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-infectious</td>
<td>Misfortune</td>
</tr>
<tr>
<td>Infectious</td>
<td>Weather</td>
</tr>
</tbody>
</table>

Predators
Table 4
Animal Health
Important diseases of farmed deer in North America (adapted from Haigh and Hudson 1993)

<table>
<thead>
<tr>
<th>Non-infectious conditions</th>
<th>Intermediate</th>
<th>Bad management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bad luck</td>
<td>Dystocia</td>
<td>Dystocia</td>
</tr>
<tr>
<td>Misadventure</td>
<td>Predation</td>
<td>Nutritional diseases</td>
</tr>
<tr>
<td>Porcupine quills</td>
<td>Trauma</td>
<td>Late calving</td>
</tr>
<tr>
<td>Lightning strike</td>
<td></td>
<td>Poisonings</td>
</tr>
<tr>
<td>Vandalism</td>
<td></td>
<td>Trauma</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infectious conditions</th>
<th>Bacterial diseases</th>
<th>Parasitic diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant catarrhal fever</td>
<td>Clostridial diseases</td>
<td>Brain worm</td>
</tr>
<tr>
<td>Blue tongue and EHD</td>
<td>Colibacillosis</td>
<td>Elaeophorosis</td>
</tr>
<tr>
<td></td>
<td>Johne's disease</td>
<td>Liver fluke</td>
</tr>
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<td></td>
<td>Leptosporosis</td>
<td>Lungworm</td>
</tr>
<tr>
<td></td>
<td>Necrobacillosis</td>
<td>Winter tick</td>
</tr>
<tr>
<td></td>
<td>Tuberculosis</td>
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<tr>
<td></td>
<td>Yersiniosis</td>
<td></td>
</tr>
</tbody>
</table>
**CALVES**

- Tag not less than 12 hr after birth.
- Match to mothers.
- Watch for orphans.
- Ensure colostrum in store.
- Observe for scours, and take prompt action if needed.

**YEARLINGS**

- Monitor for worms, (esp. lungworm), treat if needed.
- If weaning Sept 1, ensure creep available well ahead.
- Weigh at weaning.
- Clostridial vaccine at weaning and again after 30 days, ± Leptospiral vaccine in risk areas.

- Continue worm surveillance, esp. in warm climates.
- Treat for ticks if needed.
- If weaned Nov. 1, carry out vaccination as above at 30 d intervals.

- Weigh monthly.
- Continue to supplement throughout.
- TB tests at any time from 6 months of age.

- Move to yearling column.

**Fig 2.**

Calendar of events in the management of calves and yearlings on a wapiti farm

(Dark stippling indicates maximal activity)

### ADULT MALES

Sort into mobs for velveting.  
Velveting may start in older stags.

<table>
<thead>
<tr>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perform udder check and sort into mobs for calving.</td>
</tr>
<tr>
<td>Ensure exercise and avoid overfeeding.</td>
</tr>
<tr>
<td>Calving may start mid-late May.</td>
</tr>
</tbody>
</table>

Velveting continues, sort as velvet matures.  
Treat with anthelmintics at velveting if needed.  
Weigh.  
Vaccinate with clostridial vaccine at velveting.  
TB tests can start after velveting.  
Remove secondary antler growth for market if desired.

<table>
<thead>
<tr>
<th>June</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main calving season tapering off into July.</td>
</tr>
<tr>
<td>Ensure top quality nutrition.</td>
</tr>
<tr>
<td>Ideal situation to have two calving paddocks, one for early, one for late calvers.</td>
</tr>
</tbody>
</table>

Sort stags into breeding mobs.  
Weigh.  
Monitor + treat for intestinal parasites.  
Depending on location, start tick treatment.  
Remove antler regrowth either for market or as hard antler.  
Semen test mature stags at month’s end.

<table>
<thead>
<tr>
<th>Aug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monitor nutrition, ensure adequate ME intake.</td>
</tr>
<tr>
<td>Check fecal samples for parasite eggs or larvae.</td>
</tr>
<tr>
<td>Depending on location, start tick treatment.</td>
</tr>
</tbody>
</table>

For wapiti, introduce stags in early - mid Sept, depending upon desired calving dates.  
Change to back-up stag after 4-5 weeks.  
Semen test younger breeding stags mid-month.  
Dates about 3 weeks later for red deer.

<table>
<thead>
<tr>
<th>Sept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wean calves about Sept 1.</td>
</tr>
<tr>
<td>TB test can be carried out before joining to stags.</td>
</tr>
<tr>
<td>Weigh adults.</td>
</tr>
<tr>
<td>Start tick treatment if needed.</td>
</tr>
<tr>
<td>Join wapiti for targeted first calving date.</td>
</tr>
</tbody>
</table>

Remove stags from breeding mobs.  
Nov 1st for wapiti, Nov 15-20 for red deer.  
Ensure good nutrition for stags after rut.  
TB tests can be conducted.

<table>
<thead>
<tr>
<th>Nov</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separate from stags about Nov 1st (wap) or 20th (red).</td>
</tr>
<tr>
<td>TB tests can be conducted from about a week later.</td>
</tr>
<tr>
<td>Repeat tick treatment.</td>
</tr>
</tbody>
</table>

Continue to ensure adequate nutrition after rut  
TB tests can still be conducted from this point.

<table>
<thead>
<tr>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy check 6 weeks after stag removal.</td>
</tr>
<tr>
<td>Manual check possible for wapiti.</td>
</tr>
<tr>
<td>Ultrasound for red deer.</td>
</tr>
<tr>
<td>Treat for ticks if needed, or if not treated in preceding fall.</td>
</tr>
</tbody>
</table>

Continue to ensure adequate nutrition.  
Obtain midwinter weights in late January.  
Initiate supplementary feeding for stags about 30 days before casting of antlers, which may start in late February or early March, depending on stag age & isolation.  
Ensure good nutrition during antler growth.  
Record all button casting dates.

<table>
<thead>
<tr>
<th>Jan-Apr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tick treatment if needed, or if not conducted in preceding fall.</td>
</tr>
<tr>
<td>Ensure good maintenance ration.</td>
</tr>
<tr>
<td>Vaccinate with clostridial vaccine 30-60 days before expected first calf.</td>
</tr>
</tbody>
</table>

### ADULT FEMALES

Perform udder check and sort into mobs for calving.  
Ensure exercise and avoid overfeeding.  
Calving may start mid-late May.

### Calender of year-round events in the management of adult animals on a wapiti farm.

(Dark stippling indicates maximal activity)
THE PREVALENCE OF BENZIMIDAZOLE RESISTANCE IN ANTELOPE COLLECTIONS IN FLORIDA

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Department of Clinical Sciences, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14850-6407, USA

Anthelmintic resistance has been defined as a heritable change in the ability of individual nematodes in a population to survive the recommended therapeutic dose of anthelmintic drug. This becomes clinically evident when anthelmintic treatments that were previously effective fail to prevent the pathological effects of the parasitic infection in the host. Resistance of trichostrongyloid nematodes to benzimidazole anthelmintics is common and worldwide in distribution in domestic sheep, goats and horses.

Previous studies have demonstrated that trichostrongyloid nematodes are a major source of morbidity and mortality in zoological collections of captive exotic ungulates and that anthelmintics are routinely used in these species. Benzimidazole resistance has previously been demonstrated in roan antelope (Hippotragus equinus), but no reports have documented the prevalence of this problem in exotic ungulate collections. The purpose of this survey was to estimate the prevalence of benzimidazole resistance in captive antelope in Florida.

All institutions considered for inclusion in this survey were located in Florida and were selected from a list of the American Association of Zoological Parks and Aquariums accredited institutions that were known to have collections of antelope. Private collections that had referred antelope to the University of Florida Veterinary Medical Teaching Hospital were also included. Of the 11 institutions identified as potential study sites, 7 granted permission to sample their collections. The study was conducted from August, 1992 to November, 1992. A group consisting of six male domestic sheep housed in an indoor enclosure at the University of Florida were selected to provide a source of benzimidazole susceptible controls.

Fecal samples from the antelope herds sampled as well as the sheep controls were examined for trichostrongyloid genera present using a fecal larval culture technique. Haemonchus spp. was found to be the predominant trichostrongyloid species in all the samples. A thiabendazole egg hatch assay was used to detect benzimidazole resistance.

Evidence of benzimidazole resistance was found in 6 of the 7 collections sampled, yielding an estimated statewide prevalence of between 42.1 and 99.6 percent (95 percent confidence limits). This study demonstrated that benzimidazole resistance is common in antelope in Florida.
Florida. Current parasite control practices used in these collections, particularly high treatment frequencies, limited rotation of anthelmintic classes, and chronic underdosing of anthelmintics probably contribute to the development of benzimidazole resistance.

Trichostrongyloid nematodes in captive exotic ungulates represent one of the few infectious diseases for which we have reliable antemortem diagnostic tests, an understanding of the basic epidemiology of the disease, and effective therapeutic agents to control the infection. Continued use of anthelmintics in poorly designed programs, relying solely on suppression of worm populations with drugs, may lead to the loss of these therapeutic agents and consequently an increase in animal morbidity and mortality. Because benzimidazole resistant worms were so prevalent in zoological collections in Florida, the continued use of these drugs should be reserved for cases where the efficacy of a particular drug has been confirmed for that collection.

LITERATURE CITED

VACCINATION OF ZOO BIRDS AGAINST AVIAN BOTULISM WITH MINK BOTULISM VACCINE

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Introduction

Following 7 consecutive summer outbreaks of avian botulism in and around the Denver Zoological Gardens, a vaccine trial was initiated in the spring of 1992 using a commercially available mink botulism vaccine (Botumink®, United Vaccines, Inc., P.O. Box 44220, Madison, Wisconsin 53744 USA). Waterfowl, pheasants and cranes were included, representing species in which losses to the disease had occurred over the 7-year period.

Materials and Methods

Two hundred seventy-three individuals of 42 species were inoculated with 1 ml of the product subcutaneously on the craniodorsal thorax between the wings. Table 1 lists the species included in the trial. All birds were given the same dose regardless of size; thus a small merganser received the same dose as a large crane. The area was swabbed with alcohol prior to each injection. Vaccinations began in mid-April and were still in progress when the eighth summer botulism outbreak began in early July. A second vaccination was administered at approximately 1 month.

The product used (Botumink®) is a bacterin-toxoid prepared from a pure culture of Clostridium botulinum Type C, inactivated with formalin and combined with an aluminum adjuvant. It is labeled for use in mink, and has been sold commercially in over 35 countries for the past 25 years. The recommended dose in mink at least 6 weeks old is 1 ml injected subcutaneously under the loose skin of the armpit. Adults are revaccinated annually with the same dose at the time of kit vaccination.

Heparinized plasma samples were collected from 91 birds at the time of first vaccination, from 78 of these individuals at the time of second vaccination 1 month later, and from a subset of 22 of these birds after an additional month. All samples were frozen at -80°C pending decision on the most appropriate laboratory assay procedure.

