Abstract

Starting in 1966 with the identification of the viral nature of intracytoplasmic inclusions in erythrocytes of geckos, more and more viruses have been identified as potential pathogens in reptiles. Many diseases originally thought to be bacterial in origin are now being recognized as viral diseases. Pneumonia that was first seen in a colony of vipers (Bothrops moojeni) was initially thought to be caused by Pseudomonas. Subsequent investigations identified the presence of a myxovirus and transmission studies have confirmed the pathogenic effect of these viruses. There is a very basic approach in determining the viral basis for a disease. The history of a die-off coupled with histopathologic findings may be suggestive. Ultimately, virus needs to be identified in tissues either by electron microscopy or by culturing the agent in tissue culture. Negative staining electron microscopy can be used to identify particles in feces, secretions, and tissue homogenates. Serology also has been used to determine exposure to a pathogen and PCR can be used to identify presence of specific nucleotide sequences in tissues or secretions of the host. Ultimately, virus needs to be cultured, purified and challenged back into naive animals to establish a causal relationship.

Introduction

Since 1966, a wide variety of viruses have been either identified in tissue section and/or isolated from members of the orders Chelonia (turtles and tortoises), Crocodylia (alligators, crocodiles, caiman, and gharial), and Squamata (Lacerta: lizards; Ophidia: snakes). An endogenous retrovirus is the only virus identified in Rhynchocephalia (tuatara). Most viruses identified in reptiles have been only circumstantially incriminated as causes of disease, and few studies fulfill Koch’s postulates. In many cases evidence of viral infection is based upon identification in tissue section by light microscopy, with more specific identification made using electron microscopy. Of the various viruses identified in reptiles, transmission studies demonstrating a causal relationship have only been documented for the gray patch herpesvirus of green turtles (Chelonia mydas) in aquaculture, ophidian (snake) paramyxovirus, and a retrovirus isolated from boid snakes with inclusion body disease. Most of the viruses identified in tissue section of reptiles have not been isolated and their role in reptile disease awaits further studies.

In this paper I will review the various diagnostic approaches used in determining the presence of viruses in reptiles.
Histopathology and Transmission Electron Microscopy

While viruses have been isolated from tissues of reptiles without concomitant histopathology, often the isolation of viruses follows light microscopic findings suggestive of viral infection. Tissue specimens at necropsy are routinely collected in neutral buffered 10% formalin and if a viral infection is suspected, additional samples can be placed in Trump’s solution (a 4% formalin/1% glutaraldehyde mixture). If there is the potential of performing immunohistochemical staining such as for ophidian paramyxovirus, tissues should be transferred and stored in 70% ethanol at 24 to 48 hr following initial fixation. Frozen tissues can be collected for demonstration of viral antigen using antibody-labeled fluorescence microscopy. This has been used to demonstrate presence of ophidian paramyxovirus in lung tissue of infected snakes. Based upon light microscopic findings such as the presence of intranuclear inclusions in chelonian herpesvirus infections, or intracytoplasmic inclusions in boid inclusion body disease, the next step would be evaluation of tissues by transmission electron microscopy (TEM). While in TEM it is ideal to have tissues fixed in an appropriate fixative such as Trump’s solution or 2.5% glutaraldehyde, formalin fixed tissue and even paraffin embedded tissue can be examined for presence of virus. Sections of paraffin embedded tissue can be cut from a block using a scalpel blade, post-fixed, and submitted for TEM. The cost of TEM ranges from $100.00 to $200.00 per sample for processing, sectioning, examination, and photography. Based upon the size, location, morphology, and morphogenesis, a presumptive categorization into a family of virus often can be made. However, a final diagnosis is dependent upon biochemical characterization or determining specific nucleotide sequences using polymerase chain reaction.

Negative Staining Electron Microscopy

Negative staining in conjunction with TEM is a useful and rapid method of examining clinical specimens for presence of virus. The principle of negative staining is that there is no reaction between the stain and the specimen. The most commonly used negative stains are uranyl acetate (0.5-1.0%) and potassium phosphotungstate (PTA) (0.5-3.0%). Depending on the nature of the tissue, different ways of processing the sample are required to detect viral particles. Fluid from vesicles can be obtained with a sterile pipette and may be placed directly on a Formvar-coated 200 mesh copper grid, while large amounts of fluid (lung washings) require centrifugation for clarification. In these cases the supernatant after low speed centrifugation (1,500 g), or the diluted pellet after high speed centrifugation (15,000 g), is placed on the grid for staining. Fecal material requires suspension and concentration and should be placed in distilled water or phosphate buffered saline (PBS). Fecal material can be mixed with PBS to give a 20% suspension in 5 ml. After centrifuging, a drop of the supernatant is placed on a grid for examination.

Cell Culture

Viruses can be isolated from: 1) swab specimens of lesions or luminal surfaces; 2) tissue biopsies; and 3) tissues collected at necropsy. When biopsies or tissues are collected at necropsy, it is important to collect samples as aseptically as possible. Tissues can be placed in a sterile petri dish.
and transported to the laboratory on ice, frozen in sterile plastic bags at -70°C for future viral isolation, or placed in cell culture media and frozen for future viral isolation attempts. Tissues should be sectioned into small pieces or ground in a tissue grinder, with cell culture media added. Processed samples are added to plastic cell culture flasks that contain actively dividing cells. Several reptile cell lines are commercially available from American Type Cell Culture (Bethesda, Maryland) and include: 1) viper heart cells; 2) iguana heart cells; and 3) Terrapene heart cells. Cells are incubated at 30°C and are checked daily for cytopathic effects (CPE). Certain reptile viruses such as ophidian paramyxovirus have been adapted to mammalian Vero cells. This has certain advantages since it is possible to achieve faster growth of mammalian cells in culture compared to reptile cells. If changes are seen such as cell necrosis or syncytial cell formation, samples can be removed from a flask, fixed and processed for electron microscopy. Suspected viral antigen can be demonstrated in infected cells using a fluorescent antibody technique. This approach has been used for demonstrating the presence of ophidian paramyxovirus in infected viper heart cells.11

Serology

Very few serologic tests have been developed or are available for diagnosing exposure of reptiles to specific viruses. Serologic studies have been performed on free-ranging reptiles and reptiles used in experimental studies in order to determine the presence of antibodies to various togaviruses.7 However, infections with these viruses do not appear to be clinically important in reptiles. A viral neutralization test has been developed to determine exposure of tortoises (Testudo hermanii and T. graeca) to a herpesvirus suspected of causing a stomatitis/pharyngitis.5 A hemagglutination inhibition (HI) assay has been developed to determine presence of antibody against ophidian paramyxoviruses.6,12 The HI assay has been most useful because of its relative simplicity and rapid “turnaround” time.2 Briefly, serum samples collected by heart puncture or tail venipuncture are diluted 1:10 in sterile physiologic saline at 56°C for 30 min to inactivate complement, then absorbed with washed and pelleted chicken erythrocytes to remove nonspecific agglutinins (12 hr at 5°C). Using microtiter methodology, serial doubling serum dilutions are made (1:10, 1:20, 1:40, 1:80, etc.) using 0.05 ml. volumes of phosphate buffered physiologic saline containing 0.1% bovine serum albumin. The latter minimizes autoagglutination of the erythrocyte suspension used later to indicate whether active virus is present or not. To each serum dilution is added an ophidian paramyxovirus suspension diluted to contain 8 hemagglutination units/0.05 ml. This has previously been determined by titration, taking advantage of the fact that the virus causes chicken erythrocytes to bind together (hemagglutinate). After allowing the virus-serum mixtures to interact for 1 hr at room temperature, 0.1 ml of a 0.3% suspension of washed chicken erythrocytes is added to each well of the microtiter plate. The plates are placed in a refrigerator for 2-3 hr to permit settling of the erythrocytes. If antibodies are present in a particular serum dilution, they will bind to the viruses and prevent them from hemagglutinating the erythrocytes (hemagglutination-inhibition). Where antibody is present, the red cells settle into a “button” at the bottom of the microtiter well, rather than forming a “mat” due to the hemagglutinating property of the viruses. The serum antibody “titer” is read as the reciprocal of the highest serum dilution which causes hemagglutination-inhibition. An HI titer >20 is considered to be positive, indicating definite exposure to virus. Snakes which survive paramyxovirus infections may have HI antibody titers exceeding 10,240.2
A single sample is only indicative of the exposure status at the time the sample was collected. In order to demonstrate an active infection, samples should be collected at 2-4-wk intervals. A change of titer greater than one dilution would indicate an active infection.

The laboratory of the author of this paper is currently performing assays for zoological and private collections throughout the United States. Blood samples are collected and placed in lithium heparin tubes. Plasma is removed, placed in a cryotube, and submitted on dry ice. Minimally, 0.2 cc of plasma should be provided. The cost of an assay is $30.00.

**PCR**

Polymerase chain reaction (PCR) is a method detecting portions of DNA by amplifying short nucleotide sequences within the DNA genome. PCR can be used for detection of viruses in: 1) cell culture; 2) tissues; 3) secretions; 4) lavages; and 5) other biologic samples. A primer of the sequence being amplified is needed. PCR requires oligonucleotide primers complimentary to the sequence being amplified. A PCR assay has been developed for detecting presence of the marine turtle fibropapilloma associated herpesvirus in tumors of sea turtles (Dr. Paul Klein, Department of Pathology and Laboratory Medicine, University of Florida, Gainesville, Florida).

**LITERATURE CITED**

THERAPEUTIC EFFICACY OF HYPERIMMUNE BOVINE COLOSTRUM TREATMENT AGAINST Cryptosporidium INFECTIONS IN REPTILES

Thaddeus K. Graczyk, Msc, PhD,1,2 Michael R. Cranfield, DVM,2,3* and Eileen F. Bostwick4

1Department of Molecular Microbiology and Immunology, and Department of Environmental Health Sciences, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD 21205 USA; 2Medical Department, The Baltimore Zoo, Druid Hill Park, Baltimore, MD 21217 USA; 3Division of Comparative Medicine, School of Medicine, Johns Hopkins University, Baltimore, MD 21205 USA; 4GalaGen, Inc., Arden Hills, MN 55126 USA

Abstract

Therapy based on the protective passive immunity of hyperimmune bovine colostrum (HBC) (raised against Cryptosporidium parvum in dairy cows immunized during gestation) was tested for heterologous efficacy in subclinical and clinical C. serpentis infections of 14 captive snakes, 13 moribund Leopard geckos (Eublepharis macularius), 5 Indian star tortoises (Geochelone elegans), and one Bog turtle ( Clemmys muhlenbergii) infected with Cryptosporidium sp. Six gastric colostrum treatments of 1% body wt at 1-wk intervals each, have histologically cleared C. serpentis in three subclinically infected snakes, and regressed gastric histopathologic changes in one of these snakes. In all snakes, each subsequent colostrum treatment significantly decreased the number of oocysts recovered in gastric lavage eluants. The treatments induced oocyst-negative gastric eluants and stools in all snakes, and improved clinical signs of infection. In geckos, seven gastric HBC treatments at 1-wk intervals each decreased the relative output of Cryptosporidium sp. oocysts and the prevalence of oocyst-positive fecal specimens. Histologically, after 8-wk therapy, 7 of 12 geckos had only single developmental stages of Cryptosporidium sp. in the intestinal epithelium, and 3, 1, and 1 gecko had low, moderate, and high numbers, respectively, of the pathogen developmental stages. Three G. elegans subjected to 5-wk HBC therapy remained positive for Cryptosporidium sp. infection as determined by examination of the feces. As the bovine colostrum treatment was safe and highly efficacious in snakes, it is recommended to administer the bovine hyperimmune colostrum to snakes clinically or subclinically infected with C. serpentis, or to use the colostrum for snake supportive therapy or prophylaxis.

Introduction

Cryptosporidium serpentis-associated reptilian cryptosporidiosis is a common and sometimes life-threatening disease of captive snakes.3-5 The high morbidity and moderate mortality caused by Cryptosporidium in ophidian collections is due to the lack of an anticryptosporidial compound that could be safely and efficaciously used for prophylaxis or therapy.2,6,10 Halofuginone, spiramycin, and paromomycin therapeutically used for reptilian cryptosporidiosis, reduced the number of voided oocysts, but did not eliminate infection as determined by histologic sections and oocyst-positive feces.2,5,6,10,17
Protective passive immunity treatment utilizing hyperimmune bovine colostrum (HBC) (raised against *C. parvum* in dairy cows during gestation) was safe and efficacious in AIDS patients infected with this pathogen. Although HBC has been therapeutically suggested for snake cryptosporidiosis, there was no data on the efficacy of any colostrum-derived product against *Cryptosporidium* infections in reptiles. The purpose of the present study was to determine the therapeutic efficacy of hyperimmune bovine colostrum treatments for *Cryptosporidium* infections in captive reptiles.

**Methods**

Hyperimmune bovine colostrum (HBC) therapy was administered to captive snakes, geckos, tortoises, and a turtle. Fourteen snakes infected with *C. serpentis* were randomly divided into a treatment group of 11 snakes and a control group of 3 snakes. All snakes intermittently voided *C. serpentis* oocysts as determined by the acid-fast stain (AFS) and the MERIFLUOR*™ Cryptosporidium/Giardia* test kit (IFA) for a 6-mo period prior to initiation of the study. Thirteen leopard geckos (*Eublepharis macularius*) originating from a private collection and infected with *Cryptosporidium* sp. were transported to the Baltimore Zoo. All geckos were anorectic and moderately to severely emaciated as noted by severe deficiency of fat in their tails; most of them were lethargic. All geckos had a history of persistent voiding of high number of *Cryptosporidium* sp. oocysts as determined by AFS. Four Indian star tortoises (*Geochelone elegans*), and one *G. elegans* and bog turtle infected with *Cryptosporidium* sp., originated from the Bronx and Baltimore Zoos, respectively. All reptiles were housed as described previously.

Hyperimmune bovine colostrum was prepared from equal volumes of first and second milking colostrum from a multiparous cow immunized against *C. parvum* during gestation as described previously. Colostrum was administered to reptiles by gastric intubation six times at weekly intervals; the volume of administered colostrum constituted 1% of reptile body wt (ml to g). Gastric lavages for detection of *Cryptosporidium* oocysts in the stomach eluants were carried out for all snakes. The lavages were done 3 days after feeding as the recent meal enhances pathogen reproduction processes and increases number of eluted oocysts.

Oocyst isolates from snakes were subjected to HBC/IFA competition binding assay to determine the binding efficacy of the HBC immunoglobulins to the oocysts.

**Results**

Gastric eluants of all snakes prior to the initiation of HBC therapy were positive for *C. serpentis* oocysts and they contained progressively fewer oocysts as the therapy progressed. Stomach eluants of one snake became oocyst-negative after the first colostrum treatment, five snakes had negative gastric eluants after the fourth treatment, and three snakes were negative after the sixth treatment. No *Cryptosporidium* oocysts were found in the fourth collection of gastric eluants of all treated snakes. Three of five colostrum-treated and euthanatized snakes, subclinically infected prior to the therapy, did not have developmental stages of *Cryptosporidium* in histologic sections of the gastric
region (Table 1). Two other euthanatized snakes had a small number of *Cryptosporidium* developmental stages on the surface of gastric epithelium (Table 1).

In geckos, no significant differences were observed in intensity of *Cryptosporidium* sp. infections, decrease of oocyst output, or prevalence of oocyst-positive fecal specimens. All geckos were positive for developmental stages of *Cryptosporidium* sp. which occurred exclusively in the small intestine.

Three *G. elegans* subjected to 5-wk HBC therapy remained positive for *Cryptosporidium* sp. infection as determined by examination of the feces. The feces of one *G. elegans* and Bog turtle became oocyst-negative after HBC therapy.

**Discussion**

Cryptosporidiosis in reptiles is a potentially devastating disease that threatens large collections and the search for prophylaxis and therapy represents a most vital and urgent need. To accurately evaluate the therapeutic efficacy of colostrum treatment, we recommend using the most advantageous diagnostic techniques developed in our laboratories specifically for snake cryptosporidiosis in its subclinical phase (i.e., gastric lavage) and immunofluorescent antibody (IFA), together with histologic detection of the pathogen developmental stages. Also, we recommend performing the HBC/IFA competition binding assay to determine the binding efficacy of the HBC immunoglobulins to *Cryptosporidium* isolates infecting reptiles.

In all *C. serpentis*-infected snakes, HBC therapy based on six treatments at 1-wk-intervals resulted in oocyst-negative stools after the fourth treatment. The stomach eluants obtained 3 days after feeding of the snakes, when the oocyst numbers should be at their peak, showed decreased oocyst numbers and were negative in all snakes after the sixth treatment. Considering the above facts, we conclude that in snakes the therapeutic efficacy of the HBC treatment was high.

We recommend gastric delivery of colostrum which is relatively simple to perform, safe for snakes, and assures an efficient use of the costly medication. As *Cryptosporidium* in snakes is confined to the gastric region, stomach administration delivers immunoglobulin-rich medium directly into the area occupied by the pathogen. Colostrum immunoglobulins that play an essential anticryptosporidial therapeutic role are labile, and therefore colostrum has to be properly stored and handled to preserve its immunologic activity.

In geckos, the efficacy of HBC was lower than observed in *C. serpentis*-infected snakes; which may be due to the intestinal location of *Cryptosporidium* sp. infection in these geckos. Besides anticryptosporidial activity of immunoglobulins present in the HBC, the colostrum itself is rich in easy-digestible proteins which when delivered orally may be beneficial for geckos with significant weight lost.

Hyperimmune bovine colostrum therapy was the least efficacious in tortoises, which again may be
due to the intestinal location of Cryptosporidium sp. infection in tortoises.

ACKNOWLEDGMENTS

The study was supported by the Morris Animal Foundation, Englewood, Colorado, grant 98ZO-28.

LITERATURE CITED

**Table 1.** The results of therapy based on the protective passive immunity of hyperimmune bovine colostrum raised against *Cryptosporidium parvum* in dairy cows during gestation applied to captive snakes clinically and subclinically infected with *Cryptosporidium serpentis* and euthanatized after the therapy. Colostrum administered six times with 1-wk-interval. Control snakes treated with saline.