Results

Empirical data suggest that Botumink® was highly safe and efficacious in the wide range of zoo birds vaccinated:

1. No vaccinated birds became sick or died due to botulism.
2. Five zoo ducks not inoculated for fear of disrupting nesting were found dead and 2 sick ones were saved by treatment. Botulism was confirmed as the cause of death by the U.S. Fish and Wildlife Service’s National Wildlife Health Research Center, Madison, Wisconsin. These sick and dead zoo birds (2 Northern eiders [Somateria mollissima borealis], 5 Barrow’s goldeneyes [Bucephala islandica]) were in an area frequented by wild ducks and geese, several of which were also found dead.

3. Numerous wild waterfowl that freely shared pens or ponds with vaccinated zoo birds died (80 ducks, 3 geese), and 13 wild ducks and 2 geese were treated in the zoo hospital and released in an area remote from the zoo. In some cases, maggot-infested carcasses were found floating in ponds with healthy zoo birds that were undoubtedly consuming these toxin-laden delicacies.

4. There were no known adverse reactions to the vaccine. Inspection of the injection site showed normal appearing skin when birds were captured for second vaccination or follow-up bleeding.

Discussion

Neither the dosage, frequency, nor site of vaccination in avian species were known to the investigators or vaccine manufacturer. The authors opted for a 2-injection regimen, but in retrospect this may not have been necessary as many birds had only one injection by the time of the explosive botulism outbreak and seemed to be protected. The interval between vaccination and production of protective antibody to botulism toxin was also unknown. Rather than being based on a "best guess" estimate of optimum time for vaccination prior to the outbreak, the starting time of this project (mid-April) was decided after the acute deaths of a group of mergansers that were later shown to have been caused by a disease other than botulism. Typically it was June or July or later before the start of the annual botulism die-off. Duration of immunity was another unknown factor, prompting addition of the second dose.

Vaccinations were repeated in the spring and summer of 1993. Previously-vaccinated birds received a single booster, while naive birds received the 2-vaccine regimen. Current plans are to incorporate yearly botulism vaccination into the preventive medical program for appropriate birds.

Data from the frozen plasma samples have yet to be generated. Discussions with the vaccine company and with a university laboratory have not identified the most suitable test to gauge host response to vaccination.

ACKNOWLEDGMENTS

The authors thank Roger G. Brady and Dr. Herbert Kammer of United Vaccines, Inc., for advice and information used in the vaccine trial and in this report. Curt Sturges, DVM, former preceptor, was instrumental in identifying and locating the vaccine company. Our greatest thanks go to Susan Haeffner, Area Supervisor, and to Mike Hudak and other Denver Zoo Keepers who assisted in this study.
Table 1. Bird species in 1992 Denver Zoo botulism vaccination trial

<table>
<thead>
<tr>
<th>Order</th>
<th>Species</th>
<th>Order</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Anseriformes</strong></td>
<td></td>
<td><strong>Galliformes</strong></td>
</tr>
<tr>
<td></td>
<td>Tufted duck</td>
<td></td>
<td>Vulturine guinea fowl</td>
</tr>
<tr>
<td></td>
<td>Cape shelduck</td>
<td></td>
<td>Golden pheasant (Ghigi)</td>
</tr>
<tr>
<td></td>
<td>Northern pintail</td>
<td></td>
<td>Great argus pheasant</td>
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<tr>
<td></td>
<td>Ruddy shelduck</td>
<td></td>
<td>Himalayan impeyan pheasant</td>
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<tr>
<td></td>
<td>Falkland flightless steamer duck</td>
<td></td>
<td>White-eared pheasant</td>
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<tr>
<td></td>
<td>Mandarin duck</td>
<td></td>
<td>Greater Bornean crested fireback</td>
</tr>
<tr>
<td></td>
<td>Northern eider</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Redheaded duck</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Ruddy duck</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Marbled teal</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Smew</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Canvasback</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Barrow's goldeneye</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>American goldeneye</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Chestnut-breasted teal</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Mute swan</td>
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<tr>
<td></td>
<td>Coscoroba swan</td>
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<td></td>
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<tr>
<td></td>
<td>Black swan</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>American merganser</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hooded Merganser</td>
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<td></td>
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<td></td>
<td>Lesser snow goose</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Red-breasted goose</td>
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<td></td>
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<tr>
<td></td>
<td>Emperor goose</td>
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<td></td>
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<td></td>
<td>Nene goose</td>
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<td></td>
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<tr>
<td></td>
<td>Egyptian goose</td>
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<td></td>
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<tr>
<td></td>
<td>Crested screamer</td>
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<td></td>
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<tr>
<td></td>
<td>Bar-headed goose</td>
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<td></td>
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<td></td>
<td>White-winged wood duck</td>
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<td></td>
<td>Lesser Magellain goose</td>
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<td></td>
<td>Red-crested pochard</td>
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<td></td>
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<td></td>
<td>Rosybill pochard</td>
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<tr>
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<td>Galliformes</td>
<td></td>
<td>Gruiformes</td>
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<tr>
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<td></td>
<td></td>
<td>White-naped crane</td>
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<td></td>
<td></td>
<td>Hooded crane</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>East African crowned crane</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Demoiselle crane</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Wattled crane</td>
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</tbody>
</table>
Prompted by diagnosis of paratuberculosis in 2 Blesbok (Damaliscus dorcas phillipsi) and a Chinese Reeve's Muntjac (Muntiacus r. reevesi) in 1991, a survey of ungulates at the San Diego Wild Animal Park for the causative agent, Mycobacterium paratuberculosis, was initiated in the summer of 1992. Fecal samples were collected from the ground from identified individual animals. Three grams of feces were placed into 30ml of HPC decontaminant/transport medium at the park. All fecal samples were sent to the laboratory, as per protocol, within 3 days of collection. At the laboratory, the samples were processed by radiometric culture for M. paratuberculosis. This technique involves filter (3um pore diameter) concentration of 10ml of supernate in the feces /HPC mixture after over night settling. The 13mm diameter polycarbonate filter was placed directly into modified BACTEC 12B radiometric culture medium (Becton Dickinson Diagnostics, 383 Hillen Road, P.O. Box 20086, Towson, MD 21204, U.S.A.). This commercially available medium contains 14C palmitic acid as the principal carbon substrate and the 14CO2 resulting from microbial metabolism is detected by a gas ionization detector called a BACTEC 460 (Becton Dickinson Diagnostics, 383 Hillen Road, P.O. Box 20086, Towson, MD 21204, U.S.A.). The medium was modified by addition of 1.0ml egg yolk suspension (Difco Laboratories, P.O. Box 331058, Detroit, MI 48232), 1.0ug/ml mycobactin (Allied Monitor, P.O.Box 71, Fayette, MO 65248, U.S.A.)and three antibiotics (vancomycin 10ug/ml, amphotericin B 20ug/ml, and nalidixic acid 30ug/ml). Vials of medium were incubated at 37 degrees C and growth was monitored weekly on the BACTEC 460. When the growth index reached a value >100, an aliquot from the vial was inoculated into a blood agar plate and an acid-fast stain was performed. If no growth was observed on the blood agar plate after 48 hours incubation, ruling out non-mycobacterial contaminants, and if acid-fast bacteria typical of mycobacteria were observed on the stained smear, we reported the presumptive isolation of a mycobacterial species. Most isolates were detected after 4-6 weeks of incubation whereas cultures were declared negative if no growth was detected after 7 weeks incubation. The mycobacterial isolates were identified using a PCR amplified DNA probe (IDEXX Laboratories, Inc.,One IDEXX Drive, Westbrook, ME 04092, USA) for M. paratuberculosis. When the isolates tested negative with this probe, we attempted to establish their identity using RNA probes for other mycobacteria (AccuProbe, GenProbe, 9880 Campus Point Drive, San Diego, CA 92121, USA). Non-M. paratuberculosis isolates were sequentially tested using AccuProbe kits for M. avium, M. intracellulare, and the TB complex (M. tuberculosis and M. bovis), respectively.

Over 80% of the 1500 ungulates at risk at the park were sampled. Over 1600 fecal and tissue specimens were tested by radiometric culture from June 1992 to May 1993. Fifty isolates of mycobacteria were obtained; 31 (62%) were identified as M. paratuberculosis. No
mycobacterial isolates were found to be in the TB complex, 3 (6%) were *M. intracellurar*, and 6 (12%) were identified as *M. avium*. Ten (20%) mycobacterial isolates did not test positive with any of the nucleic acid probes employed and have been sent to a reference laboratory for further identification. Species and number of animals positive for *M. paratuberculosis* by radiometric culture are shown in Table 1. Several of these animals share a common enclosure. Also, *M. paratuberculosis* was isolated from pond water in one of the enclosures that had multiple positive animals.

Results of our survey reveal that the infection is limited to only one-third of the park. Infection prevalence in this area is estimated at 10%. Measures have been instituted to prevent spread of the infection to other parts of the park. In addition, the more heavily infected species of animals have been removed from the enclosures (Table 1.). Surveillance of animals at the park by fecal culture continues. Movement within and out of the collection is restricted to animals from negative areas/exhibits. DNA "finger print" technology is being used to further identify each isolate and will contribute to the better understanding of the epidemiology of this disease outbreak.

Table 1. Species and number of animals positive for *M. paratuberculosis* by radiometric culture at the San Diego Wild Animal Park

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Animals</th>
<th>Culture Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern White Rhinoceros (<em>Ceratotherium s. simum</em>)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>*Blesbok (<em>Damaliscus d. phillipsi</em>)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>*Angolan Springbok (<em>Antidorcas m. angolensis</em>)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>*Reeve’s Muntjac (<em>Muntiacus r. reevesi</em>)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Gemsbok (<em>Oryx g. gazella</em>)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Dybowski’s Sika (<em>Cervus n. hortulorum</em>)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Addra Gazelle (<em>Gazella d. ruficollis</em>)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>*British Red Deer (<em>Cervus e. scoticus</em>)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Japanese Serow (<em>Capricornis c. crispus</em>)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Goral (<em>Nemorhaedus g. arnouxianus</em>)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Lowland Nyla (<em>Tragelaphus angasi</em>)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Arabian Oryx (<em>Oryx leucoryx</em>)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Axis deer (<em>Cervus a. axis</em>)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Red Lechwe (<em>Kobus l. leche</em>)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>West Caucasian Tur (<em>Capra i. caucasica</em>)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Siberian Ibex (<em>Capra i. siberica</em>)</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*=Removed from exhibit*
AN OUTBREAK OF RABIES AMONG AFRICAN WILD DOGS (Lycaon pictus) IN THE MASAI MARA, KENYA

K.A. Alexander, DVM*
National Museums of Kenya, Department of Molecular Genetics, P. O. Box 40658, Nairobi, Kenya

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A pack of African Wild Dogs (Lycaon pictus) ranging to the north of the Masai Mara National Reserve in southwestern Kenya was monitored from 1987 to 1989. During a six week period (1 August - 14 September, 1989), 20 of 22 members of this pack died. It was possible to retrieve five carcasses for necropsy and histopathological examination. Gross findings varied among individuals and included multiple bite wounds; synovitis; lymphadenopathy; submandibular, cervical and vocal cord edema; blood in bronchi, bronchioles, stomach and intestine; and anterioventral lung lobe consolidation.