<table>
<thead>
<tr>
<th>Snake species</th>
<th>Number of fecal specimens</th>
<th>Positivity for <em>Cryptosporidium</em></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Oocyst-positive</td>
<td>Stomach</td>
<td>Intestinal contents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AFS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>IFA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>imprint</td>
<td>contents</td>
<td>histologic sections</td>
<td></td>
</tr>
<tr>
<td><em>Pituophis melanoleucus</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14</td>
<td>3</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Lampropeltis t. conati</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8</td>
<td>2</td>
<td>5</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Lampropeltis t. conati</em></td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Epicrates inornatus</em></td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Epicrates c. cenchria</em></td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Elaphe o. obsoleta</em>&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Elaphe o. obsoleta</em>&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup>Acid-fast stain, <sup>b</sup>Immunofluorescent antibody, <sup>c</sup>Severe clinical infection, <sup>d</sup>Control snakes
A REVIEW OF REPTILIAN AMEBIASIS AND CURRENT RESEARCH ON THE DIAGNOSIS AND TREATMENT OF AMEBIASIS AT THE BALTIMORE ZOO

Mary C. Denver, DVM,1* Michael R. Cranfield, DVM,1,2 Thaddeus K. Graczyk, MSc, PhD,1,3 Peter Blank, BA,1 Anthony Wisnieski,4 and Vicky Poole, BS4

1Medical Department, Baltimore Zoo, Baltimore, MD 21217 USA; 2Division of Comparative Medicine, School of Medicine, Johns Hopkins University, Baltimore, MD 21205 USA; 3Department of Molecular Microbiology and Immunology, and Department of Environmental Health Sciences, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD 21205 USA; 4Herpetology Department, Baltimore Zoo, Baltimore, MD 21217 USA

Abstract

Entamoeba invadens is an important gastrointestinal pathogen in large reptile collections and may cause large losses in short periods of time. Turtles, crocodilians and some species of snakes and lizards have been identified as carriers and should not be housed with susceptible species. Disinfection of tools between exhibits is important in reducing the likelihood of an epizootic, and for controlling a current epizootic. Detection of Entamoeba cysts and trophozoites in carrier animals can be difficult due to low numbers of organisms passed in the feces. It is also difficult to differentiate Entamoeba trophozoites from other reptilian intestinal amoebas. Treatment options currently recommended in the literature are based on clinical experience rather than scientific data. The current research project at the Baltimore Zoo is directed towards improving efficiency of detection of carrier and subclinical cases via improved culture and sampling methods, and determining safety and efficacy of three different drugs (metronidazole, diloxanide furoate and iodoquinol) at three doses in snakes and turtles.

Introduction

Many species of amoebae can be found in the gastrointestinal tract of reptiles. Some may be commensals and others are considered pathogens. Cases of reptilian amebiasis with morbidity and mortality are usually attributed to the organism, Entamoeba invadens, although there are multiple species of reptilian Entamoeba.6,9 Some of the described Entamoeba sp. are morphologically distinct from Entamoeba invadens, while others are very similar.3 Amebiasis can be a serious and devastating disease in a large reptile collection due to its ability to infect reptiles across taxonomic orders, unlike most viral diseases.2 Although morbidity is variable, the organism is difficult to eradicate from the environment or the carrier animals once it enters a collection, and losses may continue for long periods of time. Detection of amebic cysts or trophozoites in fecal samples from clinically affected animals is considered diagnostic. Detection of carrier animals is difficult with currently available microbiologic techniques. Snakes and lizards are considered to be the most susceptible to fatal amebiasis. Although turtles, crocodilians and reptile-eating snakes and lizards are considered to be resistant to the disease and potential carriers, it is also possible for them to develop clinical disease.
Amebiasis causes morbidity and mortality by invading the epithelium of the gastrointestinal tract, especially the colon, and lysing the cells. The extent of damage to the intestinal mucosa is variable, but may result in thickening or obstruction of the gastrointestinal tract, or perforation. Secondary bacterial infection is usually concurrent, worsening the clinical course of disease. Trophozoites may also enter the mesenteric circulation and invade the liver, causing abscesses or other extra-intestinal signs of amebiasis. Thrombic emboli may form causing necrosis of large portions of the liver or gastrointestinal tract. Reptiles may show no clinical signs, and be found unexpectedly dead, or may become anorectic and dehydrated. Mucous and or blood may be seen in the feces, but it is not consistent.

The organism has a direct life cycle and the mode of transmission is fecal-oral contamination with cysts from enclosure mates or from fomites brought in from other enclosures. The reptile ingests a cyst which excysts within the lumen of the gut and becomes a trophozoite, the replicating phase. The reproducing trophozoites invade the gut wall or encyst and are passed out in the feces. Trophozoites are not thought to be infective as they desiccate rapidly in the environment. It may be difficult for technicians to detect the trophozoites or the cysts on routine fecal examinations in carrier animals. It is somewhat more obvious in clinically affected cases. Culture techniques\(^1\)\(^8\) may improve detection compared to direct and flotation fecal examinations. The most important method of preventing an epizootic within a collection is to avoid housing probable carrier animals in mixed species exhibits with susceptible animals. Quarantine procedures, properly followed, and appropriate disinfection of tools and equipment between exhibits are also very important.

The quadrinucleate cysts of *E. invadens* (*Ei*) are morphologically similar to those of the human amoebas *E. histolytica* (*Eh*, pathogenic) and *E. dispar* (*Ed*, non-pathogenic) and can be distinguished in vitro by organism temperature preferences in culture. *Ei* grows best at 25°C and will not grow above 33°C.\(^7\) In human medicine, *Eh* and *Ed* can be distinguished from each other by the presence of anti-amebic antibodies (elicited by *Eh* only), the presence of occult blood in feces (*Eh*), or the presence of ingested erythrocytes within the trophozoites (*Eh*). In light of this information regarding the difficulty in differentiating human *Entamoeba* sp., there is no reason to assume that *Ei* is the only pathogenic species of reptilian ameba, that all cases of amebiasis in reptiles are caused by *Ei*, or that all strains of pathogenic ameba are equally pathogenic in all species of reptiles. Other morphologically indistinguishable species may be present, whether causing disease or not.

Treatment recommendations for reptiles have been based on human treatment regimens, of which metronidazole is a cornerstone of chemotherapeutics for invasive amebiasis. Metronidazole dosages and frequencies for reptiles that have been recommended in the literature range from 275 mg/kg orally once, to 50 mg/kg orally s.i.d. for 5 days and many dosages and frequencies in between. Metronidazole is most active against the trophozoites but does not eliminate all cysts from the gut lumen. Metronidazole at high doses can cause hepatotoxicity with neurologic signs. Dimetridazole is also reported to be effective but is not currently available in the United States. Tetracyclines or erythromycin may be used in patients that are intolerant of metronidazole, but are less effective than metronidazole and are rarely used in reptilian medicine for that reason. Emetine class drugs were used historically to treat invasive amebiasis in humans and reptiles, however, significant cardiotoxic
side effects and neuromuscular toxicity have virtually eliminated the use of this group of drugs.  

In human clinical cases, metronidazole is often given concurrently with or immediately followed by lumen active agents; diloxanide furoate, paromomycin or iodoquinol (diiodohydroxyquin) to reduce cyst passage in the feces. There are anecdotal reports of the use of these agents for the treatment of amebiasis in reptiles although no clinical studies have been performed determining safety or efficacy of these drugs. Diloxanide furoate is the drug of choice for eradication of Eh from the intestinal lumen (95% clearance rate in humans), however, it is not readily available in the United States. Use of diloxanide furoate in reptiles at 0.5 g/kg as a single oral dose has been published, but no clinical studies have been cited to support this dosage. Paromomycin is an oral aminoglycoside which may cause mild gastric irritation and fungal overgrowth. It is only used in mild or asymptomatic infections in humans. Iodoquinol is probably as effective as diloxanide furoate, but requires twice as long a treatment period and has been reported to cause optic nerve atrophy.

**Study Design**

The Baltimore Zoo Medical and Herpetology Departments have undertaken a multifaceted research project involving early diagnosis and treatment of pathogenic amebiasis in reptiles. The first phase of the study is designed to improve diagnostic techniques to detect carriers and subclinical cases of Entamoeba sp. Direct microscopic examination of samples obtained by cloacal flushing with saline was compared to culture techniques for detection of cysts and trophozoites in samples. Single sampling events were compared to multiple sampling events for detection of cysts and trophozoites. Organisms were then transferred into susceptible hosts (juvenile corn snakes, *Elaphe guttata*) in order to identify pathogenic strains. Once pathogenic strains are identified, molecular techniques will be utilized to find markers to attempt to differentiate between pathogenic and non-pathogenic strains or species of Entamoeba. Pathogenic strains will be transferred into susceptible species and efficacy of metronidazole, diloxanide furoate and iodoquinol will be evaluated in infected reptiles. Metronidazole, diloxanide furoate and iodoquinol have also been evaluated at several doses in healthy reptiles to determine safety. The three drugs will also be evaluated for efficacy and safety in box turtles and wood turtles which are infected with Entamoeba of unknown species or pathogenicity.

The safety trials consisted of evaluating metronidazole at 50 mg/kg s.i.d. for 5 days, repeated twice 2 wk apart; metronidazole at 250 and 500 mg/kg p.o. every 2 wk for three treatments. The two lower doses are based on reports in the reptile literature. Iodoquinol was evaluated at 30, 60 and 120 mg/kg p.o., s.i.d. for 14 days. The lowest dose is the recommended human pediatric dose. The length of treatment was based on a recommendation from Barbara Bonner, DVM (pers. comm.) Diloxanide furoate was evaluated at 20, 40 and 80 mg/kg p.o., s.i.d. for 10 days. Again, the lowest dose is based on the human pediatric dose. Length of treatment is the same as that recommended for humans. The higher doses of all drugs were evaluated for toxicity purposes. Juvenile black rat snakes (*Elaphe obsoleta*) were used to evaluate safety as some of our adult black rat snakes had developed hepatotoxicity with neurologic signs when treated with metronidazole at 250 mg/kg every 2 wk for
three treatments. Three snakes were evaluated per drug dose. Three control snakes were given saline at 1% body wt for 10 days.

**Results**

Multiple sampling events are better for the detection of carrier animals. Results of the diagnostic evaluations to date indicate that 24°C is the best temperature at which to culture the organism, that no benefit is gained from culturing for more than 3 days and that samples deteriorate over time after 3 days. It is best to centrifuge the tubes before removing a sample for microscopic analysis for detection or for transfer to susceptible hosts in order to have the greatest chance of detecting or transferring cysts. If the culture tubes are not centrifuged, there is a high degree of variability within the culture medium of where the cysts are most likely to grow. Inconsistent sampling technique introduces a number of variables into detection of oocysts. We have been unable to detect a pathogenic strain to evaluate efficacy of the drugs.

No snakes developed clinical signs of toxicity during the study. At the end of the treatment period, the snakes were euthanatized and necropsied. No gross abnormalities were noted. Histopathology is pending.

**Conclusion**

*Entamoeba* spp. can be a devastating disease in a reptile collection. Proper separation of carrier animals from susceptible animals reduces the likelihood of an epizootic in a collection. Sanitation and proper tool disinfection are equally important. Early detection of cysts and trophozoites prior to presentation of clinical signs could save many animals lives. Culture techniques may offer increased frequency of detection over standard fecal smears or floatation in carrier or subclinical animals. Metronidazole, iodoquinol and diloxanide furoate tested at three different dosages in black rat snakes appear to be safe in that species.

**LITERATURE CITED**

REPTILE ANESTHESIA: UPDATE ON DRUGS AND MONITORING TECHNIQUES

Juergen Schumacher, Dr med vet

Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN 37901 USA

Abstract

The field of reptile medicine and surgery has expanded considerably over the past decade. In addition to a more thorough understanding of reptile pathophysiology, a variety of surgical procedures, including microsurgical techniques are now routinely performed. In order to successfully anesthetize a reptile, a thorough understanding and knowledge of their unique anatomy and physiology is essential. It is also necessary to be familiar with the cardiopulmonary and anesthetic effects of the anesthetic agent(s) being used. The anesthetic effects of drugs used for the immobilization of reptiles show species and individual differences. Additional factors that may influence the response to anesthetic agents include season, age, reproductive status, wild vs. captive animals and health status of the animal.

In many cases, especially when handling large or potentially dangerous species, or in order to minimize stress from handling or to facilitate sample collection, it is essential to restrain and anesthetize reptiles for a variety of diagnostic procedures. In order to reduce mortality and morbidity it is necessary to select a safe and appropriate anesthetic regimen for the species and for the procedure being performed. The veterinarian should select drugs or drug combinations he or she is familiar with and apply his or her knowledge directly to the reptilian patient. Although the response to and the effects of certain drugs may be very different from those seen in domestic mammals, the standards and principles employed in domestic animal anesthesia should be directly applied to the reptilian patient. Several reviews of reptile anesthesia have been published recently.1,3,5

Anesthetic Agents

A variety of different agents have been used in reptile anesthesia with variable success. Many injectable agents are associated with prolonged induction and recovery times as well as profound respiratory depression. For this reason opioid agents and barbiturates are rarely used in reptile anesthesia and most anesthetic regimen are based on the administration of the dissociative agent ketamine HCl combined with other drugs such as the benzodiazepines (e.g., diazepam and midazolam) or opioid agents (e.g., butorphanol and buprenorphine). Ketamine alone is associated with poor muscle relaxation and poor analgesia and if used at high dosages sometimes prolonged recoveries. Ketamine has been used in all orders of reptiles and is most useful for sedation or induction of anesthesia to facilitate endotracheal intubation. Maintenance of anesthesia with an inhalational agent such as isoflurane is recommended. The administration of the dissociative agent tiletamine combined with zolazepam (Telazol) will improve muscle relaxation when compared with
ketamine alone but recovery times may be prolonged. At lower dosages (2-6 mg/kg i.m.) it is adequate for minor diagnostic procedures or for tracheal intubation.

Recently, ultra-short acting induction agents and reversible agents have been investigated for their use in reptile anesthesia. While published data on their cardiopulmonary effects are limited at present, these drugs have the potential to improve anesthetic management of the reptilian patient. Propofol, a non-barbiturate short acting induction agent is commonly used in human and veterinary anesthesia. The main characteristics of this drug are rapid induction and recovery times and minimal cumulative effects, even after prolonged administration. The pharmacokinetics of propofol do not appear altered in patients with renal disease, making it especially useful in reptile patients, commonly diagnosed with impaired renal function. The cardiopulmonary depressant effects of propofol are comparable to those of the barbiturates but they are more transient. A major disadvantage of propofol is the need for i.v. administration making it unsuitable in very small or potentially dangerous reptiles. A recent study investigated the cardiopulmonary effects of propofol administered intraosseously in green iguanas for induction and maintenance of anesthesia. At the dosages used in this study (5 mg/kg and 10 mg/kg i.o.) administration of propofol resulted in rapid inductions but was associated with marked respiratory depression and endotracheal intubation and assisted ventilation and supplemental administration of oxygen was recommended. Further studies are needed to investigate the cardiopulmonary effects of propofol in other reptile species and determine effective and safe dosages.

Reversible agents such as the \( \alpha_2 \)-agonist medetomidine are currently investigated for their potential use in reptile anesthesia. Medetomidine offers the advantage over other agents that it is reversible with the specific antagonist atipamezole. In addition, when combined with other anesthetic agents such as ketamine the dose of ketamine can be reduced, thereby resulting in faster recovery times. However, medetomidine has been associated with pronounced cardiopulmonary depressant effects in dogs and cats, including bradycardia and respiratory depression. A study in leopard and yellow foot tortoises showed medetomidine (100 \( \mu \)g/kg i.v.) combined with ketamine (5 mg/kg i.v.) produced rapid induction times and reversal with atipamezole (400 \( \mu \)g/kg i.v.) was complete within 30 min. In the same study Aldabra tortoises were immobilized with medetomidine (25-80 \( \mu \)g/kg i.v.) and ketamine (3-8 mg/kg i.v.) showing similar results. The above dosages were adequate to allow minor procedures and facilitate endotracheal intubation. All tortoises showed a decrease in heart rates when compared to baseline values. Studies investigating more reptile species and determining effective dosages are needed.

Although some of the newer drugs warrant further investigation, at present isoflurane is the anesthetic agent of choice for prolonged procedures requiring a surgical plane of anesthesia. Recovery times are rapid and isoflurane offers the advantage over other inhalational agents that it has limited organ toxicity and limited effects on renal and hepatic function.
Monitoring depth of anesthesia and cardiopulmonary performance is more difficult in reptiles than in mammals and avian species. Some reflexes are difficult or impossible to assess during anesthesia and depth of anesthesia is often determined by the response of the patient to the surgical procedure. Monitoring of cardiopulmonary performance is often difficult due to the size of the patient and the lack of proper monitoring devices or the inaccessibility of suitable vessels for blood pressure measurements or blood gas analysis. In most reptiles, the use of a Doppler flow device allows monitoring of heart rate and rhythm. The device may be placed either directly over the heart or a suitable artery. Monitoring direct arterial blood pressures by placement of an arterial catheter always requires a cut-down procedure and is impractical for most species of reptiles as a routine monitoring tool. The use of pulse oximetry for monitoring relative arterial oxygen saturation is a standard monitoring practice in human and veterinary anesthesia. Recently, different pulse oximeter probes have been made available which allow their use in even small patients. However, the use of pulse oximeters is limited in reptile anesthesia. Pulse oximeters are calibrated for human patients and calculate arterial oxygen saturation based on the human oxygen hemoglobin dissociation curve. Thus when using pulse oximeters in reptile patients, it must be considered that the saturation values are not absolute numbers. Pulse oximetry is useful in monitoring trends of arterial oxygen saturation.

Arterial blood gas analysis is impractical in most reptiles, especially in small species due to the difficulty of arterial access. Obtaining a blood sample via cardiocentesis and determining blood gas values is of limited value since the obtained sample is in most cases a mixed arterial/venous sample. In larger reptile species measurement of end-tidal CO₂ concentrations is useful for evaluating respiratory performance. In smaller specimen dead-space within the apparatus will compromise results.

Since reptiles are poikilothermic animals, careful monitoring of ambient temperature and initiation of corrective measures, if necessary, is important to assure normal metabolism and excretion of anesthetic agents in reptiles.

Further work is necessary to investigate the cardiopulmonary effects and safety of the newer anesthetic agents in reptile species. Monitoring devices need to be calibrated and validated for their use in reptile anesthesia.

LITERATURE CITED

EVALUATION OF VITAMIN D CONCENTRATIONS IN \textit{Uromastyx} spp. WITH AND WITHOUT RADIOGRAPHIC EVIDENCE OF DYSTROPHIC MINERALIZATION

Bonnie L. Raphael, DVM, Dipl ACZM,* Stephanie B. James, DVM, and Robert A. Cook, VMD

Wildlife Health Sciences, Wildlife Conservation Society, Bronx, NY 10460 USA

Abstract

Vitamin D metabolism is a complex process involving a balance of nutritional, environmental and behavioral factors.\textsuperscript{1,2,6} The roles of calcium, phosphorus, ultraviolet radiation, pre-formed vitamin D and vitamin D precursors in reptile health are the focus of study for many investigators. In reptiles, imbalances of the myriad of factors contributing to biologically correct vitamin D metabolism has been documented to result in clinical syndromes such as fibrous osteodystrophy, dystrophic mineralization, and hypocalcemic tetany.\textsuperscript{3}

\textit{Uromastyx} spp. are herbivorous agamid lizards that have evolved to live in arid, hot climates. In the Wildlife Conservation Society’s collections of \textit{U. ornatus} (\textit{Uo}) and \textit{U. aegyptius} (\textit{Ua}), a disease process, which consists of mineral deposits in skin and periarticular regions, has been observed. Affected animals may develop multiple mineralized nodules which surround joints, diffusely invade skin and ultimately impair mobility. Analysis of the mineralized material in one case revealed the presence of calcium hydroxyapatite and calcium pyrophosphate dihydrate. Uric acid deposits, seen in articular gout, are not a component of this syndrome.

The goal of this investigation was to determine plasma 25-hydroxyvitamin D (25-OH-D) concentrations in captive \textit{Uromastyx} spp., and to correlate those levels with radiographs and clinical signs. The hypothesis was that animals with radiographic lesions would have discernible differences in measurable vitamin D concentrations from animals without lesions. It was hoped that the data would aid in formulation of treatment protocols for clinically affected animals.

Twenty-two \textit{Uromastyx} spp. (\textit{Uo} = 8, \textit{Ua}=14) were evaluated. Animals from four WCS departments (Bronx Zoo Department of Herpetology \textit{n} = 2, Bronx Zoo Children’s Zoo \textit{n} = 10, Prospect Park Wildlife Center \textit{n} = 1, and Central Park Wildlife Center \textit{n} = 3) and one private individual (\textit{n} = 6) underwent physical examination, radiography and blood collection from the ventral tail vein. Of the 22, seven (\textit{Uo}=3, \textit{Ua}=4) were considered clinically affected with one or more of the following: dermatitis, dystrophic mineralization, loss of range of motion in joints, and hypocalcemia. The affected animals also had radiographic lesions consisting of mineralization of the skin and/or multifocal periarticular mineralization.

25-OH-D was determined in samples from all 22 animals (7 affected, 15 unaffected) at Boston University using previously described methods.\textsuperscript{4} The mean concentrations and standard deviations (SD) are reported in Table 1.
For all *Uromastyx* examined, vitamin D₃ concentrations were lower (5-43 ng/ml) than those reported for clinically normal herbivorous lizards housed outdoors (>400 ng/ml).¹ However, none of the animals had evidence of metabolic bone disease or fibrous osteodystrophy. Dystrophic mineralization was seen in *Uromastyx ornatus* having lower vitamin D levels than unaffected animals. The finding of dystrophic mineralization associated with hypovitaminosis D is consistent with previously reported observations in green iguanas.⁵ In *Uromastyx aegyptius*, however, no difference in vitamin D concentrations were seen between affected and unaffected animals.

This study suffers from inconsistencies in blood sampling times vs. appearance of clinical lesions. In some cases, animals had advanced lesions at the time blood was collected for the vitamin D levels. It was beyond the scope of this study to collect dietary or husbandry data, so it is not possible to determine whether the low vitamin D levels reflect insufficiencies in ultraviolet radiation, over supplementation with other fat soluble vitamins, and/or low environmental temperature.

The impetus to perform this study was to help clinicians develop treatment protocols for *Uromastyx* sp. that are affected with varying degrees of dystrophic mineralization. When presented with an animal that has mineral deposits in aberrant sites the quandary has been whether to treat the individual for hypo or hypervitaminosis D, hypo or hypercalcemia and/or renal failure. Based on this investigation it appears that it is reasonable to treat clinically affected *Uromastyx* spp. with injectable vitamin D₃ as part of an initial therapeutic regimen. However, this treatment should be done in conjunction with husbandry measures to assure that the animal has access to appropriate ultraviolet radiation sources and adequate thermal conditions to allow for conversion of previtamin D₃ to vitamin D₃.

**ACKNOWLEDGMENTS**

The authors gratefully acknowledge the cooperation and contributions of Bill Holmstrom, Bruce Foster, Tom Probst and Jim Breheny during the collection of samples for this study, and Michael Holick and Tai C. Chen of the Boston University Medical Center for analysis of the samples. This study was funded by a grant from the Species Survival Fund of the Wildlife Conservation Society.

**LITERATURE CITED**


Table 1. 25-(OH)-D concentrations (x ± SD) in *Uromastyx aegyptius* and *Uromastyx ornatus* with and without dystrophic mineralization (x ± SD).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>25-(OH)-D (ng/ml)</td>
<td>21.6 ±13.6</td>
<td>21.3 ± 18.8</td>
<td>21.6 ±13.0</td>
<td>11.8 ±7.2</td>
<td>6.5 ± 2.1</td>
<td>13.5 ± 7.5</td>
</tr>
<tr>
<td>Sample size</td>
<td>n = 14</td>
<td>n = 3</td>
<td>n = 11</td>
<td>n = 8</td>
<td>n = 2</td>
<td>n = 6</td>
</tr>
</tbody>
</table>
PERIARTICULAR HYDROXYAPATITE DEPOSITION DISEASE IN TWO RED-BELLIED SHORT-NECKED TURTLES (Emydura albertisii)

Christian J. Wenker, Dr med vet, Madeleine Bart, med vet, Franco Guscetti, Dr med vet, Jean-Michel Hatt, Dr med vet, MSc, and Ewald Isenbügel, Prof Dr med vet

1Division of Zoo Animals and Exotic Pets, Veterinary Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland; 2Institute of Veterinary Pathology, Veterinary Faculty, University of Zurich, Winterthurerstrasse 268, 8057 Zurich, Switzerland

Abstract

Two red-bellied short-necked turtles (Emydura albertisii) were presented with multiple periarticular nodules, 1-6 mm in diameter, on all limbs, located predominantly on digital joints. Clinical investigations and subsequent necropsy revealed that these nodules were periarticular deposits of calcium hydroxyapatite (Ca_{10}(PO_{4})_{6}(OH)_{2}). Similar lesions were described in the literature in two previous reptile cases as false gout or pseudogout. Because pseudogout in humans is always associated with articular deposits of calcium pyrophosphate dihydrate (CPPD), it is suggested to correspond to human nomenclature and use the term periarticular hydroxyapatite deposition disease (HADD) also in reptiles. The present report describes the clinical and pathologic features of HADD in two together-housed turtles. The etiology of the condition could not be determined but the simultaneous appearance of the lesions points to biochemical imbalances caused by nutritional or husbandry deficiencies.

Introduction

The crystalline arthropathies are a heterogeneous group of biochemical disorders that share the common feature of crystal accumulation in and around joints. In human medicine, they are classified as gout, which is the deposition of monosodium urate crystals in the synovial tissue following sustained hyperuricaemia, as pseudogout, which is the articular deposition of calcium pyrophosphate dihydrate crystals (CPPD), and as hydroxyapatite deposition disease (HADD).\textsuperscript{1,12,13} The latter is usually periarticular, but intraarticular calcification can occur in a specific syndrome (Milwaukee shoulder-knee syndrome). HADD has been found in patients with chronic renal failure and secondary to connective tissue disorders.

In animals, CPPD cases have been reported in three dogs and one horse.\textsuperscript{5,7-9} In reptiles, hydroxyapatite deposition has been described in an Egyptian spiny-tailed lizard (Uromastix sp.) and a red-eared slider turtle (Chrysemys scripta elegans).\textsuperscript{4,6} In contrast to the human nomenclature and classification these reptile cases were designated as false gout or pseudogout, respectively. The purpose of this report is to describe two simultaneous cases of periarticular hydroxyapatite deposition in two red-bellied short-necked turtles, and to discuss possible etiologic factors of the disorder.
Case Report

Two juvenile, male, red-bellied short-necked turtles were presented for examination at the Division of Zoo Animals and Exotic Pets of the University of Zurich. This strictly carnivorous species lives in inshore waters in Papua New Guinea, and has a characteristic red-colored plastron, especially in juveniles and mature males. The turtles were brought to the clinic because both had multinodular periarticular swellings on all legs. The nodules had been first noticed less than 2 wk before and the alterations had since rapidly progressed. Both turtles were recently acquired from a pet store and housed together in an aqua-terrarium. Water temperature was held at around 26°C, recommended in the literature is 22°C. The turtles diet consisted of shrimps, commercial pellets, flies, and ground meat.

On physical examination, the animals were alert, eating and swimming normally. The larger one weighed 100 g, and the other 49 g. Abnormal findings included multiple, 1-6 mm in diameter, firm, white nodules, located predominantly around digital, but also around radiohumeral, radiocarpal, femorotibial, and tibiotarsal joints of all legs. Radiographic examination revealed amorphous, radiopaque densities at the sites of these periarticular nodules. Insertion of a hollow needle revealed a homogenous, strongly viscous white material. No microorganisms could be cultured from this sample. Cytologic examination showed non-cellular agglomerations of crystal-like clumps.

The owner declined any treatment, but permitted further investigations. The turtles were anesthetized with ketamine-hydrochloride (60 mg/kg body wt i.m.) and blood was collected by cardiocentesis for hematology and serum chemistry. Then the animals were euthanatized and submitted for postmortem examination. Heart, lung, liver, kidney and limb tissues were collected for routine histopathology, periarticular tissue was processed routinely for transmission electron microscopy, and two samples of the nodules were submitted for crystallographic analysis.

Because blood reference parameters for this species are not available, comparisons were made with general reference ranges of chelonians (Table 1). All values were within normal limits except for elevated glucose and urea values in the smaller turtle. The serum calcium-phosphorus ratio was 1.7 and 2.1, respectively. Hematology revealed a leucopenia in the smaller turtle. Except for the nodular swellings on the limbs and an additional similar nodule, 5 mm in diameter, subcutaneously on the ventral aspect of the neck of the larger animal, no gross pathologic lesions were found. The tophaceous periarticular swellings consisted of a solid yellowish mass which adhered to the surrounding subcutaneous and muscular tissues. Histologically, these nodules contained homogenous mineralized material which was delimited by a granulomatous inflammatory reaction with variable numbers of histiocytes and giant cells of the foreign-body type. In Ziehl-Neelsen and periodic acid-Schiff (PAS) stains, these granulomas were negative for acid-fast bacteria or fungal organisms, respectively. Electron microscopy of the deposits revealed tiny, needle-like, short, nanometer-sized crystals. On crystallographic examination the specimens examined consisted of 100% calcium hydroxyapatite (Ca_{10}(PO_4)_6(OH)_2). On the basis of these findings a diagnosis of periarticular hydroxyapatite deposition disease was made.
Discussion

Differential diagnoses of periarticular swellings in reptiles include arthritis, viral papillomas, periarticular abscesses, mycobacterial infections, dermatophilosis, subcutaneous nematodes and filarial parasites, degenerative joint disease, articular forms of gout, metabolic bone disease, pseudogout, and neoplasms.\textsuperscript{2,14} Radiographically, radiodense deposits are indicative of concretions with high calcium contents. In contrast to urate deposits, which usually can be easily removed at necropsy, the hydroxyapatite deposits adhere to the surrounding tissues.\textsuperscript{14} Furthermore, the apatite crystals can be differentiated by electron microscopy from the 2-20 $\mu$m long urate rods and the 2-10 $\mu$m long rhomboidal or rectangular CPPD crystals by their nanometric size and needle-like appearance. This is consistent with the radiographic, histopathologic, and electron microscopic findings in the two red-bellied short-necked turtles, and was confirmed by crystallographic detection of calcium-hydroxyapatite which is known as hydroxyapatite deposition disease (HADD) in humans. Because pseudogout in mammals including humans is always related to articular deposition of a different crystal, calcium pyrophosphate dihydrate (CPPD), it is suggested to correspond to this nomenclature and use periarticular HADD to describe this feature in reptiles.

In human cases, clinical diagnosis is confirmed by electron microscopic examination of synovial fluid samples because the crystals themselves are tiny – only 7-25 nm in diameter.\textsuperscript{12} In addition to radiography, this would also be the method of choice for the clinical diagnosis of reptile samples because with routine cytologic light microscopy only non-birefringent clumps are detected and they could be mistaken for debris.

At variance with previously reported single cases in reptiles, two animals which were kept together showed HADD and were affected at the same time. This opens interesting epidemic aspects of the disease. Husbandry- and nutrition-related biochemical disorders, infectious and hereditary causes have to be considered. The low calcium-phosphorus ratio in these animals supports the hypothesis of a renal or nutritional origin of the disease. Ground meat is known to be low in calcium and to have a calcium-phosphorus ratio of 0.04\textsuperscript{13} and most captive diets have to be supplemented with calcium to avoid nutritional secondary osteodystrophy. No evidence of renal insufficiency could be determined: Uric acid levels were within normal ranges in both turtles. Urea levels were moderately elevated in the smaller turtle but the kidneys were found to be histologically inconspicuous. The parathyroid glands were grossly not considered to be enlarged and therefore they were not investigated histologically. Markedly elevated glucose levels in the smaller turtle were considered to be stress-related. Leucopenia in the smaller animal could be a sign for a viral infection. However, no morphologic lesions indicative of an infectious disease were detected histologically. A relationship of the two turtles could not be determined. In humans with articular gout, apatite crystals were found together with urate crystals in samples of synovial fluid. A secondary apatite formation due to inflammatory processes primarily caused by articular gout is discussed for these patients (Prof. Asper, University Hospital, Zurich, Switzerland, personal communication). However, no urate crystals were detected in the two samples of the turtles by crystallography. Further investigations on the etiology of HADD in reptiles are needed.
ACKNOWLEDGMENTS

Special thanks go to the laboratory staff of the Institute for Clinical Chemistry, University Hospital, Zurich, Switzerland (Prof. Asper) for performing the crystallographic work.

LITERATURE CITED


Table 1. Hematology and serum chemistry of two red-bellied short-necked turtles (Emydura albertisii) with periarticular hydroxyapatite deposition disease.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Turtle 1, 100 g</th>
<th>Turtle 2, 49 g</th>
<th>Reference range Cheloniaa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/L)</td>
<td>0.84</td>
<td>0.65</td>
<td>0.35-1.13</td>
</tr>
<tr>
<td>Red blood cell count (10⁶/µl)</td>
<td>0.83</td>
<td>0.68</td>
<td>0.15-0.98</td>
</tr>
<tr>
<td>White blood cell count (10³/µl)</td>
<td>-</td>
<td>2.5</td>
<td>6-48</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>34</td>
<td>28</td>
<td>20-35</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>4.7</td>
<td>3.7</td>
<td>2.9-6.6</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>111.7</td>
<td>353</td>
<td>32.4-147.7</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>21.3</td>
<td>52.1</td>
<td>10.1-44.8</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.16</td>
<td>0.32</td>
<td>-</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>0.05</td>
<td>4</td>
<td>1.5-11.9</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>6.37</td>
<td>13.6</td>
<td>5.2-68.1</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>3.72</td>
<td>6.47</td>
<td>2.5-10.8</td>
</tr>
<tr>
<td>Calcium:phosphorus ratio</td>
<td>1.7</td>
<td>2.1</td>
<td>-</td>
</tr>
</tbody>
</table>

aBeynon, et al. (1992)

CRYSSTALLINE INCLUSIONS ASSOCIATED WITH VOMERONASAL ORGAN

26

1999 PROCEEDINGS AMERICAN ASSOCIATION OF ZOO VETERINARIANS
PATHOLOGY IN RED-EYED TREE FROGS (Agalychnis callidryas)

Tracey S. McNamara, DVM,1* Peter C. Charles, PhD,2 Yvonne Kress, MS,2 Karen Weidenheim, MD,2 and William Holmstrom, BS3

1Department of Pathology, Wildlife Health Sciences, Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, NY 10460 USA; 2Department of Pathology, Albert Einstein College of Medicine of Yeshiva University, 1300 Morris Park Avenue, Bronx, NY 10461 USA; 3Department of Herpetology, Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, NY 10460 USA

Abstract

The vomeronasal organ (VNO), located in the ventral portion of the nasal septum, plays an important role in chemoreception. Odorant particles from inhaled air and the oral cavity reach the VNO via the incisive duct. Once there, the particles come into contact with two kinds of epithelium. The lateral aspect of the VNO is lined by pseudostratified columnar respiratory epithelium. The medial portion is lined by specialized sensory epithelium composed of basal, supporting, and bipolar nerve cells.2 The neurons deliver chemical messages via the vomeronasal nerve to the olfactory bulb. Pheromones detected in this manner are believed to serve as cues for maternal and male and female sexual behavior.1 Any pathology of the VNO could potentially interfere with normal reproductive behavior and therefore warrants investigation.

Nine red-eyed tree frogs (Agalychnis callidryas) from the collection at the Wildlife Conservation Society died over a 5-yr period with similar lesions in the VNO. The frogs were from a single breeding colony. All but one were captive bred. One frog was obtained from a now defunct dealer and in all likelihood was imported from the wild.

All nine frogs shared a dramatic histologic finding on routine hematoxylin and eosin (H&E) sections. Triangular to polyhedral inclusion bodies were seen in the nuclei of cells in the VNO chemosensory epithelium. The inclusions were brightly eosinophilic, refractile, and had a crystalline appearance. In most cases the inclusions had a base width of approximately 2 microns and were readily visible. In others, the inclusions were smaller, less distinct and were only discerned by focusing in and out of the plane of section.

The inclusions were colorless on unstained sections, intensely eosinophilic on H&E sections and were intensely basophilic with Toluidine blue stains. The inclusions did not stain with periodic acid-Schiff (PAS) or Gomori’s methenamine silver (GMS) stains.

Five out of nine cases had mild to marked, acute to chronic inflammation associated with the VNO. Transmission electron microscopy was performed on two cases. Ultrastructural evaluation revealed greater variety in the size, shape and location of the inclusions than was appreciable at the light microscopic level. The majority of inclusions were intranuclear, but some were found in the cytoplasmic compartment as well. Triangular shapes predominated but rectangular and rhomboid
forms were also present. Multiple inclusions were found in some nuclei. These were attached and arranged in a haphazard manner reminiscent of toy building blocks. Individually or when multiple, the inclusions were surrounded by a fine limiting membrane. Internally the inclusions were composed of a highly ordered pattern of closely spaced parallel dense lines approximately 60-70 angstroms diameter. In addition to these, smaller discrete globular amorphous bodies were seen projecting and/or separate from the larger inclusions. When separate, these were surrounded by a multilayered membrane ranging from 66-160 nm in thickness.

A third identifiable structure within the nuclei of cells with inclusions consisted of honeycomb arrays of roughly spherical particles measuring 22 nm in diameter. These particles studded the surface of the inclusions and were also dispersed throughout the nucleoplasm. Additional ultrastructural changes included mitochondrial swelling, dilatation of smooth endoplasmic reticulum, the formation of myelin figures and/or cellular apoptosis. These changes were seen only in cells containing inclusions. Adjacent non-affected cells were normal.