Histological features also varied among individuals and included severe suppurative bronchopneumonia, suppurative vasculitis, valvular endocarditis, myocarditis and lymphoid depletion of the lymph nodes, tonsils, and spleen. Histological examination of two available brain samples revealed eosinophilic intracytoplasmic inclusions (Negri bodies), supporting the diagnosis of rabies viral encephalitis. An additional brain sample tested positive for rabies via fluorescent antibody test (IFA). Of four serum samples collected from individuals demonstrating clinical signs, two tested positive for rabies antibodies using a serum neutralization test. Clinical signs observed were similar in all affected individuals. Only the paralytic form of rabies was apparent, and clinically ill individuals were often the recipients of aggression from pack mates.

A serologic screen for rabies antibodies was performed on samples collected between 1987 and 1990 from 18 apparently normal African Wild Dogs in the study site. These samples, collected from individuals from five different packs, were all seronegative.
RABIES IN THE SPOTTED HYENA (Crocuta crocuta): AN UNPROVOKED ATTACK ON A HUMAN SETTLEMENT IN THE MASAI MARA, KENYA

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In November, 1992, an adult spotted hyena (Crocuta crocuta) attacked humans in two settlements located just north of the Masai Mara National Reserve in Kenya. This was the third such attack since 1990 in this area. Six people were severely bitten during this incident and one boy died as a result of wounds inflicted. The hyena was killed by the family members, who destroyed the carcass to the extent that the animal could not be accurately weighed or sexed.

A brain sample was collected by incising the skin and musculature over the joint of the occipital bone and atlas. With the head bent, a plastic straw was inserted through the occipital foramen and directed towards one eye. The straw was then pinched and withdrawn from the foramen. The sample contained in the straw was then preserved in a 50% glycerol solution. With this technique, the rachidian bulb, basis of cerebellum, hippocampus (i.e. Amon's horn region) and cortex were sampled. This procedure is described by Barrat and Blancou (1988). The brain sample was found positive for rabies when tested with a fluorescent antibody test (IFA).

A serologic screen for rabies antibodies was conducted using serum samples collected from 1989 - 1992 from spotted hyenas in this region (n=72). Hyenas were immobilized with Telazol® (Fort Dodge Laboratories Inc. Fort Dodge, Iowa 50501) using a remote injection system. Anesthesia was maintained from 35 to 55 minutes with a dose of 3-4 mg/kg. All animals were found seronegative for rabies antibodies using a Rapid Fluorescent Focus Inhibition test.

The area of private land just outside the Masai Mara National Reserve is populated by the Masai Tribe, their domestic animals and a high density of wildlife species. Rabies has been diagnosed in this region in domestic dogs, cats, cattle and wildlife species such as the African Wild Dog (Lycaon pictus). As spotted hyenas frequently scavenge around human settlements and tourist lodge garbage dump sites, they frequently come in contact with domestic dogs (Canis familiaris). The dynamics of rabies in this ecosystem is not yet fully understood but based on preliminary data, the domestic dog is suspected to be the main reservoir species.
AORTIC STENOSIS AND ATRIO/VENTRICULAR DILATATION IN A GREEN IGUANA (Iguana iguana)

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Introduction

The circulatory system in non-Crocodilian reptiles is unique to those found in avian and mammalian species. The incompletely divided ventricle receives blood from both the right and left atria. A muscular ridge and blood current flows distribute deoxygenated blood primarily into the pulmonary artery and oxygenated blood primarily into the right systemic aortic arch. With its opening situated between that of the pulmonary artery and right aortic arch, the left aortic arch carries blood of mixed oxygenation to the systemic circulation, dependent upon blood volume, pressures, and flow. Though differences in anatomy and physiology exist, reptilian cardiovascular disease may be investigated by methods utilized in small animal medicine: auscultation, thoracic radiography, electrocardiography, and echocardiography.

Case Report

A 1.1 kg adult male green iguana (Iguana iguana) was presented to the Veterinary Medical and Surgical Group, Ventura CA for the complaints of decreased activity, inappetence, change in skin color, and increased respiratory rate of two days duration. The iguana had a history of a surgical tail amputation one year prior to presentation and had been owned by our client for approximately four months. No previous husbandry or medical information was otherwise available. Current diet included a variety of fruits and vegetables, Science Diet feline maintenance kibble, and periodic bird gravel. The iguana roamed the house freely with access to a heated perch and to limited interactions with a domestic cat.

Physical examination revealed skin discoloration consisting of shades of orange and brown, muffled heart sounds, increased respiratory rate, mildly increased bronchovesicular sounds, and generalized muscle atrophy. Radiographically, a rounded soft tissue density filled two-thirds of the cranioventral thorax. On ultrasound examination using a real time scanner and both a 7.5 MHz CW transducer and a 7.5 MHz PW fluid offset transducer (Ausonics Opus I, Sydney, Australia), the soft tissue density was identified as an enlarged heart chamber surrounded by pericardial effusion. Poor contractility of the enlarged ventricle was noted. Differential diagnoses under consideration included dilated cardiomyopathy, cardiac neoplasia, soft tissue abscess, or parasitic nodule. The owner declined centesis to further define the condition.

Supportive care was initiated, including an intramuscular injection of a multivitamin preparation (0.15 ml/kg once, Injacom 100, Roche Vitamins and Fine Chemicals, Nutley NJ 07110), oral trimethoprim/sulfa (15 mg/kg q 12 hours, Sulfamethoxazole and Trimethoprim, Phoenix Pharmaceutical, Inc, St. Joseph MO 64506), syringe-fed baby food and water, and increased warmth and humidity to the home environment.

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Similar clinical signs and physical examination findings were present two days later. Electrocardiogram demonstrated widened QRS complexes, increased Q-T interval, and a mean electrical axis of approximately +60°. Extrapolating from normal feline electrocardiogram values, these findings were consistent with increased electrical conduction times and ventricular enlargement. Mild to moderate pericardial effusion was again visualized on echocardiography. Wall to wall measurement of the ventricle was 4.63 cm. Hyperechoic shadowing within the beating free walls of the heart was compatible with sluggish blood flow or with a soft tissue mass. Furosemide (5 mg/kg q 24 hrs, Lasix Syrup 10%, Hoechst-Russel, Somerville NJ 08876) was added to the supportive treatment regimen. Angiography was declined by the owner.

Sixteen days after initial presentation, little improvement was noted on physical examination. The respiratory rate remained increased at approximately 40 breath per minute. Muscle mass continued to decline. On auscultation, a faint murmur was heard. Thoracic radiographs were repeated, showing an increased size in the heart shadow. On repeat cardiac ultrasonography, persistent pericardial effusion, a decreased wall-to-wall ventricular measurement of 3.64 cm, and unchanged mild intraventricular hyperechogenicity were present. Blood collected from the caudal tail vein was submitted for complete blood count (Avian & Exotic Animal Clin/Path Labs, Redondo Beach CA 90278). White blood cell indices were within normal limits, with slight to moderately degranulated heterophils. Working diagnosis at this time was congestive heart failure related to a dilated, poorly contracting ventricle. Frequency of diuretic therapy was increased to q 12 hours.

Twenty days after initial presentation, the owner was contacted by telephone. She had discontinued all medications due to the iguana's poor condition, which included periods of dyspnea, discoloration, and dysphagia. Recheck examination, further diagnostics, and therapeutics were declined.

On physical examination seven weeks after initial presentation, the iguana was extremely weak, discolored, orange, and hypothermic. Mucous membranes were pale. Absence of lung sounds ventrally and muffled heart sounds were auscultated. Increased pericardial effusion, rounded cardiac chambers, and poorly moving valves were seen on ultrasonographic evaluation. Euthanasia and necropsy were requested by the owner due to severe lethargy and deterioration. Euthanasia was accomplished by Isoflurane (AErrane, Anaquest, Madison WI 53713) overdose.

Gross necropsy showed severe muscle atrophy, straw-colored pericardial effusion, an enlarged heart (7 x 5 x 3.5 cm), caudodorsally displaced lungs, gas-dilated stomach and proximal intestinal loops, diffusely pigmented pancreas, and renal pelvic urates bilaterally. The pericardial fluid was classified as a modified transudate, with mesothelial cells, a 1.020 specific gravity and 2.0 gm/dl total protein. The intact heart and samples of tissues from all major organ systems were fixed in 10% formalin and submitted for histopathological evaluation (PET/PATH American Histolabs, Gaithersburg MD 20879).
Gross pathologic findings of the heart were as follows. The right atrium and right ventricular segment were extremely dilated with extensive attenuation of the ventricular wall (<1mm thick) compared to the left portion of the ventricular free wall and incomplete septum (3-5 mm). Thinned chordae tendinae and accentuated trabeculae were present in the ventricular chamber. As they exited the heart base, the left and right systemic branches of the double aorta were markedly stenotic (2 mm diameter). The pulmonic trunk was dilated with an aneurysm of the right branch.

Histopathologic findings were extreme attenuation of right ventricular myocardial fibers without degenerative or inflammatory changes and relatively normal myocardial fibers of the left ventricle and septum. Generalized mesothelial reaction was present. Both aortic arches had narrowed lumens with irregular thickened intimal surfaces, focal mild calcification, and mild patchy inflammatory changes. Pulmonary vasculature was extremely distended with thinned walls. At its atrial junctions, the lung showed smooth muscle hypertrophy.

Increased numbers of hematopoietic cells and mild fatty change were seen throughout the hepatic lobules. Kidney tissue had moderate segmental interstitial fibrosis with some tubular and glomerular drop out. A few tubules had gouty tophi with distended lumens and lymphocytic foci. Stomach, small and large intestine, coelomic fat, spleen, and skeletal muscle were within normal limits.

Discussion

Generalized cardiomegaly of this iguana was evaluated diagnostically via radiography, electrocardiogram, ultrasonographic imaging, necropsy, and histopathology. Unusual cardiovascular changes were found. No significant myofiber changes were seen microscopically to suggest a primary cardiomyopathy to account for the gross cardiac dilatation. The right atrio/ventricular myocardium was primarily affected. The thin-walled saclike ventricular myocardium most likely resulted secondary to chronic high pressures leading to overstretching. Chronicity may suggest a congenital etiology which might be explained by the stenosis of the double aorta. The aortic intima was thickened and irregular with only mild focal calcification.

Narrowing of the left ventricular outflow tract impeded normal systemic ejection. Incomplete dual circulation would likely allow this systemic stream to preferentially shift to pulmonic branches where lower resistance initially exists. With greater blood volumes to move, right ventricular pressures increase and may chronically lead to dilatation of right heart chambers, jet stream ectasia and aneurysm of the pulmonary artery segment, and distended thinned pulmonary vasculature, as was seen in this case.