The nature and significance of these inclusions remains unclear. Crystalline inclusions of both viral\textsuperscript{3,7} and non-viral\textsuperscript{4-6,8} origin have been described in nearly all compartments of the cell\textsuperscript{3} in both vertebrates and invertebrates. The presence of honeycombed structures, in association with the inclusions, degenerative cellular changes in only those cells containing inclusions, and the associated inflammation suggest these findings are not innocuous.

Additional studies are being pursued. These include: infrared spectral analysis of the crystals that may provide information as to their chemical composition; immunostaining specific for the neurons of the VNO that may better define the cell type involved; and polymerase chain reaction (PCR) and immunostaining against nuclear polyhedrosis viruses, insect pathogens known to cause similar inclusions.

ACKNOWLEDGMENTS

We sincerely thank Mr. Alfred Ngbokoli for the extensive slide preparation required in this study.

LITERATURE CITED


ANESTHESIA OF RED PACU (Piaractus brachypomus): COMPARATIVE EFFICACY OF MS-222 AND EUGENOL

Kurt K. Sladky, MS, DVM,1,2* Cliff R. Swanson, DVM, MS, Dipl ACVA,3 Michael K. Stoskopf, DVM, PhD, Dipl ACZM,1,2 and Gregory A. Lewbart, MS, VMD1,2

1Environmental Medicine Consortium, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 USA; 2Department of Companion Animal and Special Species Medicine, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 USA; 3Department of Anatomy, Physiological Sciences and Radiology, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 USA

Abstract

Tricaine methanesulfonate (MS-222), a benzocaine derivative, is the most commonly used anesthetic agent in fish, worldwide, and the only United States Food and Drug Administration (U.S. FDA) approved anesthetic for use in food fish. However, it requires a 21-day withdrawal period in fish prior to human consumption, and there is evidence that chronic exposure in fish, amphibians, and humans can cause reversible retinal deficits. In an effort to find a safe, unregulated alternative, clove oil, the active ingredient of which is eugenol (4-allyl-2-methoxyphenol), has been demonstrated to have anesthetic properties in several fish species.1,3,5,6 Advantages of eugenol relative to MS-222 are that it is considered an unregulated substance by the U.S. FDA, is commercially available, and is inexpensive. However, its safety and efficacy as an anesthetic have not been systematically evaluated. The objectives of this study were to compare the anesthetic efficacy and associated physiologic changes of MS-222 and eugenol across three concentrations of each agent in red pacu (P. brachypomus), and to describe analgesic properties of both agents.

Fifteen cultured red pacu (P. brachypomus) of uniform age and similar weight were used in a within-subjects, complete crossover design. Each subject was exposed to each of the following six anesthetic concentrations expressed as mg/L of H2O: MS-222 (Finquel®, Argent Chemical Labs, Redmond, WA 98052 USA) at 50 mg/L (MS50), 100 mg/L (MS100) and 200 mg/L (MS200); and 100% eugenol (Sigma Chemical Co., St. Louis, MO 63178 USA) at 50 mg/L (E50), 100 mg/L (E100), and 200 mg/L (E200). There was a washout period of 2-4 wk between each anesthetic exposure. Mixed venous/arterial blood samples were collected on each subject from the caudal artery and vein, prior to, and immediately after anesthetic induction for comparative purposes, and the following parameters were measured using an iSTAT clinical analyzer (Sensor Devices Inc., Waukesha, WI 53186 USA): sodium, potassium, glucose, ionized calcium, hematocrit, hemoglobin, pH, P CO2, T CO2, P O2, S O2, HCO3, and base excess. Rate of respiration, as assessed by opercular movement, was recorded prior to, and at each stage of anesthesia and recovery. Stages of anesthesia and recovery were monitored, and behavioral reactions to the insertion of a hypodermic needle during blood sampling were documented prior to, and during anesthesia, in order to subjectively assess analgesia. Data are presented as mean ± SD.
Mean induction times were longer for fish being exposed to MS-222 (MS50 = 600 ± 0; MS100 = 572.3 ± 63.6 and MS200 = 361.3 ± 127.1 sec) relative to eugenol (E50 = 309.3 ± 135.9; E100 = 209.7 ± 137.1 and E200 = 186.3 ± 124.6 sec), and there was a linear trend indicating shorter induction times at higher concentrations of each anesthetic. Time to recovery followed the opposite pattern with more rapid recoveries following exposure to MS-222 (MS50 = 215.3 ± 117.2; MS100 = 318.3 ± 155.9 and MS200 = 452.3 ± 145.9 sec) relative to eugenol (E50 = 537.9 ± 126.9; E100 = 568.3 ± 78.0 and E200 = 600 ± 0 sec), and there was a trend toward prolonged recovery times as anesthetic concentration increased. Subjects exposed to eugenol at higher concentrations required post-anesthesia resuscitation (6 of 15 at E100; 11 of 15 at E200). More individuals reacted to post-anesthesia needle insertion under eugenol anesthesia compared to MS-222. Mean percent change of mixed venous/arterial PO2 dropped by approximately 80%, while PCO2 consistently increased by greater than 90% with anesthesia. Mean blood glucose, sodium, and potassium concentrations, as well as hematocrit consistently increased with anesthesia for both MS-222 and eugenol. Mean blood pH consistently decreased with anesthesia for both MS-222 and eugenol.

Anesthesia with either MS-222 or eugenol contributes to hypoxemia, hypercapnia, respiratory acidosis, and hyperglycemia in the red pacu. Relative to MS-222, eugenol is an effective anesthetic compound in red pacu, characterized by rapid inductions and prolonged recoveries, but the margin of safety appears to be narrow. However, care must be taken when using higher concentrations of eugenol for induction, as medullary collapse is possible and may occur rapidly. In addition, it is unwise to assume that eugenol has analgesic properties until further studies are conducted.

ACKNOWLEDGMENTS

This project was supported by a grant from the Department of Companion Animal and Special Species Medicine Research Fund, College of Veterinary Medicine, North Carolina State University.

LITERATURE CITED

PLASMA IONIZED CALCIUM CONCENTRATION IN HEALTHY GREEN IGUANAS (Iguana iguana)

Patricia M. Dennis, MSL, DVM,* R. Avery Bennett, DVM, MS, and Brad Lock, DVM

Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 USA

Abstract

Calcium is crucial to many physiologic processes, including enzymatic functions and the electrical activity of muscles and nerves. Typically, the calcium status of animals is assessed by the measurement of total serum or plasma calcium concentrations. The nonionized portion of this total calcium consists of calcium in complexed or protein-bound form. Ionized calcium is the biologically active form of calcium. It is normally kept within a narrow range by a variety of homeostatic mechanisms. Measurement of ionized calcium may provide a more accurate assessment of the calcium status of an animal.

Ionized calcium was measured in the plasma of 30 healthy green iguanas (Iguana iguana) to establish a normal range. The animals were housed outside at two separate locations in northern Florida. Blood samples were collected during July and August. Health was assessed by physical examination, complete blood count, and plasma biochemistry profile. Plasma calcium, glucose, phosphorus, uric acid, total protein, albumin, globulin, aspartate transaminase, sodium, potassium, ionized calcium, total carbon dioxide, and pH were measured. The average value of ionized calcium measured in whole blood was 1.47 ± 0.10 mmol/L. The average plasma calcium was 12.8 ± 0.93 mg/dl.

LITERATURE CITED

METABOLIC SCALING OF ENROFLOXACIN IN GREEN IGUANAS (*Iguana iguana*)

Lara K. Maxwell, DVM* and Elliott R. Jacobson, DVM, PhD

University of Florida, College of Veterinary Medicine, Gainesville, FL 32610 USA

Abstract

The equation MR=aM^b is used to express the relationship between metabolic rate (MR) and body wt (M) in animals. The mass constant (a) and mass exponent (b) differ between taxonomic or ecologic groups. Within the broad category of allometric scaling, metabolic scaling relates pharmacokinetic parameters to metabolic rate. Metabolic scaling has been used for both people and animals and has become a fairly common technique in human and veterinary medicine for determination of drug dosages and frequency of administration. This technique is used both interspecifically, for species in which no pharmacokinetic information is available for a particular drug, and intraspecifically, for species that exhibit a wide range of body sizes. Metabolic scaling of drug dosages may be applicable to many animal species. Intraspecific metabolic scaling may be a particularly useful tool in reptile medicine as many reptiles show a tremendous variation in size with age, sex, and maturity, with green iguanas experiencing a 600-fold increase in size from a 10-g neonate to a 6000-g adult.

The use of metabolic scaling has become more common in exotic animal practice for which little pharmacokinetic or physiologic data may be available about a particular species. Data derived from pharmacokinetic studies in one species are used to mathematically determine an appropriate dose and interval of administration for a species in which no pharmacokinetic information is available. This methodology depends upon the metabolic allometric equation of the unknown species approximating that of the known species. Thus, the mass exponent and the mass constant in the equation relating metabolic rate for body mass should be approximately the same for both unknown and known species. If the mass constant and mass exponent in the unknown species fail to approximate that of the known species, then inappropriate dosing will occur. Further, physiologic and biochemical pathways may differ between different species, making metabolic scaling impractical. Allometric scaling may be most accurate when used intraspecifically, with pharmacokinetic information available for that species and adjustments made for size differences.

Allometric scaling of drug dosages in exotic animal medicine is based on the assumption that pharmacokinetic parameters, such as half-life and clearance, scale to body mass to the same power as metabolic rate scales to body mass. In theory, knowledge of the mass constant and exponent of the equation relating metabolic rate and body mass in green iguanas, combined with pharmacokinetic information, should allow the veterinarian to account for size differences in iguanas and formulate an appropriate dose. However, use of this dosing procedure has not been studied in reptiles and may not be more effective than traditional methods in which dosing is directly proportional to body wt and dosing interval is not adjusted to body mass. Scaling based on
metabolic rate is often not as effective as is using allometry to scale each pharmacokinetic parameter separately for each individual drug. Furthermore, the kinetics of some drugs do not seem to lend themselves to prediction by either allometric or metabolic interspecific scaling in mammals.

The objective of this study was to examine the relationship between pharmacokinetic parameters of enrofloxacin (Baytril, Bayer, Leverkusen, Germany) and body size in green iguanas. Enrofloxacin is a broad-spectrum fluoroquinolone antibiotic that is primarily cleared by the kidneys in mammals. Iguanas were maintained at 30°C (86°F.) for 1 wk prior to and during the study and were fed a commercial iguana food (Zeigler Bros., Gardners, PA, USA). In the preliminary portion of this study, 10 green iguanas, ranging in size from 480-3,824 g, were given injectable enrofloxacin at 5 mg/kg intravenously by jugular catheter. Ten blood samples were taken between time 0 and 96 hr post-administration. The plasma concentrations of both ciprofloxacin and enrofloxacin were measured using liquid chromatography with ultraviolet or mass spectroscopy detectors at either North Carolina State University or University of California at Davis. The plasma concentration vs. time profile curve was analyzed non-compartmentally using Kinetica (InnaPhase, Champs sur Marne, France).

Clearance, half-life, mean residence time (MRT), volume of distribution at steady state (Vdss), and Vdarea were plotted against body mass on a double log plot with linear regression of the resulting curve. Both volume of distribution parameters were significantly correlated with body size with an R² = 0.92 and 0.93 for Vdss and Vdarea, respectively, and scaled to body mass with a slope = 0.81 and 0.78. Drug half-life, clearance, and MRT were not as strongly correlated to body mass, with R² = 0.43, 0.55, and 0.58, and slope = 0.38, 0.40, and 0.41.

The standard metabolic rate of green iguanas scales to body mass by approximately the 3/4 power (unpublished data). Preliminary results from this study indicate that metabolic scaling can be used to predict some pharmacokinetic parameters, such as volume of distribution, as both scale to a similar exponent. Metabolic scaling may not be effective in predicting other parameters, such as half-life, clearance, and MRT, as these either do not scale to body size or scale to a different exponent than does metabolic rate. Dose is dependent on volume of distribution, whereas half-life, clearance, and MRT determine the dosing interval. Therefore, although metabolic scaling can be used to adjust the dose of enrofloxacin in various-sized iguanas, its use may not be justified in adjusting the dosing interval to body size.

ACKNOWLEDGMENTS

The University gratefully acknowledges and thanks Morris Animal Foundation for its financial, technical, and administrative assistance in funding and managing the research through which this information was discovered. Without the Foundation’s support and encouragement, this presentation would not be possible. We also thankfully acknowledge support from Bayer Corporation and Pet Care Trust. Additionally, Santa Fe Community College Teaching Zoo graciously provided care for the study animals.
LITERATURE CITED

BENZIMIDAZOLE TOXICITY IN BIRDS

Lauren L. Howard, BS,1* Rebecca Papendick, DVM,2 Ilse H. Stalis, DVM,2 Jack L. Allen, DVM,3 Meg Sutherland-Smith, DVM,4 Jeffery R. Zuba, DVM,3 Daniel Ward, MS5 and Bruce A. Rideout, DVM, PhD2

1Virginia-Maryland Regional College of Veterinary Medicine, 2405 Capistrano Street, Blacksburg, VA 24060 USA; 2Department of Pathology, Zoological Society of San Diego, PO Box 120551, San Diego, CA 92112-0551 USA; 3Department of Veterinary Services, San Diego Wild Animal Park, 15500 San Pasqual Valley Road, Escondido, CA 92027-7017 USA; 4Department of Veterinary Services, San Diego Zoo, PO Box 120551, San Diego, CA 92112-0551 USA; 5Support Laboratory for Study Design and Statistical Analysis, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA 24060 USA

Abstract

Medical records and necropsy reports of 402 pigeons and doves from the Zoological Society of San Diego were examined to determine if birds treated with fenbendazole (FBZ; Panacur, Hoescht Roussel Agri-Vet Comp, Somerville, NJ 08876-1258 USA) or albendazole (ABZ; Valbazen, Pfizer Animal Health Group, Lee’s Summit, MO 64081 USA) had a higher prevalence of toxicity related signs compared to nontreated birds. Birds presented for non-medical reasons (quarantine, relocation, pre-shipment examinations, and routine parasite screening) were exclusively used to avoid confounding variables involved in comparing sick birds to healthy birds. Compared to 5% average weight gain in nontreated birds, birds given FBZ had an average weight loss of 12% (P = 0.0004), and birds given ABZ had an average weight loss of 13.3% (P = 0.0028). Percent of birds with marked leukopenia (WBC <1,000/ul) was higher in FBZ treated birds (62.5%) and ABZ treated birds (100%), compared to nontreated birds (2.1%). Bone marrow hypoplasia was found in more FBZ treated birds (33.9%) and ABZ treated birds (83.3%) than nontreated birds (1%). Small intestinal crypt epithelial changes were not found in nontreated birds, but were identified in 24.5% of FBZ treated birds and in 58.3% of ABZ treated birds. Percent survival was lower in FBZ treated birds (50%, P = 0.0001) and ABZ treated birds (66.7%, P = 0.0001) than in nontreated birds (92.4%). FBZ treated birds also had shorter average survival times following treatment or examination (281.1 days) than nontreated birds (1,231.5 days). FBZ effects appear to be dose related. Birds treated with FBZ at 100 mg/kg showed significantly greater average weight loss (17.7%) and lower survival (11.1%) compared to birds given FBZ at 50 mg/kg, with 7.7% weight loss and 68.4% survival. Birds treated with FBZ at 100 mg/kg also had shorter average survival times following treatment (27 days) than birds treated with FBZ at 50 mg/kg (387 days). These findings are consistent with a toxic etiology. The results of this study suggest that birds of the order Columbiformes are susceptible to toxicity following FBZ or ABZ administration.
DETECTION OF SALMONELLAE IN THE GREEN IGUANA (Iguana iguana) USING THE POLYMERASE CHAIN REACTION TECHNIQUE

Mark A. Mitchell, DVM, MS 1,2* Simon M. Shane, BVSc, MBL, PhD, Dipl ACPV,2 Alma Roy, MS, MT (ASCP)3 and Thomas N. Tully, DVM, MS, Dipl ABVP (Avian)1

1Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 USA; 2Department of Epidemiology and Community Health, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 USA; 3Louisiana Veterinary Medical Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 USA

Abstract

As reptiles continue to gain in popularity as pets and exhibition animals there is a growing concern regarding zoonotic diseases. Reptiles have long been known to carry Salmonella spp. and reptile associated salmonellosis in humans increases relative to exposure. Hatchling aquatic turtles, Pseudemys scripta elegans, were associated with over 300,000 incident cases of salmonellosis in children annually until interstate commerce was banned in 1974. More recently, increased import and exhibition of reptiles have been correlated with an increase of human salmonellosis.2,6 Salmonella spp. has been diagnosed as a primary disease agent in reptiles, but is most often considered to be a normal component of the intestinal tract. Microbiologic techniques are used to isolate the organism, but diagnostic sensitivity can be affected by a number of factors.3 The time required to isolate and confirm the identity of Salmonella spp. is 72-96 hr with conventional microbiology. The delay makes management of cases and therapy more difficult. The application of a genus specific technique, such as the polymerase chain reaction, is rapid and sensitive and could improve the diagnostic capability and management of cases. Depending on sample quality, PCR can provide a diagnosis within 24-48 hr. The purpose of this study was to determine if available oligonucleotide primers used to diagnose Salmonella spp. in domestic species would be of value in identifying Salmonella serotypes in reptiles. Two distinct oligonucleotide sequences were evaluated in this study to determine their applicability to future studies.

Six Salmonella serotypes were isolated from green iguanas and their environment in Costa Del Sol, El Salvador, including Salmonella arizonae 48: I-Z, S. seftenberg, S. newport, S. havana, S. matadi, and S. saint-paul. Escherichia coli and Pseudomonas aeruginosa were also used to assess the specificity of the primers. Bacteria were collected from stock agar and the DNA was extracted using a commercial kit (Qiagen Inc., Valencia, CA, USA). The first oligonucleotide sequence (SGS) defined the amplified region of a 496 bp highly conserved segment of the histidine transport operon gene of S. typhimurium.4 The primer sequences for the upper and lower oligonucleotides were: lower-5' ACT GGC GTT ATC CCT TTC TCT GGT G 3' and upper-5' ATG TTG TCC TGC CCC TGG TAA GAG A 3'. The second oligonucleotide sequence defined the amplified region of a 457 bp invasion protein (InvA) gene of S. typhimurium.5 The primer sequences for the upper and lower
oligonucleotides were: lower- 5' TGC CTA CAA GCA TGA AAT GG 3' and upper-5' AAA CTG GAC CAC GGT GAC AA 3'. Polymerase chain reaction mixtures were prepared including a primer set (20 μM SGS or InvA), 6 μl MgCl, 10 μl dNTP, 1 μl Taq polymerase, 10 μl 10x buffer, and 65 μl of sterile water. Seven microliters of the DNA template extracted from each of the Salmonella samples was added to a PCR mixture. Each Salmonella serotype was tested in triplicate for each of the primer sets. The samples were placed in a 9600 thermal cycler (Perkin-Elmer, Saint Quentin, France) and run for 30 sec at 94°C, 30 sec at 60°C, and 45 sec at 72°C for 33 cycles with an additional 10-min extension cycle at 72°C. The amplified samples were subjected to electrophoresis on a 1% agarose gel containing ethidium bromide. The gel was visualized and photographed using a UV transilluminator. A positive result for the SGS amplicon was indicated by a 496 bp band on the gel and a negative result by absence of band formation. A positive result for the InvA amplicon was indicated by a 457 bp band on the gel.

All six Salmonella serotypes produced 496 bp and 457 bp bands on their respective gels. No band was observed for the E. coli and P. aeruginosa control samples. These findings suggest that these genes are conserved at the generic level and would be useful to identify common Salmonella serotypes in the green iguana.

Further studies are required to evaluate the effectiveness of the PCR technique in comparison to standard microbiologic culture to identify Salmonella spp. infection in reptiles. Currently, three successive negative fecal cultures are required to confirm the negative status of a green iguana. The polymerase chain reaction is more rapid and sensitive than culture and may reduce time for diagnosis.

LITERATURE CITED

RECOGNIZING THE IMPACT OF ENVIRONMENTAL STRESSORS ON CORTICOID LEVELS IN CAPTIVE GIANT PANDAS

Lori Jackintell, MS,1 Megan Owen, MS,1* Nancy Czekala,1 and Don Janssen, DVM2

1Center for Reproduction of Endangered Species and 2Department of Veterinary Services, Zoological Society of San Diego, 1354 Old Globe Way, San Diego, CA 92112 USA

Abstract

Institutions that hold populations of captive species in hopes of breeding them must consider the impacts of physiologic stress on reproductive status. Physiologic stress can be measured in an individual by assessing levels of corticoids.1-4 Measuring physiologic stress is particularly important when the populations are endangered and have a poor track record for captive propagation. Pinpointing the potential source of stress and subsequent elevation of stress is of primary importance and needs to be considered from a variety of sources.

The aim of this study was to characterize the urinary cortisol response to environmental stressors, behavior, and health care management and to also investigate the relationship between stressor and behavioral expression of stress in giant pandas (Ailuropoda melanoleuca). We considered potential stressors from environmental sources (ambient noise); husbandry and veterinary requirements (including anesthesia), and the health of the animal in question. To further evaluate the impact of these stressors on the physiologic condition of the animals, we also looked at the correlation between behavioral stress and elevated cortisol levels.

The current study reflects data gathered primarily from two giant pandas (a 20-yr-old wild caught male, and a 7-yr-old captive bred female) housed at the San Diego Zoo (SDZ). Additional hormonal data were collected at the Giant Panda Breeding Center in Wolong China. Behavioral data was collected at the SDZ for 2 hr, twice daily, 5 days/wk. Activity budgets were determined using scan sampling, at 1-min intervals. The ethogram contains over 100 behaviors related to feeding, locomotion, stress, courtship/social interactions, stereotypies, etc. Stress behaviors include the following: pacing, door-directed behavior, and scratching. Ambient noise levels were recorded continuously using a Cel Instruments 573 sound meter. Urine samples, when possible, were collected daily, during the period of September 1996 through April 1999 at SDZ and between April 1997 through July 1997 at Wolong. Samples were kept frozen at –20°C until analysis. Urinary cortisol levels were determined by radioimmunoassay as previously described.4 Duplicate 10 μl aliquots from each urine sample were directly analyzed using cortisol antibody (Lot #R2-P, ICN Biomedicals Inc. Costa Mesa California). Cross-reactivity was 100% with cortisol, 11.4% with 21-desoxycorticosterone, 8.9% with 11-desoxycorticisol, 1.6% with corticosterone and <1% with other urinary metabolites. Detection limits were 7.8 to 1000 pg/tube. Variation in urine concentration were corrected relative to creatinine (Cr) concentration. Samples with Cr value <0.05 mg Cr/ml were not used.
In this preliminary study, mean basal urinary cortisol concentrations ranged from 50 ng/mg Cr (at Wolong) to 100 ng/mg Cr (at SDZ). There was a 5-10 fold increase in cortisol levels following routine health exams (650-1,335 ng/mg Cr), artificial insemination (647 ng/mg Cr), and initial medical treatment for epistaxis with propanolol (elevated levels returned to baseline 2 days following treatment). Elevated cortisol levels (200-600 ng/mg Cr) were occasionally noted following the occurrence of mucous stools. Additionally, preliminary analysis of the relationship between ambient noise levels (decibels) and cortisol levels in the San Diego female have shown a positive interaction between the two (Mann-Whitney U: z = -2.75; n = 30; P = 0.006). There was also a positive correlation between the percent time spent engaged in stress related behaviors and cortisol levels (Elevated cortisol: 3.76 ± 1.02 SE; Normal cortisol: 1.76 ± 0.42 SE), but this correlation was not significant (Mann-Whitney R: z = -1.41; n1=53; n2=61; P = 0.15) Correlation between behavioral stress and ambient noise level was also positive. But weak (Low db: 4.17 ± 2.06 SE; High dB: 5.12 ± 1.52 SE). This correlation was not significant (Mann-Whitney U: z = -1.19; n = 30; P = 0.23).

LITERATURE CITED

ANESTHESIA, HEMATOLOGY AND DISEASE INVESTIGATION OF FREE-RANGING CRABBEATER SEALS (*Lobodon carcinophagus*)

Michael J. Lynch, BVSc, Albert D.M.E. Oosterhaus, PhD, Debby V. Cousins, PhD, Paul Selleck, PhD, and Pamela Williams, BSc

1Australian Antarctic Division, Channel Highway, Kingston, Tasmania 7050, 1Present address: Melbourne Zoo Veterinary Department, PO Box 74, Parkville, Victoria, 3052; 2Department of Virology, Erasmus University, 3000 DR Rotterdam, Netherlands; 3Australian Reference Laboratory for Bovine Tuberculosis, Department of Agriculture, South Perth, Western Australia, 6151; 4Australian Animal Health Laboratories Geelong, Victoria, 3220; 5Victorian Veterinary Pathology Services, South Yarra, Victoria 3141

Abstract

Thirteen free-ranging adult crabeater seals (*Lobodon carcinophagus*) were immobilized during October 1997 with combinations of pethidine hydrochloride (150 mg/ml solution) and midazolam hydrochloride (20 mg/ml solution) given by i.m. injection via dart. Both drug solutions were prepared from pure substance at the Royal Hobart Hospital, Hobart, Tasmania. Weights of animals were estimated and a state of light anesthesia was the desired level of immobilization. Initial doses of pethidine administered ranged between 1.29-2.2 mg/kg and midazolam between 0.29-0.37 mg/kg. Four animals required supplemental doses of anesthetic agents in order to achieve adequate immobilization. These were delivered intramuscularly by hand injection 30-35 min after initial drug administration. Of these animals, one received only midazolam at 0.16 mg/kg while the other three received pethidine and midazolam at doses of 0.31-0.58 mg/kg and 0.15-0.16 mg/kg respectively. There was one mortality which occurred in an animal that received a supplemental dose of pethidine (0.42 mg/kg) and midazolam (0.15 mg/kg). All other animals were revived using 0.5 mg flumazenil (Anexate, Roche Products, Australia) and 4 mg naloxone (Narcan, Boots Company, Australia) given intramuscularly by dart or hand injection or intravenously into the extradural venous sinus. Time to initial recovery was defined as the ability to move in a coordinated fashion. For animals that received antagonists intramuscularly this ranged from 4-11 min. Animals receiving antagonists intravenously recovered in 1-2 min.

Blood samples were collected from the extradural venous sinus of six animals, placed in plain and EDTA tubes and maintained at 10°C until processing within 4 hr of collection. Blood films were stained using the May-Grunwald Giemsa method. White cells were counted in 10 fields, a mean number of cells per field calculated, and from this an estimated white cell count was calculated. Differential white cell counts were performed manually. Microhematocrit tubes were spun at 1200 rev/min before the PCV was read. PCV values ranged between 41-52% and estimated total white cell counts were between 7-13 × 10^9/L. Neutrophils ranged between 69-81 %, lymphocytes 9-23 %, monocytes 2-7 % and, eosinophils 0-4 %. Serum collected was frozen in liquid nitrogen for less than 4 wk until biochemical analysis. Serum biochemistry parameters measured were total protein, albumin, globulin, sodium, potassium, chloride, bicarbonate, urea, creatinine, calcium, phosphorus, glucose, cholesterol, total bilirubin, ALP, ALT, GGT, AST, CPK, and LDH. All were similar to
those reported for other pinniped species.\textsuperscript{1,2,3}

All blood samples were tested for their ability to neutralize canine distemper virus, phocine distemper virus-1, porpoise morbillivirus, dolphin morbillivirus and phocine herpes virus-1. Three seals had neutralizing antibodies to morbillivirus that in two animals were probably induced by a canine distemper-like virus. One seal had virus-neutralizing antibodies to phocine herpesvirus-1. All blood samples were tested by ELISA for antibodies to influenza A, \textit{Mycobacterium bovis} and \textit{M. avium}. No antibodies to these agents were detected.

LITERATURE CITED

DETECTION OF CIRCULATING BASIC FIBROBLAST GROWTH FACTOR (bFGF) IN SERUM OF ANIMALS WITH KNOWN MALIGNANCY: A PILOT STUDY OF FELIDS AND URSIDS

Christopher Bonar, VMD,1,2* Shawna R. Cornelius,1 Erwin A. Kruger,1 Albert H. Lewandowski, DVM,2 and William W. Li, MD1

1The Angiogenesis Foundation, 124 Mount Auburn Street 207N, Cambridge, MA 02138 USA; 2The Cleveland MetroParks Zoo, 3900 Brookside Drive, Cleveland, OH 44109 USA

Abstract

Angiogenesis, new blood vessel growth, is a critical step in the growth and metastasis of solid tumors.1 Basic fibroblast growth factor (bFGF) is a potent angiogenic cytokine expressed by tumors, and is present at elevated levels in the serum of human cancer patients, and it correlates to prognosis and survival.2-6 Because bFGF has a highly conserved homology across species, we hypothesized that felids and sloth bears, both highly prone to malignancies, would also demonstrate bFGF levels in their serum as an indicator of cancer. The purpose of this study was to determine if bFGF is detectable in felids and ursids, and correlative with malignancy.

Methods

We examined serum samples from 15 animals [4 Siberian tigers (Panthera tigris altaica), 2 Persian leopards (Panthera pardus saxicolor), 1 Indian leopard (Panthera pardus), 1 snow leopard (Panthera uncia), 1 jaguar (Panthera onca), 1 African lion (Panthera leo), 3 sloth bears (Melursus ursinus), and 2 domestic cats (Felis catus)]. Four of the animals had known malignancy (uterine leiomyosarcoma [1 Indian leopard]; bronchoalveolar carcinoma [1 African lion]; metastatic adenocarcinoma of the liver [1 sloth bear]; vaccine-related fibrosarcoma [1 domestic cat]). Eleven healthy animals served as controls. An ELISA assay (Quantikine, R & D Systems, Minneapolis, Minnesota USA) was used to quantitate serum bFGF levels.

Results

Basic FF levels were detected in three of four animals with known malignancy (African lion = 8.98 pg/ml; sloth bear = 21.0 pg/ml; domestic cat = 35.46 pg/ml). No bFGF was detected in 9 of 11 healthy control animals. Two animals thought to be healthy had detectable bFGF (Siberian tiger = 71.29 pg/ml; snow leopard = 2.27 pg/ml) and were later diagnosed with metastatic mammary adenocarcinoma and soft tissue sarcoma respectively. At a 12-mo follow-up, all truly healthy animals remained alive and without evidence of cancer.
Discussion

In this pilot study, the angiogenic cytokine, bFGF, was detectable in the serum of felids and sloth bears with a variety of malignancies. In contrast, animals without neoplasms did not have detectable bFGF in their serum. Further studies are warranted to determine if bFGF may serve as a biologic marker for malignancy for possible application in: 1) early cancer detection; 2) disease prognosis; and 3) selection of novel antiangiogenic therapeutics for the treatment of cancers of felids, ursids, and other animals.

LITERATURE CITED

COMPARATIVE CARDIOPULMONARY AND ANESTHETIC EFFECTS OF KETAMINE-MEDETOMIDINE AND KETAMINE-XYLAZINE IN COUGARS (*Felis concolor*)

**Juergen Schumacher, Dr med vet, Jennifer Erdtmann, DVM, Christal Pollock, DVM, and Ralph Harvey, DVM**

1Departments of Comparative Medicine and 2Small Animal Clinical Science, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN 37901 USA

Abstract

Ten (five female, five male) captive-born cougars (*Felis concolor*) weighing 45 ± 11 kg (x ± SD) were immobilized to determine the cardiopulmonary effects of an i.m. ketamine (Ketaset, Ft. Dodge Animal Health, Ft. Dodge, Iowa 50501 USA) (2.2 ± 0.2 mg/kg) - medetomidine (Domitor, Pfizer Animal Health, Exton, Pennsylvania 19341 USA) (43.4 ± 5.4 µg/kg) combination. In a second trial, 6 mo after completion of the first trial, eight (four female, four male) of the same animals were immobilized with i.m. ketamine (8.4 ± 2.9 mg/kg) – xylazine (Butler Co., Columbus, Ohio 43228 USA) (1.8 ± 0.4 mg/kg). Immediately following immobilization cougars were placed in lateral recumbency and respiratory rates, heart rates, functional oxygen hemoglobin saturation (SpO2) and end-tidal CO2 concentrations were determined every minute for the first 5 min and every 5 min thereafter for 30 min. Arterial blood pressures were determined every 5 min throughout the period of immobilization. Arterial blood samples were collected at 1, 15 and 30 min of immobilization.

Induction time with ketamine-xylazine was significantly longer (19 ± 8 min) than with ketamine-medetomidine (9 ± 3 min). No significant changes in heart rate were seen during the period of immobilization and between groups. A significant decrease in respiratory rates when compared to values obtained at 1 min of immobilization was seen in both groups after 5 min of immobilization and thereafter. Arterial blood pressures were well maintained and no significant changes were seen over time and between groups. Pulse oximeter readings indicated functional oxygen hemoglobin saturation (SpO2) to be < 90% during the first 2 min of immobilization in the ketamine-medetomidine group, indicating hypoxemia. For the remainder of the event SpO2 readings were >90%. No periods of hypoxemia were seen in the ketamine-xylazine group with pulse oximeter readings >90% throughout the period of immobilization. End-tidal CO2 concentrations were < 35% throughout the anesthetic event in both groups. Arterial blood gas variables indicated no periods of hypoxemia (PO2 < 60 mmHg) in both groups during the immobilization period. Reversal with atipamezole (Antisedan, Pfizer Animal Health, Exton, Pennsylvania 19341 USA) given half i.v. and half s.c. (five times medetomidine dose) or 248 ± 0.1 µg/kg in the ketamine-xylazine group, administered 30 min after immobilization was significantly more rapid (4 ± 2 min) and smoother in the ketamine-medetomidine group than in the ketamine-xylazine group (15 ± 9 min).

It is concluded that the cardiopulmonary effects of ketamine-medetomidine and ketamine-xylazine at the dosages used in this study are similar, although ketamine-medetomidine may cause more
pronounced respiratory depression. Quality and time to induction and recovery, as well as muscle relaxation were better in the ketamine-medetomidine group.
IMMOBILIZATION OF FELIDS USING ORAL DETOMIDINE AND KETAMINE

Edward C. Ramsay, DVM, Dipl ACZM,1* Daniel Grove, BS,1 Michele Miller, DVM, PhD,2 and Juergen Schumacher, Dr med vet1

1Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, TN 37901-1071 USA; 2Disney’s Animal Kingdom, Walt Disney World Co., Lake Buena Vista, FL 32830-1000 USA

Abstract

Orally delivered detomidine (0.5 mg/kg; Pfizer Animal Health, Pfizer, Inc., West Chester, Pennsylvania 19380, USA) mixed with ketamine (10 mg/kg; Fort Dodge Laboratories, Fort Dodge, Iowa 50501, USA) was found to reliably produce lateral recumbency within 15 min in domestic cats. Lateral recumbency lasted for approximately 60 min.1 Cardio-pulmonary data indicate this regimen to be reasonably safe as well as effective. The adverse effects noted in domestic cats were salivation, occasional vomiting, and sinus bradycardia (D. Grove and E. Ramsay, unpublished data).

The above detomidine-ketamine regimen has been used in several exotic felids. Seven servals (Felis serval) were given 0.5 mg/kg detomidine and 10 mg/kg ketamine orally. Success of administration was considered <100% in five animals. All servals attained sternal recumbency but only six became laterally recumbent. Mean time (± SD) to sternal recumbency was 9.4 min (± 2.7 min) and mean time to lateral recumbency was 11.3 min (± 3.1 min). Three cats required supplemental administration of drugs (ketamine i.m. or isoflurane) before they were safe to handle. One serval, which became laterally recumbent in < 5 min, showed apnea and required endotracheal intubation and ventilation until reversal. Reversal of the servals with yohimbine was smooth and complete in most cats. One cat, which had received supplemental ketamine i.m., was beginning to spontaneously recover and became very excited following yohimbine administration. This excitement was controlled with i.m. diazepam.

An adult lion (Panthera leo; body wt. = 127 kg) receiving 0.5 mg/kg detomidine and 11.4 mg/kg ketamine orally showed mild ataxia at 16 min and became sternally recumbent at 28 min. This cat was quiet for darting and required only minor supplementation (50 mg tiletamine and 50 mg zolazepam i.m.) for safe examination. Use of approximately 0.37 mg/kg detomidine with 6.7 mg/kg ketamine in a litter of juvenile lions was less successful, in part due to the inability to separate the individuals being dosed and the littermates repeatedly stimulating (awakening) each other. These dosages were also lower than those found effective in domestic cats. One tiger (Panthera tigris; body wt. = 110 kg [estimated]) received 0.5 mg/kg detomidine orally as a premedication. This animal became sternally recumbent at 10 min and did not rise to be darted at 28 min post-detomidine administration.

The above data indicate that orally-delivered detomidine and ketamine is an effective method to sedate exotic felids. Administration of supplemental immobilizing agents is usually required to
achieve complete immobilization, for safe handling of dangerous animals.