In this patient, low output heart failure ensued with its associated clinical signs of weakness, pallor, and severe weight loss. Advanced effects of congestive heart failure on other organs were not appreciated at necropsy, though cardiomegaly, hypocontractile ventricle, and fluid accumulation in the extravascular pericardial space occurred antemortem. Pericardial effusion may have been a sign of early congestive heart failure.
Therapeutics in cardiac failure address pump failure, volume overload, and compensatory vasoconstriction. Standard treatment protocols include positive inotropic agents, diuretics, and vasodilators to improve contractility, to reduce fluid accumulation and venous congestion, and to enhance forward blood flow, respectively. Ventricular volume decreased in response to diuretic therapy in this iguana. Additional medical therapy and pericardiocentesis may have improved cardiac function.

Angiography may have further defined this clinical condition antemortem. Throughout clinical work-up and treatment, cardiomyopathy was suspected, but a ventricular mass related to neoplasia or bacterial infection could not be ruled out entirely. Ultrasonographic evaluation showed a mild hyperechogenicity within the ventricular wall, compatible with sluggish blood flow or with soft tissue density. Contrast media would highlight an intraluminal mass and/or define dilatation of chambers and vessels.

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SEASONAL CHANGE IN SERUM PROLACTIN CONCENTRATIONS IN THE CAPTIVE MALE BLACK BEAR, *Ursus americanus*

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American black bears, *Ursus americanus*, have a mating season from late spring to early summer. Our previous study in black bears suggested that spermatogenesis initiated during mid-denning period with the elevation of testosterone and estradiol-17β which may be synthesized in Leydig cells and Sertoli cells, respectively. However, it is not known what stimulates the steroid synthesis in the bear testis at the time of initiation. Therefore, we examined preliminarily the possibility of prolactin, one of polypeptide hormones from pituitary glands as a factor which stimulates testicular steroid synthesis. Our objectives were to establish prolactin assay with bear sera and to describe seasonal changes in serum prolactin concentrations under the captive condition. Serum samples were collected 9 times during a year from 3 captive mature male black bears which were in denning between November and March and were active during the other period. Prolactin radioimmunoassay using porcine prolactin conjugated with 125I and goat anti-porcine prolactin as a primary antibody was established for bear serum samples with a parallelism between standard of porcine prolactin and prolactin concentrations of several diluted bear sera and pituitary extracts. Furthermore, prolactin response for TRH injection was obtained from the 3 male bears in June. The sensitivity of assay was 0.08 ng/tube. Coefficiency of variation for intra- and inter-assay were 5.5% (n=6) and 5.7% (n=6), respectively. Serum prolactin concentrations from the bears changed seasonally with the lowest in December (Mean ± SD = 1.1 ± 0.1 ng/ml) and the highest in May (17.6 ± 4.7 ng/ml) preceding the peak of testosterone concentrations in June. We conclude that prolactin is an useful indicator of testicular steroidogenic activity and may play an important role as a modulator of steroid synthesis, particularly during the pre-mating season in the bear testis.
MYCOBACTERIOSIS IN HAIRY-FOOTED HAMSTERS (Phodopus sungorus)

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Introduction

Bacteria in the genus Mycobacterium are widely distributed in nature, and several species have been documented in captive exotic animals. Infection occurs via portals of entry such as umbilical vein, alimentary or respiratory tracts, and cutaneous wounds. Cell-mediated resistance to infection is largely dependent on the ability of macrophages to inhibit the growth of intracellular mycobacteria. Disease, drugs, endogenous hormones, or malnutrition can compromise cell-mediated immunity (CMI). Inherently dysfunctional CMI can exist in some animals.

Mycobacterial infections were diagnosed in six hairy-footed hamsters (Phodopus sungorus) examined over a period from 1988 to 1993 at the National Zoological Park (NZP). Herein, we describe clinical and pathological features of mycobacteriosis in these hamsters.

Case Reports

One hamster was found dead, months after being separated from cage mates in an effort to prevent intraspecies aggression. At necropsy, this animal was in poor nutritional condition, alopecic, and had a renal tumor. Histopathologic examination revealed granulomatous hepatitis with acid-fast bacillary bacteria (AFB); the renal tumor was diagnosed as a carcinoma.

Another hamster, presented to the NZP Department of Animal Health with unilateral conjunctivitis; physical examination also revealed unilateral, subcutaneous abscessation in the thoracic-axillary region. Examination of cytologic preparations of the exudate from this lesion revealed pyogranulomatous inflammation with intracellular and extracellular AFB. Culture of the exudate yielded Mycobacterium avium serotype 4. Histopathologic examination revealed mycobacterial pneumonia and hepatitis, and an ovarian embryonal tumor.

Four of the six hamsters had multifocal to coalescing, occasionally ulcerated, ventral abdominal and inguinal cutaneous lesions. These animals were euthanized and post mortem examinations were done. On cut surface, the affected dermis and subcutis were markedly thickened, firm, nodular, and pale yellow to white. In one animal, a white caseous exudate was present in the dermis. The ulcerated lesions were covered by a mixture of plant material and debris originating from the substrate (sphagnum moss and soil). Impression smears of the lesions revealed numerous macrophages intermixed with many neutrophils; low numbers of AFB were within some macrophages, and rarely extracellularly.
Mycobacteriosis was not suspected in the first three hamsters of this group, and consequently, tissues were not submitted for culture. Attempts to culture mycobacteria from the last case were unsuccessful.

Histopathologically, the cutaneous lesions were characterized by a granulomatous to pyogranulomatous dermatitis and panniculitis. Multiple nodular aggregates of closely packed neutrophils closely surrounded variably sized, extracellular vacuoles (30 to 200 μm in diameter). Few to moderate numbers of bacillary to filamentous AFB were within some of the extracellular vacuoles, scattered macrophages, and free in the interstitium.

Conclusions

In four of the six hairy-footed hamsters in this report, granulomatous cutaneous lesions containing AFB were limited to the ventral abdominal and inguinal areas. Features of these lesions are characteristic of those reported in other species with "atypical mycobacteriosis", caused by opportunistic, saprophytic Mycobacterium species. Two of these animals were litter mates, and the other two were cage mates. Intraspecies aggression among hairy-footed hamsters is not uncommon, and the supine defensive and/or submissive posture predisposes the hamsters to cutaneous wounds of the caudoventral surface of the body. Cutaneous wounds in these hamsters, resulting from intraspecies aggression, provide portals of entry for many types of saprophytic or parasitic Mycobacterium species possibly present in the substrate.

The two hamsters with internal mycobacteriosis were aged animals with neoplasms that most likely caused debilitation, predisposing them to opportunistic infections.

All of the hamsters in this report were female. The role that sex phenotype played in intraspecies behavior and/or cell-mediated immunity (CMI) is not clear. Other possible variables that warrant investigation are: (1) species- and/or familial-associated impaired CMI; (2) the roles of female physiology and anatomy in hormonally-induced impaired CMI and/or mycobacterial mastitis; (4) the presence of mycobacteria in exhibit substrate; and (5) the profile of the normal flora of this species's oral cavity.

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EVALUATION OF EXPERIMENTAL MENINGEAL WORM INFECTIONS IN THE LLAMA

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Introduction

The meningeal worm, *Parelaphostrongylus tenuis*, is a generally benign entity in its natural host the white-tailed deer, *Odocoileus virginianus*.\(^1\) In contrast, the meningeal worm causes fatal neurological disease in aberrant wild cervid hosts including elk, *Cervus elaphus*, and moose, *Alces alces*, when these species become sympatric with white-tailed deer.\(^2\) Fatal infections in reindeer, *Rangifer tarandus*, and sable antelope, *Hippotragus niger*, have been reported from the National Zoological Park's Conservation and Research Center in Front Royal, Va.\(^3\) Although the distribution of the parasite is restricted to eastern North America,\(^1,2\) a llama, *Lama glama*, purchased in Virginia later died due to meningeal worm infection in New Mexico, which is west of the endemic area of the parasite.\(^4\) Deaths in lamoids due to meningeal worm infections had been previously reported in Texas,\(^5\) Ohio,\(^6\) and Minnesota.\(^7\) It is unknown, however, if death precedes the prepatent period for meningeal worm in the llama. As llamas are routinely shipped across the country between zoos as well as animal dealers and private individuals, the ability to transmit meningeal worm into non-endemic areas could exert a large negative impact on native western wildlife and zoo ruminants. The purpose of this study was to determine if the life cycle of the meningeal worm can be completed in the llama.

Materials and methods

One female and five male adult llamas which had received no anthelmintic treatment for 60 days were chosen for experimental infection. All were born and raised in Oregon. Two male yearling white-tailed deer from Washington were used as control animals. All animals were housed in the maximum isolation section of Oregon State University's Veterinary Medical Animal Isolation Laboratory. A total of 44 infective L-3 larvae were obtained from five laboratory-reared snails, *Tridopsis multilineata*, which had been artificially infected with *P. tenuis*. Each llama was given five larvae directly in the first compartment of the stomach via orogastric tube. After the worms were introduced, each tube was flushed with 1280 ml water. One control deer received 6 larvae and the second deer received 7 larvae. Blood samples for complete blood counts (CBC) and biochemistry panels and cerebrospinal fluid (CSF) were collected prior to infection, at day 40, and immediately prior to euthanasia. Additional CSF samples were collected at approximately 20 day intervals beginning on day 60. Individual fecal samples were examined, using the Baermann technique, for dorsal-spined larvae weekly beginning on day 40 and daily beginning on day 60.
All animals were examined for clinical signs of neurological deficits once daily from days 0 through 40 and twice daily thereafter. The degree of neurological deficit was graded on a scale of 0 to 4. Llamas were euthanized if complete or partial paralysis caused sudden inability to stand, if sudden inability to ambulate sufficiently prevented access to food or water, or if progressive ataxia led to a minimum grade 3 neurologic deficit. White-tailed deer were euthanized at day 140, after which any surviving llamas were also euthanized. Complete necropsies were performed including sagittal brain sections and transverse sections of spinal cord at each intervertebral junction. All spinal epidural, subdural and leptomeningeal surfaces were examined and the spinal cord teased apart.