LITERATURE CITED

COMPARATIVE ANESTHETIC EFFICACY AND CARDIOPULMONARY EFFECTS OF MEDETOMIDINE-KETAMINE COMBINATIONS AND XYLAZINE-KETAMINE IN RED WOLVES (Canis rufus)

Kurt K. Sladky, MS, DVM,1,2* Michael R. Loomis, MA, DVM, Dipl ACZM,1,4 Brian Kelly, MS,5 Michael K. Stoskopf, DVM, PhD, Dipl ACZM,1,2 and William A. Horne, DVM, PhD1,3

1Environmental Medicine Consortium, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 USA; 2Department of Companion Animal and Special Species Medicine, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 USA; 3Department of Anatomy, Physiological Sciences and Radiology, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 USA; 4North Carolina Zoological Park, Hanes Veterinary Medical Center, 4401 Zoo Parkway, Asheboro, NC 27203 USA; 5United States Fish and Wildlife Service, Alligator River National Wildlife Refuge, Manteo, NC 27954 USA

Abstract

The red wolf (Canis rufus), originally endemic to the southeastern United States, is a critically endangered species. Captive propagation and reintroduction efforts undertaken by the U. S. Fish and Wildlife Service (USFWS) in eastern North Carolina have restored this carnivore species to a portion of its former range.2 Because of the endangered status of this species, and the intensive management practices being used to help maintain the health and well-being of the remaining population, the development of a safe, effective, and reversible anesthetic protocol is crucial. Although immobilization regimens have been described in several wolf species, the red wolf is not among them.3,6-10,12,15 Traditionally, the USFWS has used a xylazine-ketamine combination for immobilizing captive and free-ranging red wolves, but prolonged and rough recoveries have become a concern. Medetomidine, like xylazine, is an alpha2-agonist, but binds with higher affinity, and is more specific for the alpha2-adrenoreceptor.14 When used as a single anesthetic agent, medetomidine causes sedation and analgesia in a variety of species, however, complete anesthesia is rarely achieved.4 In order to induce anesthesia, medetomidine is typically combined with other injectable agents, such ketamine, tiletamine-zolazepam, with or without butorphanol. In addition to providing more effective induction, combinations can provide the benefit of reducing the effective dose of medetomidine. Adverse effects of medetomidine include bradycardia, hypertension, vasoconstriction, respiratory depression, hyperglycemia and hypothermia.11 Rapid and complete reversal of the sedative effects of medetomidine can be achieved by the administration of atipamazole, a specific alpha2-antagonist.

Butorphanol is a synthetic opioid agonist-antagonist, with analgesic and mild sedative properties. In dogs, it has been shown to reduce arterial blood pressure, heart rate, and arterial oxygen tension.5,13 Combining butorphanol with medetomidine can reduce the dose of medetomidine required for inducing sedation in dogs, and has the effect of counteracting the hypertension frequently observed with medetomidine combinations.1

1999 PROCEEDINGS AMERICAN ASSOCIATION OF ZOO VETERINARIANS

49
The primary objective of this study was to compare cardiopulmonary and behavioral effects associated with anesthesia induced by combinations of medetomidine (Domitor, Pfizer Animal Health, Exton, Pennsylvania 19341 USA)-ketamine (Ketaset, Ft. Dodge Animal Health, Ft. Dodge, Iowa 50501 USA), medetomidine-ketamine-acepromazine, medetomidine-ketamine-butorphanol and xylazine-ketamine. Acepromazine (Butler Co., Columbus, Ohio 43228 USA) and butorphanol (Torbugesic, Ft. Dodge Animal Health, Ft. Dodge, Iowa 50501 USA) were included in the medetomidine combinations for both their sedative and vasodilatory actions, in order to evaluate effects on arterial blood pressure and anesthetic quality. The xylazine (Butler Co., Columbus, Ohio 43228 USA)-ketamine combination, used by the USFWS, provided a useful comparison group.

Thirty-two captive, adult red wolves (19 females and 13 males) were used in a between-subjects experimental design. Each subject was assigned to one of the following four experimental groups:

- **XK/Y (n = 8):** xylazine (8 mg/kg) + ketamine (2 mg/kg) induction with yohimbine (0.10 mg/kg) reversal
- **MK/A (n = 9):** medetomidine (40 μg/kg) + ketamine (2 mg/kg) induction with atipamazole (0.2 mg/kg) reversal
- **MKA/A (n = 7):** medetomidine (40 μg/kg) + ketamine (2 mg/kg) + acepromazine (0.01 mg/kg) induction with atipamazole (0.2 mg/kg) reversal
- **MBK/A (n = 8):** medetomidine (20 μg/kg) + ketamine (2 mg/kg) + butorphanol (0.2 mg/kg) induction with atipamazole (0.2 mg/kg) reversal

Each anesthetic combination was administered by hand-injection into the caudal hindlimb muscles. Once induced, a first set of physiologic parameters were collected prior to endotracheal intubation; these included heart rate, rate of respiration, body temperature, indirect arterial blood pressure (systolic, diastolic, and mean) measured oscillometrically (Dinamap, Critikon, Tampa, Florida 33614 USA), and indirect hemoglobin saturation measured by pulse oximeter (Vet Ox 4403, Sensor Devices Inc., Waukesha, Wisconsin 53186 USA). Each subject was intubated and maintained on ambient air during the 50-min procedure. It was our objective to simulate field immobilization conditions as much as possible, so although wolves were intubated, they were not maintained on oxygen or gas anesthetics. After intubation, in addition to the above parameters, end tidal CO₂ and tidal volume were measured by sidestream capnography (Microcap, Sensor Devices Inc., Waukesha, Wisconsin 53186 USA) and spirometry (Model RM121, Fraser Harlake Co., Orchard Park, New York 14127 USA), respectively. Values were measured at 10-min intervals for the duration of the procedure. At approximately 30 min, a femoral arterial blood sample was collected for immediate blood analysis using a portable iSTAT clinical analyzer (Sensor Devices Inc., Waukesha, Wisconsin 53186 USA). The following blood parameters were analyzed: sodium, potassium, ionized calcium, hematocrit, hemoglobin, pH, P_{CO₂}, T_{CO₂}, P_{O₂}, S_{O₂}, HCO₃, and base excess. All individuals were weighed prior to anesthetic recovery. At the end of the procedure, either atipamazole (Antisedan, Pfizer Animal Health, Exton, Pennsylvania 19341 USA) or yohimbine (Antagonil, Wildlife Laboratories, Ft. Collins, Colorado 80524 USA) was administered intramuscularly. Data are presented as mean ± SD.
All four combinations provided sedation within 5 min and complete immobilization within 8-10 min of initial injection. Intubation was most easily and consistently achieved with the MBK/A group, and these subjects were considered more relaxed compared with subjects in the other groups. Arterial blood pressure was markedly elevated immediately following induction in all treatment groups. The average mean arterial blood pressures taken just after induction were as follows: MK/A = 133 ± 42 mm Hg; XK/Y = 150 ± 12 mm Hg; MKA/A = 149 ± 14 mm Hg; and MBK/A = 131 ± 37 mmHg. Blood pressure remained elevated in all treatment groups except the MBK/A group, in which blood pressure decreased steadily over time. Mean heart and respiration rates were lower at all time points in the MBK/A group relative to the three other treatment groups. Mean end-tidal CO₂ was elevated in the MBK/A group relative to the other treatment groups, and in a direct comparison between the MK/A and the MBK/A groups, mean PaCO₂ was statistically elevated in the MBK/A group. There were no differences between groups in PaO₂ or SaO₂. Hemoglobin saturation levels were slightly lower in the MBK/A group during the first 20 min, but were indistinguishable from the other treatment groups for the final 30 min of the procedure. Blood pH decreased slightly in the MBK/A group relative to the other treatment groups. After anesthetic reversal, wolves in the three medetomidine combination groups, generally, were standing within 10-11 min, while the wolves in the xylazine-ketamine group did not consistently remain standing until a mean of 23 min after the reversal agent was administered. The quality of recoveries in the xylazine-ketamine subjects were characterized by prolonged recumbency and ataxia compared with the subjects in the medetomidine combination groups.

Anesthesia of red wolves using all four drug combinations was characterized by marked hypertension. Arterial blood pressure measurements have not been previously reported in other wolf species, and the mechanism underlying this hypertension is unclear. The addition of butorphanol to medetomidine-ketamine attenuated the hypertension, contributed to smooth inductions, and provided excellent muscle relaxation, while reducing the medetomidine dosage by 50%. The mild hypoxemia, hypercapnia, and acidemia observed in the MBK/A group is probably of little clinical significance in a healthy animal, but may become important in a physiologically compromised individual.

ACKNOWLEDGMENTS

We gratefully acknowledge A. Bayer, M. Morse and W. Savage for their assistance.

LITERATURE CITED

A 12-yr-old 27-kg intact male maned wolf (*Chrysocyon brachyurus*), while in his habitat display, was noted to have a mass on the lateral upper lip, below the left nare. Housed with a mate at the Wildlife World Zoo for 7 yr, both had received annual physical examinations, CBC, chemistry profile, serology testing for coccidioidomycosis, heartworm and *Ehrlichia canis* and vaccinations (Duramune KF-11, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501 USA; 1 ml s.c.; Galaxy-D, Solvay Animal Health, Inc., Mendota Heights, Minnesota 55120 USA; 1 ml s.c.; and ImRab 3, Rhone Merieux, Inc., Athens, Georgia 30601 USA; 1 ml i.m.). The affected maned wolf had had no previous abnormalities.

The wolf was anesthetized with 100 mg tiletamine/zolazepam (Telazol, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501 USA; 1 ml i.m.) by blowpipe (Telinject, Saugus, California 91350 USA), intubated, and maintained on isoflurane and oxygen anesthesia. On oral examination a 5 × 2.5 × 2 cm mass was noted on the rostral maxilla. It was a lobular, nodular, raised, pink-gray soft tissue mass, with an ulcerated lesion on one side. Two lateral incisors were incorporated into the mass and were loose. The mass was debulked, removing the affected incisors, and samples were submitted for histologic evaluation. Maxillary radiographs revealed osteolysis in the affected area, up to, but not including the associated maxillary canine tooth. The wolf was given 1.2 mg butorphanol (Torbutrol, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501 USA; 0.6 ml i.v.). Blood was drawn for CBC, chemistry, heartworm antibody testing, coccidioidomycosis antibody testing and *Ehrlichia* antibody testing. The wolf awoke from anesthesia uneventfully.

The hemogram abnormalities included (Table 1) decreased hemoglobin, hypochromia and decreased platelets. Serum chemistry abnormalities included (Table 1) an elevated blood urea nitrogen, decreased alkaline phosphatase and decreased amylase. The agar gel immunodiffusion test for coccidioidomycosis, the enzyme-linked immunosorbent assay test for heartworm, the indirect fluorescent antibody test for *Ehrlichia canis* were all negative. On histologic analysis the rostral maxillary mass was diagnosed as a low-grade fibrosarcoma. There were interdigitating whorls, bundles and fascicles of spindle cells intersecting at various angles. Vessels showed a large area of thrombosis. There were only occasional mitotic figures seen.

Five weeks later, the maned wolf was referred for a rostral maxillectomy. The wolf was anesthetized
with 140 mg tiletamine/zolazepam by blowpipe, intubated and maintained on isoflurane and oxygen anesthesia. Examination of the affected area revealed regrowth of the mass, to approximately 0.3 × 0.2 cm size. Presurgical maxillary radiographs revealed no progression of the osteolysis from previous radiographs. Thoracic radiographs were normal. An indwelling catheter was placed in the left cephalic vein, and 950 ml normosol (Normosol-R, Abbott Laboratories, North Chicago, Illinois 60064 USA) was administered during the course of anesthesia. Additional drugs administered were 1 g cefazolin (Cefazolin, Marsan Pharmaceuticals, Inc., Cherry Hill, New Jersey 08034 USA) and morphine (Morphine Sulfate Injection, Elkins-Sinn, Cherry Hill, New Jersey 08003 USA). Blood pressure was monitored using a doppler (Ultrasonic Doppler Flow Detector, Parks Medical Electronics, Inc., Aloha, Oregon USA). The maxillectomy was routine, incorporating both maxillary canine teeth. Post-surgically a 75 μg/hr fentanyl patch (Duragesic Fentanyl Transdermal System, Janssen Pharmaceutica, Inc., Titusville, New Jersey 08560 USA) was placed between the shoulder blades. The wolf awoke from anesthesia uneventfully. The entire rostral maxilla was submitted for histologic analysis.

Histopathologically, the mass was a low-grade fibrosarcoma. The sample was characterized by interdigitating whorls, fascicles and palisades of spindloid tissue, with a mitoses range from 1-2/hpf. Resection margins were histologically complete.

The wolf recovered well, and began to eat the day following surgery. The fentanyl patch fell off 4 days following surgery. Nineteen days following surgery, the wolf was anesthetized with 100 mg tiletamine/zolazepam (Telazol, 1.0 ml i.m.) by blowpipe, and supplemented with 50 mg ketamine (Ketaset, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501 USA; 0.5 ml i.m.). Oral examination showed the sutures to be in place, the incision healing well and no evidence of tumor regrowth.

Twenty-nine weeks following maxillectomy, the wolf was noted to be ataxic, dyspneic and weak. The wolf was anesthetized with 100 mg tiletamine/zolazepam (Telazol, 1.0 ml i.m.) by pole syringe (Telinject, Saugus, California 91350 USA). The wolf’s weight was 25 kg. On oral examination there was a left lateral mass extending from the upper lip mucosa to the maxilla. Due to the likely recurrence of the fibrosarcoma, and the wolf’s old age, the wolf was euthanized using sodium pentobarbital (Beuthanasia-D Special, Schering-Plough Animal Health Corp., Kenilworth, New Jersey 07033 USA).

A complete necropsy showed generalized atrophy of the skeletal musculature. There was an hepatic cystic mass on a single surface, with pale foci throughout the liver. The jejunal mucosa appeared inflamed. There was a 2858-g mediastinal tumor adhered to one lung lobe. All lung lobes had encapsulated fibrous masses throughout the parenchyma. Histopathologically, there was centrolobular degeneration in the liver, with infiltrations of some erythrocytes suggesting some focal hemorrhages. The kidney glomeruli appeared normal, but the tubules had varying degrees of degenerative changes (many were vacuolated and others were granular). This appeared to be a terminal nephrosis of undetermined cause. The rostral maxillary, mediastinal and lung masses were all well-differentiated fibrosarcomas. All contained a measurable amount of bundles and sheets of
spindle-shaped cells with some collagen formation.

**Discussion**

The maned wolf described in this report had a fibrosarcoma that acted very similarly to that found in the domestic dog. The original rostral maxillary mass grew back aggressively after the original biopsy. Presurgical thoracic radiographs did not show any metastases. A rostral maxillectomy was performed, with margins determined to be clean. The wolf recovered uneventfully from the surgery and did well for 7 mo. Because of his age and low reproductive status, it was decided not to perform further treatment should the tumor recur. Subsequently, we performed only visual examinations to ensure the wolf’s comfort and clinical status. It was not known exactly when the fibrosarcoma began to regrow on the upper lip. When the wolf did finally depict clinical signs of dyspnea and weakness, the local recurrence and lung metastases were severely aggressive.

Due to the wolf’s status as an endangered species, the authors feel the initial aggressive surgical option was warranted. Follow-up reevaluations and perhaps future surgeries might also be warranted in a younger maned wolf that is more valuable to the present gene pool of captive specimens.

**Table 1.** Abnormal hematologic and serum biochemical findings in a maned wolf.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Finding</th>
<th>ISIS Mean(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.5</td>
<td>13.8</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (g/dl)</td>
<td>30.3</td>
<td>33.5</td>
</tr>
<tr>
<td>Platelets (k/mm(^3))</td>
<td>172</td>
<td>229</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dl)</td>
<td>30</td>
<td>23</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>16</td>
<td>131</td>
</tr>
<tr>
<td>Amylase (IU/L)</td>
<td>289</td>
<td>532</td>
</tr>
</tbody>
</table>

\(^a\)International Species Information System, Apple Valley, Minnesota 55124-8152 USA
PAIN AND ITS MANAGEMENT IN GERIATRIC VETERINARY PATIENTS

Jill E. Sackman, DVM, PhD, Dipl ACVS

Ethicon Endo-Surgery, Inc., Surgical Research and Development, 4545 Creek Road, Cincinnati, OH 45242 USA

Abstract

Recognizing Pain in Veterinary Patients

Pain Localization

The localization of pain to a particular area of the body is the responsibility of the central nervous system (CNS). Pain may be poorly localized due to a low density of sensory nerve fibers in the peripheral tissue or because of pain pathways that frequently branch and converge, making it difficult for the brain to localize the sensation. Pain inflicted to tissues with a high density of pain receptors such as the skin is generally much more precisely localized than the pain associated with internal organs.

In many cases, an animal initially reacts to pain by attempting to escape from the source. If this fails to bring relief, aggression or vocalization often follows. Animals experiencing acute postoperative or traumatic pain may also respond by biting, licking, rubbing, or scratching at the source of discomfort. Chronic, low-grade pain often associated with prolonged hospitalization, radiation, and chemotherapy or with severe osteoarthritis may manifest itself by an animal’s failure to groom, lack of interest in surroundings, reluctance to move, anorexia, weight loss, constipation, or dysuria. Elderly animals experiencing chronic pain often appear withdrawn and quiet. It is important to realize that not all signs of pain may be present at one time and that no single sign is a reliable indicator of the level of pain experienced.

Geriatric Considerations

Pharmacokinetic and pharmacodynamic changes independent of disease can be seen in geriatric patients. Generally, drug absorption remains unchanged with aging, despite increased gastric pH, mucosal atrophy, and decreased gastrointestinal motility. Age-related changes in drug distribution secondary to body composition are common. Some of the most important changes are an increase in body fat, decrease in lean body mass, and decrease in total body water. Older patients also frequently have decreased hepatic albumin production.

The ability of the aging liver to metabolize drugs does not decline equally for all agents. Glomerular filtration rate (GFR) gradually declines with age. Along with a decreased GFR, there is a decrease of almost 50% in renal plasma flow, resulting in a significant rise in filtration fraction with age.
Loss of renal tubular and resorptive function also occurs with aging. The use of non-steroid anti-inflammatory drugs (NSAIDs) in elderly patients is common, and it is in this group that adverse side effects occur most frequently. Inhibition of prostaglandin-mediated renal vasodilation by NSAIDs along with the renal changes already outlined can lead to severe nephrotoxicity in elderly patients. The concurrent use of diuretics, presence of congestive heart failure, use of general anesthesia for surgical procedures (e.g., dentistry), or existence of other conditions leading to poor perfusion all can contribute to renal toxicity during NSAID use.