Results

CBC and biochemistry panels did not change significantly during the infection period. All values remained within normal ranges. CSF glucose, AST and protein values did not change significantly during the infection period. CSF CK values were occasionally mildly-moderately elevated. There was a general trend for a shift in the CSF cellular composition. As the infection progressed, the normal lymphocyte proportion was replaced with monocytes which were subsequently replaced with eosinophils. Eosinophils were first noted in the CSF between days 40-70 post-infection, and in 4/6 llamas increased through the end of the study. Llama fecal exams were consistently negative for dorsal-spined larvae. Feces were positive for dorsal-spined larvae for the second control deer on day 137 post-infection. Neurological signs were first noted in the llamas from day 44 to day 53, generally in the hind limbs first. One llama died as a result of the infection at day 90 post-infection. 3/6 llamas were euthanized due to progression of neurologic signs at days 70, 84 and 124 post-infection. On necropsy, adult meningeal worms were found in 3/6 llamas. Locations included mid-cervical spinal cord, frontal lobe, brain stem, and cerebellum. Mature male and female meningeal worms were found one control deer. Several worm pieces were recovered from the second deer. On histopathology, five of six llamas had brain and spinal cord lesions consistent with the migration of a nematode parasite. Common histopathology included cerebral meningitis (4/6), cerebellar meningitis (3/6) and medullary meningitis (3/6). No lesions suggestive of parasitic migration were observed in one llama (#295).

Discussion

Based on the results of this study we believe it to be highly unlikely that the meningeal worm successfully complete its life cycle in a llama host. In this study, a 66% (4/6) mortality rate occurred prior to the onset of patency in the control deer. No larvae were found in the lungs of the two llamas that survived the infection until the end of the study, nor were any live, healthy adults capable of reproduction recovered from their CNS. Larvae were not recovered from llama feces at any time through day 146 post-infection. No CNS lesions indicative of parasitic migration were found at necropsy in llama 295 at day 146 post-infection. However, the eosinophilia noted in the CNS was subsiding at the time of euthanasia likely indicating a previous infection was subsiding. These data suggest that...
meningeal worm infection will either cause death in the llama prior to achieving patency, or that the worms themselves will fail to survive and complete their life cycle due to host unsuitability. Consequently, we believe the risk of llamas transporting patent meningeal worm infections to a non-endemic area to be minimal. When combined with clinical neurological deficits in llamas from endemic areas, elevated CSF eosinophil and CK values may suggest meningeal worm infection as a differential diagnosis but should not be considered to be specific indicators.

LITERATURE CITED


USE OF STABLE ISOTOPE RATIOS TO DISCRIMINATE DIETARY COMPONENTS OF FREE-RANGING ANIMALS: AN EXAMPLE WITH CHESAPEAKE BAY CANVASBACKS

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Introduction

Canvasback duck (*Aythya valisineria*) use of the Chesapeake Bay, an important traditional wintering area for the species, has declined markedly over the past four decades as evidenced by decline in the wintering population. Degradation of aquatic habitats has led to major changes in foods available. Most notable has been the loss of submerged aquatic vegetation, the historic and preferred food of canvasbacks, and a dietary shift to low-benefit shellfish, in particular, the ubiquitous Baltic clam (*Macoma balthica*). The effects of this dietary change on the status of canvasbacks has not been well studied, but it might affect body condition, winter survival, winter distribution and subsequent breeding performance. An additional component in the winter nutrition of canvasbacks is the feeding of corn by urban residents who wish to attract ducks to their waterfront properties. This activity raises the compelling question of subsistence feeding by canvasbacks on a terrestrial source of food in light of the diminished natural estuarine food supply. Our study employs stable isotope chemistry as a potential tool to investigate the food habits of canvasbacks on Chesapeake Bay and specifically to evaluate the importance of corn versus benthic animal foods in the canvasbacks' winter diet.

Stable isotope chemistry provides a new and potentially powerful method to investigate trophic and nutritional relationships. Originally devised by geochemists as a technique to trace global elemental cycling, only recently has this technology been used by ecologists to investigate ecosystem function. The technique is based on the finding that the isotopic signature of an animal's tissue reflects that of its food plus enhancement by fractionation during metabolism. Thus different food sources and trophic levels potentially can be identified by determination of the isotopic signature of an animal's tissue versus that of sources of available food. Ideally, if an animal consumes only a few dietary items and those items can be discriminated isotopically, then the greatest potential exists for success of this method to determine contributions from those foods, for example carbon, to specific tissues. This technique is gaining greater acceptance in applied biology because of its potential to unravel difficult food habits/nutrition questions as demonstrated in recent studies of gulls; cormorants; elephants; and snow geese.

In the present study, we conduct feeding trials with captive-reared canvasbacks to determine isotopic signatures of ducks held on historic plant and current animal diets that depict prey switching by this species because of habitat alteration on Chesapeake Bay. These feeding trials are designed to determine turnover rates and enhancement values of carbon and
nitrogen isotope ratios in selected tissues of canvasbacks (whole blood and pectoral muscle). Results of the pen study will permit evaluation of the components of the diet of wild canvasbacks.

Materials and Methods

A. Diet
Ducks were housed in individual outdoor pens designed specifically for dietary studies. During winter, feeding trials were conducted with individual canvasbacks using experimental diets of the tubers of wild celery (Vallisneria americana) to represent the historic plant diet, and Baltic clams or a mixture of Baltic clams and whole corn to represent the current diet. Six ducks were maintained on each of commercially available corn-based and soybean-based diets to provide a measure of variability in isotopic signature. Feeding trials were conducted for 12 weeks and body tissue samples were obtained on a periodic basis.

B. Collection of Body Tissue Samples
A volume of 0.1 ml whole blood was collected weekly from the medial metatarsal vein, placed in sterile, plain glass blood collection tubes, and held in an oven at 40 C until dry (about 48 hours).

Pectoral muscle tissue samples were collected at the beginning, midpoint (6 weeks) and end of the feeding trials (12 weeks) by percutaneous cannulated biopsy needle (B-D Precision-Cut: 14G/3inch) yielding 5-10 mg (wet weight) of muscle tissue. The ducks were anesthetized using isoflurane and the site of biopsy was prepared by removing 2-3 down feathers, scrubbing the skin area with betadine solution, and applying an alcohol rinse. Following biopsy, digital pressure was applied for hemostasis and the site sprayed with topical nitrofurazone wound dressing. The ducks were monitored through recovery and returned to their pens for observation. Biopsy samples were placed in sterile, plain glass blood collection tubes and held in an oven at 40 C until dry (about 48 hours).

C. Stable Isotope Analysis
Our use of stable isotopes follows the prescribed laboratory protocols. About 99% of all stable carbon isotopes have a mass of 12 and about 1% a mass of 13. Nitrogen exists as two stable isotopes: $^{14}$N (99.6%) and $^{15}$N (0.4%). Ratios of stable isotopes are measured by an isotope ratio mass spectrometer and the isotopic composition is compared to a reference. The carbon standard is a Cretaceous belemnite from the PeeDee formation of South Carolina (PDB). Atmospheric nitrogen gas ($N_2$) is the standard for nitrogen. Isotope values are measured as deviations relative to the standard, such that, for example for carbon:

$$\delta^{13}C = \left( \frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000$$

where R is the isotope ratio ($^{13}C/^{12}C$) of the sample and standard, respectively. Isotope values are expressed in parts per thousand ($\permil$). Samples with more positive values are said to be enhanced or enriched, and those with more negative values depleted relative to the standard.
Stable isotope values for canvasback diets and tissues were analyzed for $\delta^{13}C$ and $\delta^{15}N$ using standard protocols for gas chromatograph/mass spectrometer isotopic analysis at the Organic Geochemical Laboratory, University of Virginia. Organic carbon was oxidized to carbon dioxide (CO$_2$) and species of nitrogen were converted to nitrogen gas (N$_2$). Carbon dioxide and nitrogen gas was cryogenically separated from other gaseous products. Isotopic ratios were measured on a dual inlet, triple collector VG PRISM stable isotope ratio mass spectrophotometer. The precision of the analysis was estimated at $\pm 0.10\%$.

**Results**

Isoflurane anesthesia was rapid in induction and recovery and had no detectable side effects on canvasbacks. Pectoral muscle biopsies were conducted with no apparent adverse effect. Blood loss was minimal, and there were no sign of transient or permanent dysfunction of the breast muscles from the procedure.

Isotopic values of canvasback foods easily discriminate in a two variable analysis (carbon and nitrogen; Fig. 1). Corn, a monocot that uses the C$_4$ or Hatch- Slack photosynthetic pathway, is enhanced in carbon isotope value compared to benthic animal foods, such as Baltic clams available from Chesapeake Bay. With only 5 of 12 weeks of samples analyzed, our preliminary findings show isotopic values of canvasback tissues to asymptote toward food values with metabolic enhancement as predicted (Fig. 2). One exception is the nitrogen values for the whole corn diet that show no apparent change over time. Isotopic values for blood and pectoral muscle are similar and seem to be following similar time constants.

**Discussion**

Because isotopic values of other possible benthic foods of canvasbacks in Chesapeake Bay are predicted to be consistent with values obtained for Baltic clams, and because wild celery and other submerged aquatic vegetation are virtually unavailable in the present-day Bay environment, isotopic values of canvasback tissues enhanced in carbon above -18 $\%$ provide a positive indication that these ducks must be receiving corn in the diet. Thus, measures of stable isotope ratios provide a powerful tool to assess this dietary input of corn with wild ducks.

Results of our preliminary findings with penned canvasbacks suggest that isotopic values of canvasback tissues will reflect diets from Chesapeake Bay over the 12-15 week winter period. Our one exception is the non responsive nitrogen signature from a strictly whole corn diet. This outcome likely relates to the protein deficiencies inherent to a diet restricted solely to whole corn which will lead to eventual malnutrition if maintained for long periods. To avoid this problem, we switched these ducks to a balanced corn-based diet later in the study.

Tissue sampling techniques, although invasive, are remarkably uncomplicated and lend themselves to use in field situations. Blood seems to be ideal for measurement; it is easy to obtain and only a 0.1 cc volume is needed for analysis. Breast muscle appears to have the same turnover rate as blood and our interpretation based on limited data suggests it provides no additional information. Clearly, large numbers of blood samples obtained from
live-trapped birds in late winter, can be used to pattern isotopic values and thus feeding habits of canvasbacks in various parts of the Bay. These values will integrate their dietary history over a period of about 3 months, a period that approximates the average wintering time for canvasbacks on Chesapeake Bay.

We believe the technique also holds promise in providing important insights into the historical food habits of canvasbacks on Chesapeake Bay through analysis of tissues from preserved carcasses, such as museum skins, that were collected before the period of habitat degradation. These measurements might provide new insights into the dependency of canvasbacks on plant versus animal resources during a period when a much greater diversity of foods were available in the Chesapeake Bay.

LITERATURE CITED

Figure 1. Carbon and nitrogen isotopic values for foods provide penned canvasbacks.
Figure 2. Changes in carbon and nitrogen stable isotope values of whole blood from captive-reared canvasbacks fed various diets. Predicted asymptotic value for each diet, based on metabolic enhancement, is shown at right.
PHYSIOLOGICAL OPTICS AND OCULAR ANATOMY OF THE ASIAN ELEPHANT
(Elephas maximus)

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The objectives of this study were to 1) measure resting refractive state, amplitude of accommodation, and corneal curvature and 2) describe the anatomy of mature Asian elephants (Elephas maximus). Measurements were made of six mature female Asian elephants using streak retinoscopy, neutralizing videoretinoscopy, and photokeratoscopy, respectively. Anatomical description was made from three eyes obtained from 3 elephants.

The net spherical refraction was +0.23 D, with considerable individual variation. No difference was observed between cyclopleged and noncyclopleged or right or left eyes. Nine of 12 eyes had ≥ 0.5 D astigmatism. Mean corneal power measured by photokeratoscopy was 21.3 D (S.D. = 1.8D). There was a tendency toward with-the-rule astigmatism (mean: 1.2 D) that was not statistically significant (p = 0.06). Videoretinoscopy of 2 animals showed the range of accommodative ability to be approximately 3 D.

The mean axial length of the preserved eyes was 38.75 mm; the lens had an axial diameter of 10 mm. The cornea was comprised of 5 distinct layers and the posterior sclera was markedly thickened. A thin meridionally oriented smooth ciliary muscle was identified. Muscle fibers were observed associated with posterior trabeculae of the uveal meshwork. A fibrous tapetum was present. Puncta and nasolacrimal duct could not be identified.

Conclusions from the optical study included documentation of wide individual variation in resting refractive state, prevalent astigmatism, and modest accommodative range. The anatomical study documented the presence of a thin ciliary muscle apparatus, unusually thick sclera, and a fibrous tapetum.
POLYMERASE CHAIN REACTION, HISTOLOGICAL, AND ULTRASTRUCTURAL DIAGNOSIS OF DISSEMINATED TOXOPLASMOSIS IN A SQUIRREL (*Sciurus carolinensis*)

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*Toxoplasma gondii* parasitizes a wide spectrum of animal species, however infection is rarely disseminated in mature, immunocompetent animals. Serological, immunohistochemical, light and electron microscopic techniques have been the primary means of diagnosing toxoplasmosis in the past. Recently, application of molecular techniques have confirmed diagnosis of *T. gondii*. Polymerase chain reaction (PCR) analysis can support histological findings and also provides increased diagnostic sensitivity and specificity. A fulminant case of toxoplasmosis was diagnosed in a mature feral male squirrel (*Sciurus carolinensis*) by morphological and molecular methods. The squirrel was found dead in a residential area in Massachusetts and was necropsied approximately 48 hours after death. Histologically, there was widespread necrosis of organs including lung, pancreas, adrenal gland, skeletal muscle, urinary bladder, heart, hair skin, spinal cord, trachea, lymph nodes, spleen and liver. Admixed with cellular debris in affected organs were numerous individual zoites and cysts containing zoites. Crescent-shaped zoites measured 1 μm in length and cysts varied from 8 to 10 μm in size with a thin (<1 μm) wall. Ultrastructurally, cysts were seen containing zoites with nuclei located toward the posterior end and multiple rhoptries were visible anteriorly. The organisms were identified as *Toxoplasma gondii*. Observed characteristics distinguished the organism from the phenotypically similar protozoan *Neospora* sp. which has numerous rhoptries and a centrally positioned nucleus. PCR analysis of paraffin-embedded brain and cardiac muscle followed xylene/alcohol deparaffinization and proteinase K digestion of tissues. *In vitro* DNA amplification of a sequence within the B1 gene, unique to *Toxoplasma*, was accomplished using flanking primers to the sequence of interest and 1 μl of DNA-containing tissue lysate for 40 cycles of replication. The 194 base pair product was visualized with ethidium bromide after agarose gel electrophoresis. A Southern blot was performed using a 32P-labeled DNA probe internal to the amplified sequence. Toxoplasmosis infection was widespread in this case and no other infectious agents or underlying disease conditions were detected. Diagnosis of *T. gondii* may be difficult if characteristic cysts are absent or when necrosis is extensive. When present, *Toxoplasma* cysts may be confused with similar cysts formed by related apicomplexan parasites and may be difficult to distinguish by more common diagnostic techniques. Both PCR and immunohistochemistry can substantiate microscopic diagnosis of toxoplasmosis; however, the latter is limited according to the quality of the tissue sample, fixation techniques and availability of reagents. In contrast, PCR can be performed on small tissue samples, including paraffin-embedded material, has less stringent tissue fixation requirements and affords greater sensitivity and specificity.
An adult female African lesser flamingo (*Phoeniconaias minor*), one of 80 flamingos of that species on display at Sea World, Orlando, Florida, was presented with severe bilateral lameness. Other flamingos in the exhibit appeared normal. Physical examination revealed chronic bilateral pododermatitis involving the metatarsal pads and a moderate to marked reduction of the pectoral muscle mass. The bird weighed .89 kg. Average weight of the other flamingos was approximately 1.25 kg. Complete blood cell count and serum biochemistry profile were unremarkable, except for a moderate elevation in serum creatinine kinase values (1162 IU/L). Bilateral cultures of the foot lesions revealed *Streptococcus similis* in both feet, *Enterococcus faecalis* in the left foot and *Enterococcus avium* in the right foot. Based on *in vitro* sensitivity testing, chloramphenicol was the antibiotic chosen for initial therapy. Pododermatitis lesions were treated by debridement and bandaged with a topical triple antibiotic ointment. Because the bird was weak and eating poorly, she was fed a gruel of normal flamingo diet and delivered by gastric gavage. Three days following the initiation of therapy, the flamingo was placed into a sling for support. The bird died on the seventh day of treatment. The cause of death was aspiration of gastric contents which occluded the tracheal lumen. Necropsy examination revealed multifocal firm pale lesions as large as five centimeters in diameter throughout the liver. Cytological examination found these areas primarily consisted of pleomorphic hepatocytes which were variable in shape, had elevated nuclear:cytoplasm ratios, showed increased cytoplasmic basophilia, and often exhibited mitotic activity suggestive of neoplastic infiltration. Representative tissues were collected in 10% buffered neutral formalin. Histologically much of the liver architecture was disrupted and distorted by an unencapsulated, widely infiltrative neoplasm. Horseradish peroxidase immunohistochemistry performed on the tumor applying monoclonal antibodies to epithelial membrane antigen (EMA) (DAKO, Carpinteria, CA) revealed the neoplastic cells were of epithelial origin, supporting the diagnosis of complex hepatocellular carcinoma. A thin rim of residual hepatic parenchyma forming the peripheral margin was characterized by chronic periportal inflammation and diffuse hepatocellular iron pigment deposition. Neoplastic cells formed dense packets, separated by fine strands of pre-existing connective tissue stroma, or often composed large (up to two millimeters in diameter) cystic spaces, lined by one or more layers of densely spaced cells displaying marked karyomegaly and anisokaryosis. Mitotic figures were numerous, averaging 4-5/high power field. Extensive areas of hemorrhage and tumor necrosis, occasionally several centimeters in diameter, also were present. Other tissues examined were negative for tumor metastasis, however incidental findings included disseminated trematode ova to various organs, such as the kidney, lung, pancreas and liver, and the presence of adult cestodes in the small intestine. Reports of tumors in Phoeniconaias...
are rare, especially arising from the liver. This report describes what we believe to be a unique hepatic neoplasm in the lesser flamingo, diagnosed by light microscopy and supported by immunohistochemistry.
EFFECT OF DIETARY IRON ON THE ACCUMULATION OF IRON IN THE LIVER OF EUROPEAN STARLINGS

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Introduction

It has been documented that some birds in captivity store excess quantities of iron in tissues, particularly the liver. Broad surveys of birds have verified excess iron storage in virtually all groups although the highest levels were found in the orders Passeriformes, Coraciiformes, Anseriformes, and Strigiformes. Pathologies associated with iron storage disease have been most commonly reported in passerines, particularly among mynahs and birds of paradise. Recently Kincaid and Stoskopf (1987) reported iron storage syndrome in seven species of tanagers. At Brookfield Zoo, several cases of iron storage disease have been verified among tanagers in the final stages of the disease, the syndrome has manifested itself by distension of the abdomen accompanying accumulation of fluids and hypertrophy of the liver, difficulty breathing and in satiable appetite. Storage of excess iron has been suspected in many older cases and in a few it is suspected to have contributed directly to condition leading to death.

Iron is found in every cell in the body. It is involved in a wide range of processes including cell respiration, DNA synthesis, and is a component of many enzymes. It serves as a cofactor to the enzymes needed for the electron transport chain, from which ATP is generated. Iron in the body is predominantly found bound to the proteins hemoglobin and myoglobin, which serve to carry and release oxygen.

Iron uptake in the body is usually very poor in most animals. Only 5-15% of dietary iron is absorbed by humans so there is rarely a toxicity problem. However, it may be possible that some birds, especially fruit eating birds, which may have a difficult time finding good sources of iron in their free-ranging diet, have developed the ability to better absorb iron from their natural diet.

The quantity of iron present in the body is regulated through absorption. Ingested iron is absorbed in the intestine where it is bound to the protein ferritin and stored in the mucosal cell or passes into the serum bound to transferrin for transport to various sites in the body. If iron is not transferred to the serum, it is not absorbed and is lost as intestinal mucosal cells are sloughed. Heme iron, or iron which incorporated with hemoglobin is more readily absorbed than inorganic iron.

Dietary components that increase iron storage include ascorbic acid, and sugars such as lactose, fructose, and glucose. Minerals such as cobalt, zinc, cadmium, copper, and manganese, compete with iron for absorptive binding sites. Copper is especially important...
because with a deficiency of this mineral, movement of stored iron from the liver by copper containing ceruloplasmin, is reduced and high levels of iron storage can occur.  

The body guards its supply of iron through a continuous recycling process. Iron is carried from the plasma to the bone marrow, site of red blood cell production. In the bone marrow it is incorporated into the hemoglobin of the red blood cell. After several months of life the red blood cells are broken down in the reticuloendothelial cells of the liver, spleen, and the bone marrow. The iron is either stored or returned to the plasma to be used in body processes once again. The largest iron storage sites are the liver, spleen and bone marrow, where iron is stored as the compounds hemosiderin and ferritin. Since much of the iron in the body is recycled, most of that found in the excreta originates from unabsorbed food and sloughed cells. Small quantities are lost through skin cells and larger quantities through egg laying.  

Toxic levels of iron storage in the liver are seen as increased amounts of hemosiderin. The mechanism of excess storage in birds has not been established. In human pathology, two basic mechanisms of hemosiderosis have been recognized. One, idiopathic hemosiderosis, is an apparently heritable condition of abnormal iron deposition from an iron-balanced diet. In contrast, in secondary hemosiderosis the body accumulates iron in response to a specific cause such as certain anemias or chronic ingestion of excessive iron in the diet. Among birds both mechanisms have been proposed by different authors. Some diets contain excess iron as offered, but there is little quantitative information about what a bird selects from what is offered thus actual iron intake is not known for most species. If accumulated in heightened concentrations, iron is toxic to liver cells. Toxicity can lead to cell death and fibrosis. Clinical signs of chronic low iron overload include reduced feed intake and reduced growth. Sings of chronic high level overload include hepatotoxicity and hemorrhagic necrosis of the GI-tract. Acute toxicosis can result in low blood pressure, metabolic acidosis, and cardiac failure.  