Analgesic Drugs – Narcotics

*Morphine*

Morphine is considered to be the prototypic narcotic analgesic. Two advantages of morphine are analgesia and sedation. The effects of morphine can be reversed by antagonists such as naloxone. Adverse effects of morphine administration include respiratory and CNS depression. Care should be taken when administering morphine to geriatric patients with compromised renal or hepatic function, because drug elimination may be significantly prolonged. At higher doses, morphine can be excitatory for cats. Morphine given in lower doses or combined with a tranquilizer appears to reduce the untoward effects in cats.

*Fentanyl citrate*

Fentanyl (Sublimaze®) is a highly potent, synthetic phenylpiperidine derivative with action similar to that of morphine but with 100 times the potency.

Fentanyl has a short duration of action with peak effects lasting only 30-45 min. Like morphine and meperidine, the effects of fentanyl can be reversed with narcotic antagonists such as naloxone. Although fentanyl is an excellent analgesic, its use in veterinary medicine has largely been precluded (with the exception of continuous i.v. infusion or transcutaneous patches) because of its short half-life.

Transdermal application of fentanyl has recently become popular in veterinary medicine for the treatment of perioperative pain. Permeability of the stratum corneum varies widely; it is affected by body site, skin temperature, skin blood flow, skin color, and the presence of skin damage or disease. Application of fentanyl citrate patches (Duragesic®) designed to deliver 50 μg/hr to dogs resulted in an average of 1.6 ng/ml at a steady-state concentration. In humans plasma levels of between 1 and 2 ng/ml are considered to be analgesic. Transdermal fentanyl patches work particularly well in geriatric patients because they do not cause sedation or significant respiratory depression. Patches should be placed at least 24 hr prior to surgery to allow for adequate plasma levels to develop. Fentanyl is absorbed from the patch continuously for 72 hr.

*Oxymorphone hydrochloride*
Oxymorphone (P/M Oxymorphone HC1) is a semisynthetic narcotic analgesic that is approximately 10 times as potent an analgesic as morphine. It has duration of action ranging from 4-6 hr. Adverse effects are similar to those of morphine; however, it appears to cause less respiratory depression and gastrointestinal stimulation.

**Epidural opioids**

For postoperative epidural analgesia in veterinary patients, morphine is used most commonly. Subarachnoid or epidural administration of preservative-free morphine (Duramorph PF® or Astromorph/PF®) has been quite efficacious. Epidural morphine is an excellent technique for providing postoperative analgesia after pelvic limb surgery. Administered at the lumbosacral space, it has been used after thoracic surgery but depends upon cephalad migration of the drug within the cerebrospinal fluid. The dose of epidural morphine should be administered 30-60 min prior to recovery from anesthesia. The duration of analgesia is from 6-24 hr.

**Butorphanol**

Butorphanol (Torbugesic®) belongs to a group of synthetic analgesics with combined agonist and antagonist properties. Butorphanol is considered to be a weak antagonist at the μ-receptor but a strong agonist at the k-receptor. Butorphanol is three to five times more potent an analgesic than morphine. The antagonist activity of butorphanol is nearly 50 times less than that of naloxone. The respiratory depression produced by butorphanol is similar to that of morphine; however, it has a “ceiling effect” beyond which higher doses fail to increase the depression further. Butorphanol is also a well-established antitussive and has been used as an anti-emetic in cancer patients. Similar to other narcotics, butorphanol is metabolized by the liver and has a plasma half-life to 3-4 hr in dogs. Butorphanol has a relatively short half-life and is a better analgesic for visceral than somatic pain. Clinically, butorphanol is a safe drug and appears to act as a good analgesic for mild to moderate pain.

**Buprenorphine**

Buprenorphine (Buprenex®) is a mixed agonist/antagonist, which is very popular in Europe as an analgesic and sedative drug. This drug differs from butorphanol in that its association and dissociation with the opioid receptor occur slowly. Because of its slow receptor association, it may take up to 30 min after i.v. injection for buprenorphine to take effect. Buprenorphine has a longer duration of action than butorphanol because of its tight binding to and slow dissociation from, opioid receptors. Because it binds tightly to its receptor, buprenorphine’s effects can be difficult to reverse with naloxone.

**Naloxone**

Naloxone is primarily used to reverse respiratory depression and given at 0.4 to 0.8 mg either
intramuscularly or intravenously. There is rapid reversal of opioid compounds. Antagonist activity will last from 1-4 hr depending on the initial dose given. When naloxone is used to reverse a pure agonist, readministration to prevent “renarcotization” may be necessary, because many opioids have a longer half-life than naloxone.

**NSAIDS**

NSAIDs include a wide variety of different agents or different chemical classes. Most of the drugs have three major types of effect: anti-inflammatory, analgesic, and antipyretic. In general, all of these effects are related to the primary action of the drugs: inhibition of arachidonate cyclooxygenase (COX) and thus inhibition of the production of prostaglandins and thromboxanes. There are two types of COX: COX-1 and COX-2. COX-1 is a constitutive enzyme produced in most tissues, including platelets, and is involved in cell-to-cell signaling. COX-2 is induced in inflammatory cells when they are activated and is believed to be responsible for producing most of the prostanoid mediators of inflammation. Most of the NSAIDs inhibit both COX-1 and COX-2 to varying degrees. The untoward effects of most NSAIDs are secondary to their inhibition of COX-1. Selective COX-2 inhibitors are currently being developed.

*Side Effects of NSAIDs*

Adverse drug reactions are common with the use of NSAIDs, especially when they are used chronically and at high doses for musculoskeletal pain. Adverse gastrointestinal events are the most common untoward effects of NSAIDs in animal and human patients. Gastric ulceration, nausea, emesis, and diarrhea commonly occur in dogs given NSAIDs. A direct irritant effect on gastric mucosa may contribute to damage, as it is known that tablets are more likely to cause problems than capsules, suspensions, or solutions. “Slow-release” and “enteric-coated” preparations also cause fewer problems. NSAID-induced gastric ulceration, however, is predominantly due to the drug inhibition of prostaglandin synthesis. Prostaglandins normally are responsible for inhibiting gastric acid secretion as well as stimulating mucus production and mucosal blood flow. Concurrent administration of the prostaglandin analogue, misoprostol (Cytotec®) has been shown to diminish gastric ulceration in dogs when associated with NSAID use.

*Salicylates*

Salicylates commonly used in clinical veterinary practice include aspirin and bismuth subsalicylate (Pepto-Bismol®). Salicylates are effective in relieving pain associated with peripheral inflammation such as muscle and joint disease but have virtually no effect on deep or visceral pain. Salicylates have antipyretic effects because of their ability to reduce prostaglandin-induced fever.

Clinical use of aspirin has centered on the management of inflammatory and degenerative joint diseases. Aspirin may be used in the cats as long as it is administered no more than every 36-48 hr.

*Carprofen*
Carprofen (Rimadyl®) is a new NSAID approved for use in the dog. It has been shown to be anti-inflammatory, antipyretic, and analgesic. Carprofen is a reversible inhibitor of COX-1 and COX-2 and a moderately potent inhibitor of phospholipase A2. The mean half-life of elimination is approximately 8 hr after a single oral dose. Carprofen is the first propionic acid NSAID approved in the United States for use in dogs as a first-line therapy for degenerative joint disease.

Naproxen

There are a considerable number of propionic derivatives on the market. Naproxen (Naprosyn®) has effective anti-inflammatory, analgesic, and antipyretic qualities and is an effective COX inhibitor. Naproxen has approximately 20 times the potency of the related drug ibuprofen. In humans, naproxen has been used successfully to treat musculoskeletal diseases. The toxicity of naproxen is similar to that of other NSAIDs. Because naproxen has a long duration of action in the dog, it can be administered once daily.

Meclofenamic acid

Meclofenamic acid (Arquel®) inhibits COX and may also block cell surface receptors for prostaglandins. The drug has gained popularity for treating musculoskeletal pain in the dog that is refractory to aspirin. Toxic side effects similar to those of other NSAIDs have been observed in the dog.

Flunixin meglumine

Flunixin meglumine (Banamine®) is an NSAID with both analgesic and antipyretic effects. Flunixin is considered to be one of the most potent COX inhibitors available. The analgesic potency of the drug is greater than that of phenylbutazone, meperidine, or codeine.

Flunixin is recommended for relief of persistent, severe inflammation and pain associated with degenerative joint disease that is nonresponsive to milder analgesics. Because flunixin is such a potent COX inhibitor, it also frequently causes severe gastric irritation. Its use in the dog should not exceed 1 mg/kg once daily for 3 days.

Phenylbutazone

Phenylbutazone (Butazolidin®) has analgesic, anti-inflammatory, and anti-pyretic effects similar to those found in the salicylate family. As a member of the pyrazolone family, it has been associated with significant toxic side effects in humans and, less commonly, in animals.

Clinically, phenylbutazone has been used in the dog for treatment of musculoskeletal diseases and is considered to inhibit COX more effectively than aspirin but less effectively than meclofenamic acid or naproxen. Phenylbutazone is known to cause gastrointestinal irritation, renal disease, hepatic disease, and rarely, bone marrow suppression with prolonged use in the dog.
Corticosteroids

When given therapeutically, corticosteroids have powerful anti-inflammatory and immunosuppressive effects. They inhibit both the early and late manifestations of inflammation (i.e., not only the initial heat, pain, and swelling but also the later stages of wound repair). They affect all types of inflammatory reactions whether they are caused by pathogens, toxins, or physical stimuli or are immune mediated.

Despite their significant side effects, corticosteroids are very useful in “dampening the fire” of acute inflammation. Useful corticosteroids for treating inflammation include hydrocortisone, prednisolone, and prednisone. The anti-inflammatory effects of prednisone and prednisolone are five times greater than those of cortisone. Corticosteroids are frequently used as short-term (3-5 days) anti-inflammatory drugs in the treatment of acute exacerbation of chronic musculoskeletal pain.

Selective Nerve Blocks

The use of local anesthetics to selectively block peripheral nerves following surgery allows for the relief of pain without the side effects associated with systemic use. Selective blocking of intercostal nerves prior to chest wall closure after thoracotomy has been shown to provide analgesia equal to that of systemic morphine. The technique involves injecting 0.5% bupivicaine (Marcaine®) at intercostal nerves as they pass behind the rib heads for two to three intercostal spaces in front of and behind the incision site. A maximum total dose of 4-5 mg/kg in dogs and 2-3 mg/kg in cats should be used to avoid systemic toxicity. Complete blocking of the intercostal nerves with bupivicaine should provide analgesia for 4-5 hr without the respiratory depression associated with narcotic use.

Selection of the Appropriate Analgesic

Published literature can provide a useful source of information on appropriate analgesia.1-6 When selecting an analgesic drug for the treatment of pain, animals should be divided into at least two categories: those with acute pain resulting from surgery or acute trauma and those experiencing chronic, low-grade pain, frequently of musculoskeletal origin. Patients with acute pain generally benefit the most from short-term opioid use. When anxiety, as demonstrated by excessive vocalization, is involved with acute pain, low doses of tranquilizers may be added to the narcotic regimen. Patients with musculoskeletal diseases, including primary and metastatic bone neoplasia, benefit the most from NSAIDs. In general, the most effective therapeutic approach is to start with aspirin and then advance to more potent drugs such as meclofenamic acid, carprofen, or piroxicam. When administering NSAIDs in geriatric patients, the veterinarian should remember to evaluate existing renal function and hydration status to avoid a worsening of their condition.

Principles of analgesic therapy in the cat should follow the same basic parameters as those in the dog. Of the NSAIDs available, only aspirin is recommended for use in the cat. Aspirin given orally
every 48 hr is generally effective for musculoskeletal pain. Narcotics are effective in controlling acute pain in the cat. Drug doses are generally somewhat lower than those in the dog to avoid the excitatory effects sometimes observed in the cat.

LITERATURE CITED

NUTRACEUTICAL CHONDROPROTECTIVES AND THEIR USE IN OSTEOARTHRITIS IN ZOO ANIMALS

Cynthia E. Stringfield, DVM* and Janna E. Wynne, DVM

The Los Angeles Zoo, Los Angeles, CA 90027 USA

Abstract

Introduction

Recently, the result of many years of research that originated primarily in Europe, has led to the development of a new class of nutrients that act completely differently from medications historically used for osteoarthritis. These compounds are collectively termed chondroprotective agents, since they directly affect chondrocytes in a beneficial manner.

As zoo animal management and health care improve, zoo veterinarians are faced with managing an increased number of geriatric patients. Osteoarthritis is a prevalent cause of morbidity in these patients. The Los Angeles Zoo has a large percentage of geriatric animals. A review of the mammal collection in 1996 showed 20% were considered to be geriatric, and 50% were past breeding age. A review of the last 4 yr of medical entries showed 60 cases in mammals of geriatric osteoarthritis treated by clinicians with therapies including chondroprotectives. Success of treatment can be subjective, however caretakers and veterinarians felt they saw improvement in the majority of cases. The method of action for the chondroprotective agents, common product examples, the data supporting their use, and three case studies from the Los Angeles Zoo are presented.

Methods of Action and Products

Normal articular cartilage consists of chondrocytes (10%) surrounded by an extracellular matrix (90%) of water, collagen and proteoglycans. Cartilage is unique among body tissues by being avascular, aneural and alymphatic. All materials necessary for chondrocyte function must diffuse through the matrix from synovial fluid or subchondral bone. This creates diffusion dynamics for the supply of vital nutrients to cartilage that normally is only barely adequate to maintain normal turnover. Thus, any insult can easily affect the nutritional state of cartilage. Such insults result in a need for augmented synthesis that often generates extremely large demands of raw materials. As chondrocytes are lost the ability to increase production is also decreased.

Proteoglycans consist of a core protein with glycosaminoglycan side chains, linked to a hyaluronic acid (itself a glycosaminoglycan) backbone (Fig. 1). Glycosaminoglycans (GAGs) are mucopolysaccharides (repeating sugar molecules). There are six types in the body: chondroitin sulfate, keratan sulfate, hyaluronic acid, dermatan sulfate, heparin, and heparan sulfate. The two found in proteoglycan side chains are chondroitin sulfate and keratan sulfate. Hyaluronic acid is
made of glucuronic acid and glucosamine and is also a component of synovial fluid, the viscous fluid that bathes the synovial membrane of the cartilaginous joint.

Glucosamine:  
1. Is a precursor for hyaluronic acid and proteoglycans.  
2. Stimulates synthesis of collagen and proteoglycans.  
3. Has a mild anti-inflammatory effect.  
4. Has no toxicity and excellent bioavailability orally. (Pharmacokinetics have been tested in dogs, rats, and humans.)

Chondroitin:  
1. Is a building block of proteoglycans.  
2. Inhibits degradative enzymes that destroy cartilage (protects against catabolism of the joint).  
3. Prevents fibrin thrombi in synovial or subchondral microvasculature.  
4. Has an anti-atherosclerotic effect - maximizes blood circulation to tissues. (Important in common geriatric diseases such as diabetes, heart and renal disease, etc.)  
5. Has an anti-inflammatory activity on the cellular level.  
6. Has an affinity for articular tissues, is well absorbed orally.

It has also been shown in cell culture that these two nutrients work synergistically by upregulating cartilage metabolism.

There are many available products containing these supplements:

1. GAGs: Polysulfated GAGs (PSGAGs): Adequan® (Luitpold Pharmaceuticals, Shirley, New York 11967 USA)  
   Mixed GAGs: from freeze dried green-lipped mussel (*Perna canaliculus*): Glycoflex®, Synoflex® (Equine - caramel and cinnamon flavors), Nu-Cat® (Feline - has taurine and digestive enzymes). (Vetri-Science Laboratories, Essex Junction, Vermont 05453 USA)  
   Chondroitin/keratan sulfate: from bovine, shark cartilage

2. Glucosamine HCl or sulfate: derived from crab shells (chitin)

3. Glucosamine HCl/chondroitin sulfates/manganese: Cosequin® -veterinary & Cosamin®-human (Nutramax Laboratories, Baltimore, Maryland 21236 USA). The equine powder has 3:1 ratio of glucosamine to chondroitin sulfate vs. SA is 1:1 due to difference in absorption patterns between carnivores and herbivores.

Products may also contain various minerals and vitamins involved in proteoglycan synthesis (Mn) and creating collagen from amino acids (Fe, Cu, Zn, Mn, vitamin C), free radical scavengers (vitamin C, vitamin E), omega-3 fatty acids for prostaglandin synthesis, etc. Nutraceuticals require NO testing and products may or may not actually contain what the label claims.

**Studies Supporting Oral Chondroprotective Use**
Many species have been studied, primarily humans, horses, rabbits, rats, and dogs. The use of injectable PSGAGS has been well described. Oral supplements have been in use in Europe for 30 yr in arthritic people and a large body of European literature exists.

Chondroitin. Studies done with Condrosulf® (IBSA, Lugano, Switzerland), a pure chondroitin 4,6 sulfate European product, showed it to be protective for cartilage insults in rabbits. Human studies showed it to improve knee arthritis, and to be protective for erosive finger lesions in a 3-yr study. When diclofenac sodium, a non-steroidal anti-inflammatory drug (NSAID) was compared to chondroitin in a sixth trial, chondroitin effects lasted 3 mo after stopping but had a later onset, the NSAID caused prompt resolution, but signs reappeared after stopping. Another study showed concomitant use of chondroitin and diclofenac resulted in less pain than with either alone, and patients were able to decrease the NSAID by 72%.

Perna. A double blind study with humans in Scotland showed 46-76% efficacy in rheumatoid and osteoarthritis.

Glucosamine. Studies showed pain relief and increased joint flexibility. Onset of action was weeks, but then glucosamine performed the same or better than ibuprofen with no side effects. Most recently in the United States, Cosequin®/Cosamin® has been studied. In a study with horses, within 2 wk after starting Cosequin® treatment, horses with proven degenerative joint disease (DJD) showed statistically significant clinical improvement irrespective of age, joint affected or use of the horse. Another study involving navicular disease showed consistent improvement of Cosequin®-treated horses vs. placebo horses. In a study with dogs, animals pre-treated with Cosequin® were protected against experimentally induced synovitis and showed decreased lameness. It caused a decrease in post-surgical stifle osteoarthritis in another study. Two 1999 Cosamin® studies with humans (one on Navy SEALS) showed relief of DJD knee symptoms with no hematologic effects. Rabbits showed histologic decreases in moderate and severe cartilage lesions when pre-treated with Cosequin®.