"Iron storage disease" among birds may be defined as hemosiderosis (increased amount of iron deposits without visible alterations in the tissues) or hemochromatosis (hemosiderosis accompanied by pathological lesions attributable to the iron overload). Although these pathologies are similar to those reported in humans (Underwood 1977) and may also be similar to conditions found in reptiles, birds do seem to be uniquely susceptible to iron overload (Ward et al. in press).

Although many of the orders of birds that have been surveyed indicate a tendency to accumulate higher levels of iron than mammals, only certain orders seem to be especially susceptible to pathological problems associated with iron storage disease. The latter observation indicates that some genetic factor may be governing levels of stored iron, perhaps in conjunction with dietary conditions. Other factors such as prior disease, other compounds present in foods as well as the pH of gastric juices (Underwood 1977) may also be important in affecting iron absorption.
To date, reports of iron storage disease in birds have primarily been summaries of post-mortem findings in particular groups of interest. A few more recent studies have attempted to identify the taxonomic extent of the problem by surveying available cases. Experimental studies of iron with poultry have been conducted, primarily to determine effects of deficiency of interactions between disease and iron absorption. These reports have examined captive birds although many of the exotic species were imported from the wild some time (up to 20 years) earlier. In fact, the basic assumption of these studies seems to be that iron storage disease is a phenomenon of captivity. Because better information is generally lacking, diets for birds in captivity are generally formulated from recommendations for poultry (Order Galliformes). However, most reports of exaggerated iron storage syndrome involve tropical frugivorous birds (Order Passeriformes) and diets based on poultry requirements may not always be appropriate.

Clearly the problem of iron storage disease is one of importance in maintaining healthy collections of birds in captivity. As we move toward long-term maintenance of populations of often delicate species, it is critical to gain a better understanding of their nutritional and physiological needs. A number of facets of the problem need to be addressed, including more complete surveys of the taxonomic extent of susceptibility, examination of the causal factors including attempts to actually induce iron storage altering levels of dietary iron, and better understanding of factors affecting absorption such as diseases and other components of the diet. The results of these studies will better define the role of dietary iron in iron storage disease and in doing so, will provide data needed to formulate appropriate diets for captive exotic birds including mynas and toucans.

The objective of this study was to examine whether excess iron storage can be induced by diet in a model species, the European starling, a Passeriforme, by a) determining the level of dietary iron that causes iron storage disease; and b) determining an approximate time required to cause iron storage disease.

Materials and methods

Twelve fledgling and 46 nestling European starlings, collected on Zoo grounds, were handraised and offered a nutritionally complete apple mash baseline diet (Table 1) that contained 167 ppm iron. The birds were separated into non-related pairs and weighed biweekly. Dry matter intakes were recorded daily throughout the experiment.

After an acclimatization period of 2 weeks to establish all birds were maintaining or gaining weight and food intake was stable, 5 pairs (10 birds) were euthanized to establish baseline data for dry matter intake, g/day (DMI), iron intake, mg/day (FEI), body weight, g (BW), liver iron content, ppm (LFE), and liver copper content, ppm (LCU), and liver and spleen weight as a percent of body weight (LWT%BW and SWT%BW, respectively) (Table 2).

The remaining 23 pairs were randomly assigned to one of two diets similar in nutrient content with the exception of iron. Twelve pairs received a low iron diet 148 ppm iron (similar to baseline diet) and 11 pairs received a high iron diet 3035 ppm iron (Table 1).
All birds received deionized water to limit possible additional iron intake. At 8 weeks after acclimatization (week 10) half the pairs in each treatment group were euthanized for tissue collection; the remainder were sacrificed 16 weeks after acclimation (week 18).

Results and discussion

Dry matter intake was not different \((P > 0.05)\) between treatment groups, 49.9 g/day and 49.0 g/day for low and high treatments. Birds on the high iron diet consumed more iron \((P < 0.05)\) than birds on the low iron diet (149 mg/day and 7.4 mg/day respectively). Body weight was not different \((P > 0.05)\) between treatment groups, 77.2 g and 76.1 g for low and high treatments.

There was an interaction between treatment and week \((P < 0.05)\) which indicated that both treatment and time had an affect on liver iron content. As can be seen in Table 3, birds on both treatments had similar liver iron content at 10 week but the birds on the high iron diet sacrificed at 18 weeks had significantly higher values \((P < 0.05)\). There also appears to be a trend for the birds consuming the low iron diet to accumulate iron in the liver over time.

Liver copper content was not different \((P > 0.05)\) between treatment groups, 25.1 ppm and 23.7 ppm for low and high treatments respectively. Liver copper content did decrease over time \((P < 0.05)\) in all birds; 26.3 ppm and 22.5 ppm at weeks 10 and 18 respectively. Since excessive iron intake may decrease copper absorption there may be less copper available for the formation of ceruloplasmin. Consequently, excess iron may be stored since ceruloplasmin is necessary for transport of iron from the liver.

Birds on the high iron treatment had larger livers as a percent of body weight \((P < 0.05, 4.1\%)\) than birds on the low iron treatment \((3.5\%)\). Spleen weight as a percent of body weight was not different \((P > 0.05)\) between treatment groups, 0.17% for low and high treatment birds. For all birds LWT%BW and SWT%BW decreased over time \((4.1\% \text{ and } 3.5\% \text{ for weeks 10 and 18, and } 0.22\% \text{ and } 0.12\% \text{ for weeks 10 and 18 for LWT%BW and SWT%BW respectively})\).

Conclusions

It appears that European starlings may be good models for endangered passerines to study the effect of dietary iron on iron storage.

This preliminary study indicates a high iron diet (above 3000 ppm) induces excess storage of iron in the liver by 18 weeks in European starlings. A trend for increased storage in the birds consuming the low iron diet may indicate excess iron storage regardless of level of iron in the diet.

Further studies need to be conducted offering intermediate dietary iron levels more similar to natural diets. The levels of iron chosen for this preliminary study were extreme; the low level similar to manufacturer low iron diets and the high level considerably above those used in other studies that did not show an increase in liver iron content. Possibly of greater
importance is the need for a longer study period to determine the effect of time on storage of iron in the liver regardless of iron level in the diet.

Diets formulated for endangered birds that appear to be susceptible to excess iron storage should contain a moderate level of iron near the known requirement for poultry of 67 ppm\textsuperscript{12} and if possible, below the 150 ppm fed in this preliminary study. One hundred fifty ppm may be an upper limit since there may be increased storage over time even at lower levels.

LITERATURE CITED


\textbf{TABLE 1 : Baseline data}

Body weight (BW), g 71.9

\begin{center}
\begin{tabular}{lcc}
\hline
Expression as a percent of the dry matter & \\
\hline
Dry matter intake (DMI), g/day & 66.4 \\
Iron intake (FEI), mg/day & 11.2 \\
Liver iron content (LFE), ppm & 2387 \\
Liver copper content (LCU), ppm & 25.2 \\
Liver weight as a percent of body weight (LWT%BW) & 4.2 \\
Spleen weight as a percent of body weight (SWT%BW) & 0.2 \\
\hline
\end{tabular}
\end{center}
TABLE 2: Nutrient analysis of apple mash on a dry matter basis.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Level in the diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>28.1</td>
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<tr>
<td>Crude fiber, %</td>
<td>4.1</td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.8</td>
</tr>
<tr>
<td>Linoleic acid, %</td>
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<tr>
<td>Vitamin A, IU/g</td>
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<tr>
<td>Vitamin D3, IU/g</td>
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<tr>
<td>Vitamin E, mg/kg</td>
<td>208</td>
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<tr>
<td>Vitamin C, ppm</td>
<td>438</td>
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<tr>
<td>Calcium, %</td>
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<tr>
<td>Phosphorus, %</td>
<td>1.3</td>
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<tr>
<td>Magnesium, %</td>
<td>78.2</td>
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<tr>
<td>Potassium, %</td>
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</tr>
<tr>
<td>Sodium, %</td>
<td>0.3</td>
</tr>
<tr>
<td>Iron, ppm</td>
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</tr>
<tr>
<td>Copper, ppm</td>
<td>24.0</td>
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<tr>
<td>Manganese, ppm</td>
<td>11.7</td>
</tr>
<tr>
<td>Zinc, ppm</td>
<td>12.6</td>
</tr>
</tbody>
</table>

* Low iron diet 148 ppm, high iron diet 3035 ppm

TABLE 3: Liver iron content on a dry matter basis, ppm; *P<.05.

<table>
<thead>
<tr>
<th>Week</th>
<th>Low iron diet</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High iron diet</td>
</tr>
<tr>
<td>10</td>
<td>3122</td>
<td>3107</td>
</tr>
<tr>
<td>18</td>
<td>3646</td>
<td>5929*</td>
</tr>
</tbody>
</table>

*P<.05.*
VESICULAR SKIN DISEASE OF TAPIRS

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A 4 yr old female lowland tapir (Tapirus terrestris) at the Nashville Zoo was examined for skin lesions occurring over the dorsal cervical and lumbosacral regions. The lesions began as coalescing raised erythematous papules which ruptured and sloughed, releasing serosanguinous fluid and leaving exposed dermis. The animal's appetite and attitude remained normal during this period, although she would collapse on all 4 legs when her head was manually elevated. The lesions were first observed 2 weeks after the tapir was moved into indoor housing for the winter. This animal’s medical record revealed that two similar incidents had occurred within the previous year at another zoo. Diagnostic evaluation included complete blood count, serum biochemical profile, bacterial cultures of skin lesions, viral isolation attempt on aspirated vesicle fluid, electron microscopy of vesicle fluid, and biopsy of affected skin.

Complete blood count and serum biochemical profile values did not differ significantly from reference intervals for the species. Bacterial cultures yielded nonhemolytic Streptococcus sp. which was considered an environmental contaminant. Viral isolation attempts using equine fibroblasts were negative and electron microscopy failed to demonstrated viral particles.

Histological evaluation of several full thickness skin biopsies revealed multiple vesicles which were predominantly subepidermal and contained abundant fibrin, neutrophils, and eosinophils. The epithelium had severe spongiosis as well as superficial to transepidermal necrosis. Subcorneal pustules and abundant emigrating neutrophils, eosinophils, and red blood cells were present in viable epithelium. Hair follicles were unaffected. Along the superficial dermis, underlying the vesicles, was a narrow zone of collagen necrosis and a mild neutrophilic and eosinophilic infiltrate. There was severe perivascular edema surrounding dermal vessels and regionally severe hemorrhage in the superficial dermis. No microorganisms were identified within the lesions. Based on these findings, a histopathological diagnosis of subacute multifocal subepidermal vesicular dermatitis was made.

Anecdotal reports of other tapirs with similar skin lesions prompted a survey of North American zoos housing tapirs regarding dermatological disease in all Tapirus species. Of 33 institutions contacted either by telephone or questionnaire, 15 responded (45%). From
these 15 zoos, 8 (53%) housed a total of 32 animals (18 Tapirus indicus, 9 T. terrestris, 5 T. bairdii) with clinical histories and/or skin biopsy histopathology compatible with vesicular skin disease.

Among these 32 affected individuals, approximately 122 episodes of vesicular skin disease were identified. Skin lesions were most commonly observed over the dorsal cervical and dorsal trunk regions except in the Baird's tapir (T. bairdii), where lesions tended to be more laterally oriented. Many affected animals had unexplained neurologic signs (e.g., hind limb ataxia or lameness, weakness, or syncope-like episodes) associated with the skin lesions. In addition, many of the affected tapirs had histories of chronic intermittent respiratory infections preceding or interspersed with the episodes of dermatitis. Females and males of all species were affected equally, but females, once affected, tended to have more repeat episodes of skin disease than males (ave 1.6 episodes per male vs. 4.3 per female, p <0.01). The average age of onset for the Malayan tapirs was 7.8 years for the males and 4.0 years for the females. No other significant risk factors were identified.

Diagnostic and therapeutic approaches to this disease varied considerably. Diagnostics including skin biopsy and cytology, CBC, serum biochemical profile, bacterial and fungal cultures, virus isolation, and serology were performed. No etiologic agent was identified. Therapeutic protocols centered on debridement of the necrotic epidermis and application of topical antibiotics or antiseptics. Systemic antibiotics were used in more severe cases and nonsteroidal antiinflammatory drugs were often used when neuromuscular signs were present. Other therapeutics employed were systemic corticosteroids, topical sun screens, topical antifungal/antibiotic/corticosteroid combinations, and moisturizing agents. None of these regimens appeared to dramatically alter the course of the disease and most cases resolved spontaneously in hours to weeks regardless of the therapy employed.

The histologic findings observed in the Nashville Zoo tapir are consistent with bullous pemphigoid, dermatitis herpetiformis, the junctional form of erythema multiforme, and the acute hemorrhagic form of staphylococcal hypersensitivity. However, the distinctive distribution of the lesions and the apparent high prevalence of similar lesions within the captive tapir population are inconsistent with the known clinical and epidemiological characteristics of these diseases in other species. Further studies are in progress to determine if immunoglobulin deposition is present within lesions and if specific risk factors are can be correlated with disease occurrence.
ELK IMMOBILIZATION WITH POTENT OPIOIDS: A-3080 VS. CARFENTANIL

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The safe and efficacious immobilization of large wild animals has improved with the utilization of very potent opioids. We compared A-3080 (Anesta Corp. Salt Lake City, UT) and Carfentanil (Wildlife Laboratories, Ft. Collins, CO), two opioids approximately 5000 and 8000 times as potent as morphine, respectively, as elk immobilization agents.

Forty-two wild captive elk were immobilized at two different sites in northern Utah, Hardware Ranch and Deseret Land and Cattle Company, during January through March 1993. All elk received hay on a feed line throughout the winter and were baited into circular corrals to perform the study. The elk were moved through squeeze chutes to a scale where baseline weight, temperature, pulse and respiratory rate (RR) were recorded prior to immobilization. At Hardware Ranch 20 elk were darted, with either A-3080 (15 mg, n=10) or Carfentanil (4 mg, n=10). Deseret elk were darted, with either A-3080 (15 mg, n=11) or Carfentanil (2 mg, n=11). The drugs were administered in a randomized order, and investigators recording immobilization parameters (time to ataxia, time to immobilization, and time to standing after reversal) were blinded to the agent given. Immobilized elk had their temperature, pulse and respiratory rate recorded at the time of immobilization and every 5 min for 15 min. All animals were then given the opioid antagonist Nalmefene HCl, at a dose 50 times greater than the agonist via IM injection. The elk recovered in a separate paddock where respiratory rate was recorded hourly for 4 hours and at 24 hours after immobilization. Bonferroni t-test was used to compare immobilization and standing times; p < 0.05, were considered significant. Values are mean ± S.D.

The mean weight of the elk was 215.7 kg (range 200 - 245.5 kg) at Hardware Ranch and 122.16 kg (range 100 - 212.3 kg) at Deseret. The mean baseline RR was 36.8 ± 11.7 at Hardware Ranch and 40.4 ± 27.4 at Deseret. The mean baseline temperatures were 104.6 ± 1.1 at Hardware Ranch and 105.7 ± 1.3 at Deseret. The results are summarized in the table below.

<table>
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<tr>
<th></th>
<th>HARDWARE</th>
<th>DESERET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A-3080</td>
<td>Carfentanil</td>
</tr>
<tr>
<td>TIME TO IMMobilization (min)</td>
<td>2.0 ± 0.8</td>
<td>2.7 ± 0.8</td>
</tr>
<tr>
<td>TIME TO STANDING (min)</td>
<td>1.4 ± 0.3</td>
<td>2.3 ± 0.6*</td>
</tr>
<tr>
<td>LOWEST RR</td>
<td>17.0 ± 3.7</td>
<td>16.4 ± 5.6</td>
</tr>
<tr>
<td>HIGHEST TEMPERATURE</td>
<td>106.1 ± 1.0</td>
<td>106.6 ± 1.4</td>
</tr>
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</table>

* P < 0.05 compared to same site
Two deaths occurred during the study, both at Deseret. The first elk died 4 hours after having received carfentanil. Symptoms and necropsy findings suggest acute capture myopathy. The second animal had received A-3080 and was found dead on the feed row approximately two days after immobilization. The body had been predated, and no necropsy was performed. The cause of death is unknown. However, this animal had been observed 4 and 24 hours after having been darted and its respiratory rate and appearance were normal. No animals were observed to have renarcotized at any time during the study.

A-3080, given at doses approximately 3.75 and 7.5 times greater than carfentanil, produced more rapid immobilization in these studies. In addition, opioid reversal and recovery time was not made more difficult and was indeed more rapid in animals which had received A-3080. This result may have been due to the greater dose of Nalmefene HCl administered to elk immobilized with A-3080.
A-3080 STUDIES IN ELK: EFFECTIVE IMMOBILIZING DOSES BY SYRINGE AND DART INJECTION

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A-3080 is a new ultrapotent narcotic immobilizing agent. It has been successfully used to immobilize several species of non-domestic ungulates, including over 100 elk (Cervus elaphus canadensis). The main purpose of this study was to determine the effective doses (ED₅₀ and ED₉₀) for A-3080 immobilization in elk. We also wanted to investigate the discrepancies between drug requirements for immobilization by syringe or dart injection.

We defined two parameters for investigation of effective doses. Optimal immobilization was defined as immobilization occurring with an induction time of less than 3 minutes. Successful immobilization was defined as immobilization occurring with an induction time of less than 5 minutes. Any immobilization occurring after 5 minutes was considered unsuccessful. Injections were made by either hand-held syringe or by remote dart injection (Paxarms, Austin Electronic Firearms, Ltd., Timaru, New Zealand). We examined each parameter by both syringe and dart injection.

Doses for hand injection were: 0.5 mcg/kg, 1.0 mcg/kg, 2.0 mcg/kg, 4.0 mcg/kg, 8.0 mcg/kg, 16.0 mcg/kg, and 32.0 mcg/kg. Doses for dart injection were: 1.0 mcg/kg, 2.0 mcg/kg, 4.0 mcg/kg, 8.0 mcg/kg, 16.0 mcg/kg, 32.0 mcg/kg, 64.0 mcg/kg, and 128.0 mcg/kg.

Both syringe and dart injections were made with the elk caught in a squeeze chute (Powder River Livestock Handling Equipment, Provo, UT); after injection the elk were immediately released into a corral for observation. Dart and syringe injections were made using 16 gauge needles. All dart injections were made from a standard distance of approximately 20 feet.

ED₅₀ and ED₉₀ results are shown in the table below. During syringe injections, none of the elk injected with 4 mcg/kg or less of A-3080 were immobilized within 3 minutes (optimal immobilization), while all of the elk injected with 8 mcg/kg, our next dosage level, were immobilized within 3 minutes; therefore, we could not obtain a computer-generated curve for this parameter. We can only say that both ED₅₀ and ED₉₀ for optimal immobilization by syringe injection is between 4 and 8 mcg/kg in elk. For successful immobilization (induction time under 5 minutes) by syringe injection, the ED₅₀ was 2.22 mcg/kg; the ED₉₀ was 2.55 mcg/kg. For dart injection, the ED₅₀ for optimal immobilization was 35.26 mcg/kg; the ED₉₀ was 40.88 mcg/kg. For successful immobilization the ED₅₀ was 12.44 mcg/kg; the ED₉₀ was 29.65 mcg/kg.
No animal renarcotized or appeared to suffer serious complications secondary to drug exposure. A-3080 appeared to be a safe and effective agent for immobilization in elk. These data indicate that for immobilization with A-3080 in 3 minutes or less dart injection doses range from approximately 5 x to 10 x higher than hand injection doses, and for immobilization in 5 minutes or less the dart doses also range from approximately 5 x to 10 x higher than hand injection doses. To our knowledge this is the first time the differences between syringe and dart injection have been studied in a controlled manner.

IMMOBILIZATION RESULTS--COMPARISON OF EFFECTIVE DOSES DEPENDING ON INJECTION METHOD AND INDUCTION TIME

<table>
<thead>
<tr>
<th></th>
<th>HAND (mcg/kg)</th>
<th>DART (mcg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Optimal (&lt;3 min.)</td>
<td>Successful (&lt;5 min.)</td>
</tr>
<tr>
<td>ED_{50}</td>
<td>5.66</td>
<td>2.22</td>
</tr>
<tr>
<td>ED_{90}</td>
<td>9.23</td>
<td>2.55</td>
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</tbody>
</table>
During the winter of 1991-1992 a die-off of reindeer occurred on Hagemeister Island, Alaska. Assessment of age and sex of natural mortalities, necropsy of sacrificed animals, subjective evaluation of winter range and demographics of reindeer carcasses were evaluated in an attempt to determine the underlying cause. It would appear that observations of mortality on Hagemeister Island may reflect a population adjustment/check to both climatic conditions and food resources. However the observation that a large percentage of the carcasses examined were mature males as well as the observed delay in conception (aerial survey counts of cows and calves during spring 1992), which was reflected in the peak period of calving do not fully support this simplistic view. It is therefore hypothesized that the ultimate cause of reindeer mortality may have been a combination of environmental factors such as severe weather and delayed entry of females into the rut. This would result in an extended rut for mature males and therefore greater depletion of body stores. In this precarious position any severe climatic event could shift a fragile balance of energy equilibrium and result in death due to starvation. To test the hypothesis of the apparent late conception, reindeer fetuses were collected during November 1992 and analyzed with respect to crown-rump length and weight. A comparison to reindeer fetal development by Roine (1982) supports the hypothesis that conception was delayed by approximately 2-3 weeks in fall of 1992. This finding is also supported by aerial survey counts of cows and calves from 1993 with regards to peak period of calving.