Case Reports

Forty-two species of mammals at the Los Angeles Zoo have been treated with these products at dosages recommended by the manufacturers (dosed by body wt) (Table 1). Three specific examples are detailed below:

1. Thirteen-year-old (age estimated) wild-caught black howler monkey (Alouatta caraya): Noted to be “slowing down” with a hunched posture, unable to grip with tail normally. Physical exam showed scoliosis of spine; radiographs showed severe ventral bridging spondylosis through T-L region. He was started on Glycoflex® 100 mg p.o., s.i.d.and was much improved within 2 wk, using his tail to prehend and more mobile and active. This persisted for 1.5 yr when he died of chronic renal failure.
2. Ten-year-old (age estimated) wild-caught harnessed bushbuck (*Tragelaphus scriptus scriptus*): Presented for a right rear lameness and hunched and swinging gait in the rear and a gradual decline in its ability to get up in the morning and move quickly, that it would “warm out of.” One scoop of Cosequin® for two animals over grain once a day was started. After 2 wk, its keeper felt it was improved. Two months later, after attempted breeding by the male, it worsened and was examined: Radiographs of the hip and spine were normal after an initial pop of the right hip on palpation. Three days later the lameness had resolved. Mild signs gradually reappeared and it maintained for another 2.5 yr when it deteriorated and was euthanatized for humane reasons. On necropsy there was histologic confirmation of chronic degenerative arthritis in the right coxofemoral joint.

3. Seventeen-year-old Central American tapir (*Tapirus bairdii*): History of chronic intermittent right rear leg lameness of greater than 8 yr duration, at times non-weight bearing. Radiographically, no visible joint lesions were seen. He had been managed on soft substrate, with nonsteroidal antiinflammatory drugs when lameness was severe. After 2.5 wk on 1/2 scoop Cosequin® p.o., b.i.d., his lameness was significantly improved. Over the past 16 mo, his periods of lameness have been much less frequent with no non-weight-bearing episodes. After a recent move to a hard-floored area he has maintained well with one moderate and rapidly resolving period of lameness treated with a short course of NSAIDs in addition to the Cosequin®.

**Conclusion**

Osteoarthritis is an irreversible disease and the most effective treatment is prevention. Once it develops however, options for treatment have consisted of surgery, weight reduction, controlled exercise, and pain relief medication. Nonsteroidal and steroidal anti-inflammatory drugs are used as palliative therapy, but can have significant drug side effects. Studies have also shown that these drugs can down-regulate chondrocyte metabolism and actually decrease GAG synthesis. Nutraceutical chondroprotective agents might provide another treatment for, and may perhaps prevent osteoarthritis with no apparent side effects. They need to be used early in the process, since they require viable chondrocytes and some cartilage to be effective. Effects may be seen after 2 wk, with full effect taking 6-8 wk. If anti-inflammatory or analgesic medication is needed, they should be used in conjunction. They may decrease the amount required and help prevent the catabolic effects of nonsteroidal antiinflammatory drugs and corticosteroids. Intermittent therapy may be possible and needs further study.

**LITERATURE CITED**


---

**Figure 1.** The above reaction occurs in the chondrocyte.

**Table 1.** Species treated with nutraceuticals at the Los Angeles Zoo.

<table>
<thead>
<tr>
<th>Marsupials</th>
<th>Koala</th>
<th>Phascolarctos cinerus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wallaroo</td>
<td></td>
<td>Macropus robustus</td>
</tr>
</tbody>
</table>

---
Kangaroo
Macropus giganteus

Primates:
Ring-tailed lemur
Lemur catta
Bushbaby
Galago crassicaudatus
Black howler monkey
Alouatta caraya
Golden-lion tamarin
Leontipithecus rosalia
Emperor tamarin
Saguinus imperator
Moustached guenon
Cercopithecus cebus
Drill
Papio leucophaeus
Siamang
Hylobates syndactylus
Orangutan
Pongo pygmaeus

Carnivores:
Dingo
Canis familiaris
Maned wolf
Chryocyon brachyurus
Polar bear
Ursus maritimus
Wolverine
Gulo gulo
Mountain lion
Felis concolor
Lion
Panthera leo
Snow leopard
Panthera uncia
Jaguar
Panthera onca

Proboscideans:
Asian elephant
Elephas maximus
Perissodactyla:
Donkey
Equus asinus
Baird's tapir
Tapirus bairdii
Black rhinoceros
Diceros bicornis
Artiodactyla:
Babirusa
Babyrousia babyrussa
Warthog
Phacochoerus africanus
Guinea hog
Sus scrofa
Chinese water deer
Hydropotes inermis
Giraffe
Giraffa camelopardalis
Pronghorn
Antilocapra americana
Bison
Bison bison
Giant eland
Taurotragus derbianus
Bongo
Tragelaphus eurycerus
Bushbuck
Tragelaphus scriptus
Black duiker
Cephalophus niger
Red-flanked duiker
Cephalophus rufilatus
Gerenuk
Litocranius walleri
Markor
Capra falconeri
Nubian ibex
Capra ibex
Domestic goat
Capra hircus
Serow
Capricornis crispus
Mountain goat
Oreamnos americana

LEUKOENCEPHALOPATHY IN CHEETAHS

Linda Munson, DVM, PhD,1* Alexander de Lahunta, DVM, PhD,2 Scott B. Citino, DVM,3 Robin W.
Radcliffe, DVM,¹ Don L. Neiffer, VMD,⁵ Richard J. Montali, DVM,⁶ and Ilse Stalis, DVM, PhD⁷

¹Dept. VM-PMI, University of California, Davis, CA 95616 USA; ²Dept of Anatomy, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 USA; ³White Oak Conservation Center, Yulee, FL 32097 USA; ⁴Fossil Rim Wildlife Center, Glen Rose, TX 76043 USA; ⁵Pittsburgh Zoo, Pittsburgh, PA 15206 USA; ⁶National Zoological Park, Smithsonian Institution, Washington, DC 20006 USA; ⁷San Diego Zoo, San Diego, CA 92112 USA

Abstract

During the past 2 yr, 27 adult cheetahs (Acinonyx jubatus) in United States zoos developed an unusual progressive degenerative neurologic disease that resulted in the euthanasia of 24 cheetahs. Clinically, the disease is characterized by blindness, incoordination, and lack of normal responsiveness to the environment. Pathologic findings in all cases have been similar and characterized by a remarkable reactive astrocytosis in the cerebral cortical white matter with symmetric degeneration and necrosis. The lesions appear to begin in the corona radiata as a reactive astrocytosis, but more advanced lesions have demyelination, axonal loss, and leukoencephalomalacia. In terminal stages, there is marked cavitation of the cerebral cortical white matter with secondary hydrocephalus. In all lesions, the astrocytes are hypertrophied with bizarre nuclei and abundant, sometimes vacuolated cytoplasm. Inflammatory changes have been absent in most cases.

The unique character and location of lesions is unlike any previously recognized disease. These lesions were not noted in cheetahs before 1996 and have not been reported previously in any other species. The epidemiology of the disease also is unusual in that it has affected only older animals (7-yr-old or older) and has emerged in the population at multiple facilities throughout the U.S. during this 2-yr period. Many different founder lines have been affected suggesting that it is not a familial disease. Also a single confirmed case in England suggests that the disease is not due to some factor only in U.S. diets.

Attempts to identify potential causative infectious agents in the lesions such as feline corona virus, canine distemper virus, JC polyoma virus, and prions, have been unsuccessful to date. Because the lesions are most similar to those caused by mycotoxins or vitamin B deficiencies, serum and tissue levels of these compounds are being assayed in affected animals. Potential reactions to vaccines or medications also are being investigated. This important emerging disease has had a major impact on the captive breeding program and adds to the list of major health problems in this endangered species.
A DENTAL EXTRACTION SITE MANAGEMENT PROTOCOL UTILIZING A SYNTHETIC BONE GRAFT PARTICULATE TECHNIQUE

D.A. Fagan, DDS¹* and J.E. Oosterhuis, DVM²

¹The Colyer Institute, PO Box 26118, San Diego, CA 92196-0118 USA; ²San Diego Wild Animal Park, 15500 San Pasqual Valley Rd., Escondido, CA 92027-9614 USA

Abstract

The presence of a geriatric population of individuals is an increasingly common occurrence among the many captive groups of primate species. Routine dental and/or oral care of this group invariably reveals various degrees of advanced periodontal disease that requires the removal of one or more teeth. The surgical extraction of periodontally compromised teeth can present the clinician with a major surgical challenge. The likelihood of post-operative bleeding from a dental extraction site is increased by the normal suction component associated with the act of swallowing, as well as any elevation of the patients blood pressure commonly associated with pain. Proper management of all dental extraction sites, at the time of surgery, with emphasis upon preventative measures to minimize the possibility of life threatening post-operative complications, is an essential and necessary element of contemporary comprehensive oral care of exotic animals. An extraction site management protocol is the focus of this presentation.

The first concern of dental extraction site management is to minimize collateral or incidental trauma to the surrounding gingival tissues while the tooth is being removed, and to disinfect the oral cavity with a dilute solution of broad spectrum antiseptic or antibiotic solution, prior to beginning the procedure. Use a #15 scalpel blade to cut the attachment of the gingiva to the crest of the alveolar bony ridge. Detach and retract the adjacent gingival tissue surrounding the tooth in order to provide adequate visual access to the underlaying alveolar bony ridge. Using an appropriately sized (#2, 4, or 6) round burr in a high speed dental handpiece with both air and water coolant, create a narrow “moat” around the neck of the tooth into the crest of the alveolar ridge. With the aid of a straight elevator and/or a “cow-horn” forceps, apply directional “rocking” pressure to move the tooth away from the bony socket into the “moat” space on the opposite side of the tooth, thereby rupturing the underlaying periodontal ligament and gradually loosening the tooth. When sufficiently loosened, remove the tooth from its socket following standard oral surgical technique described for third molar impaction surgery in humans.

The second issue of concern is to thoroughly remove all of the infected, granulation tissue and debris from the socket site back to smooth, healthy bone, taking care not to damage the underlying anatomic structures like the mandibular nerve or the maxillary sinus. This is easily accomplished with two instruments, the double-ended Molt curette and/or a dental bone file, and on occasion a larger round burr in the high-speed dental handpiece. The copious use of water and H₂O₂ is highly recommended to assist in the cleaning of the extraction site. The socket is now properly prepared
for the third step.

Closing the extraction site is the third major issue of concern, and is accomplished in three steps. The first step is to fill the clean extraction site about 3/4 full with a “wet sand” mixture of Bioglass® (Consil, Nutramax Laboratories, Inc., Baltimore, Maryland 21236, USA), a synthetic bone graft particulate material moistened with any of the commonly available broad spectrum antibiotic solutions (enrofloxacin, amikacin, etc.). Step two is to place a firm layer of calcium sulphate (CapSet®, LifeCore Biomedical, Chaska, Minnesota 55318-3051, USA) prepared to the manufactures directions, over the Bioglass® to create a “barrier layer,” which also encourages epithelial “creep” and promotes healing. Step three is to suture the previously protected full thickness gingival flaps back over the filled socket site as firmly as possible. A post-operative program of antibiotics and analgesics for 7-10 days is recommended, and spraying the oral cavity once or twice a day with a broad-spectrum antibiotic or antiseptic solution when possible, will aid the healing process.

A post-operative radiograph of the freshly placed synthetic bone grafting materials is essential, and a matching follow-up radiograph should be scheduled for about 6 mo to document the replacement, regeneration and repair of the alveolar ridge.

Proven techniques applied in a timely fashion, have been shown to promote primary wound healing and minimize the risks of life threatening post-operative bleeding episodes associated with the removal of periodontally involved teeth in primates. These proven principles apply to all mammalian osseous surgery and/or extraction procedures.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Ron Kettenacker at Nutramax Laboratories, Inc., Baltimore, Maryland for his support of this work.
EUTHANASIA GUIDELINES, ETHICS AND REALITY

Leah L. Greer, DVM

Office of Laboratory Animal Care, University of California, 203 Northwest Animal Facility, Berkeley, CA 94720-7150 USA

Abstract

Introduction

Euthanasia is defined as “a good death” from literal Greek translation, one that occurs without pain and distress. This definition is a classic oxymoron in that death is defined as “the permanent ending of all life, total destruction.” This duality poses much discord to the cognizant species that must perform the act. To help veterinary professionals contend with this antipathetic act, guidelines have been developed in the interest of animal welfare, to ensure that “good deaths” are achieved. Following these guidelines is straightforward, as many common species are addressed. However, the decision to carry out, and means of, euthanasia are based on multifaceted criteria, such as the use of the animal, humane concern for the animal, and the human emotions of those directly and indirectly involved.

Guidelines

In 1992 the Executive Board of the American Veterinary Medical Association (AVMA) convened a panel on euthanasia which led to their fifth panel report, 1993 Report of the AVMA Panel on Euthanasia. In this report the panel updated information on ectothermic, aquatic, wildlife, and fur-bearing species, as well as those used in research. The panel is planning to reconvene in the fall of 1999 to update these guidelines. The importance of these guidelines is apparent, as they have been adopted and referenced by many scientific organizations and regulatory agencies.

A comparable report is available from the European Commission entitled Recommendations for Euthanasia of Experimental Animals. These guidelines are more extensive than the AVMA guidelines, including comprehensive lists of cited references, literature recommendations, and information on audiovisual materials.

Reports written by the American Ornithologists’ Union, the American Society of Ichthyologists and Herpetologists, and the American Society of Mammalogists, provide various guidelines for field conditions. Euthanasia is discussed in these reports, however, for the most part they refer to the AVMA guidelines.
**General Considerations**

There are several important considerations prior to selecting a method of euthanasia. Primarily, those performing the euthanasia must be experienced in handling the animal as well as trained at the method of euthanasia selected. Proper handling is imperative to minimize pain and distress in the animal and to assure the safety of personnel. The amount of control and handling needed must be determined by the size, temperament, and illness or pain the animal may be experiencing.4

Concern for other animals in the vicinity is also important. Studies have shown that distress vocalizations as well as the release of odors or pheromones can cause anxiety and apprehension in other animals. Actions should be taken to euthanatize animals in an area separate from other live animals, especially of their own species.12

Concern for the emotions of personnel performing or observing the euthanasia must also be addressed. People’s attitudes toward euthanasia vary. Dissident employees can unfortunately spread rumors to volunteers, the ill-informed public, and the unrelenting media. Emotionally supportive discussion groups and trained counselors can be useful in dealing with this difficult issue.4,8,9

**Electing Euthanasia**

The Veterinarian’s Oath states, “I solemnly swear to use my skills for...the relief of animal suffering.” There are no laws against performing euthanasia on animals. In fact, it is encouraged as long as it is done with professional judgement in the interest of the animals’ welfare. The decision for when to perform a “good death” is unrefutable for several areas of animal husbandry. Production animals are slaughtered at the optimal time for profit, laboratory animals are humanely euthanatized at the end of an experiment, and client-owned animals are euthanatized upon the owners’ decision.

Exhibition animals in zoological facilities fall under the scrutiny of the media and the public, making the responsible decision more complex. The decision is obvious when the animal is diagnosed with an untreatable, terminal condition that is causing pain and suffering. However, what if the animal appears to be suffering but has a chance to recover? Or, what if the animal is diagnosed terminal but is not experiencing pain or suffering? This is where professional judgement and ethics may be open to challenge.

The most uncomfortable ethical question that faces zoological facilities is surplus animals. Regardless if it is an aged animal, or an excess of genetically related animals, the disposition of surplus animals is controversial. The American Zoo and Aquarium Association (AZA) code of ethics states in section I-K “I pledge to make every effort to assure that exotic animals do not find their way into the hands of those not qualified to care for them properly.” The full extent of the dilemmas involved in placing surplus animals is covered elsewhere.5,10,11 Nevertheless, the media is quick to blame AZA institutions for contributing to undesirable fates such as the pet trade, roadside zoos, shooting preserves, and the fur trade, if the institutions cannot account for the animals.
from ‘cradle to grave.’ This issue was addressed in 1978 by the AAZPA (now AZA) Surplus Committee, “The Committee believes that the need to euthanatize surplus zoo animals (as defined elsewhere) is not a controversial issue, provided that euthanasia is accomplished by recognized humane methods.” This statement was also supported by the director of wildlife protection for the Humane Society of the United States at that time. However, the prospect of individual euthanasia is still too difficult for many zoo directors to face. The 1993 Report of the AVMA Panel of Euthanasia avoids the issue by stating that “the ethical considerations that must be made when euthanizing healthy and unwanted animals raises both professional and societal issues...it does not believe that the report is the appropriate forum for an in-depth discussion of this topic.”

**Selection of Euthanasia Method**

Euthanasia agents cause death by three different mechanisms (1) hypoxia, by either direct (e.g., decapitation) or indirect means (e.g., paralytics); (2) direct depression of neurons vital for life function (e.g., chemical anesthetics); (3) physical disruption of brain activity and destruction of neurons vital for life (e.g., concussion, pithing). The acceptable agents available to achieve euthanasia are categorized as inhalant, chemical, or physical methods. These agents and the criteria for their use are widely published in detail. Methods of euthanasia that are considered unacceptable and conditionally acceptable are summarized in Table 1. For complete information on agents or methods please refer to the guidelines directly.

It is imperative that death be confirmed after euthanasia and before disposal of the animal. Death should be confirmed only by trained professionals. A cessation of vital signs such as cardiovascular arrest should be confirmed when possible. Cessation of respiration alone is not sufficient, since many species may be in a state of deep anesthesia, while other species and neonates can survive hypoxic conditions for extended periods of time. Professional judgement is necessary to determine if a secondary means of euthanasia (e.g., exsanguination, bilateral pneumothorax, freezing, and double pithing) is needed to confirm death.

**Unusual Considerations**

Larvae of fish, tadpoles, or newts may be killed effectively by placing them in a concentrated solution of MS-222 or benzocaine. Methods of euthanasia recommended for adult fish are also recommended for fry and fingerlings.

Reptile eggs without an embryo may be frozen. Reptile eggs with an early life form should not be exposed to extreme temperatures because humane death is not guaranteed. These eggs should be injected with barbiturates, an anesthetic overdose, or an appropriate physical method used to destroy the brain. Bird eggs can be terminated by freezing (< 4°C for 4 hr) however, if the neural tube has developed into a brain then death must be confirmed by decapitation or an overdose of anesthetic.

Depending on the species of mammal, embryos that have developed a functional brain (30-50% of gestation) should be killed humanely. In many cases inhalation agents will not anesthetize a fetus.
If the fetus is to be removed from an anesthetized dam an increased amount must be administered for a longer period to ensure the anesthetic has crossed the placenta. The insensitized fetus may then be killed by decapitation or removal of the heart if small in size. If the fetus is from a larger species it should be killed by methods acceptable in adults.8,9 Neonates should be humanely killed by methods similar to their adult species. However, neonatal animals appear to be resistant to hypoxia, therefore, agents utilizing this method should not be used unless exposure is long enough to ensure death or a second method of death is used.4

Invertebrate species such as cephalopods, or mollusks can be humanely euthanatized by terminal anesthesia. The agent of choice is 7.5% magnesium chloride.6

As vertebrate animals vary widely in size and physiology, the method chosen to euthanize an animal should be chosen from published material for the most similar species. General guidelines are to use an overdose of acceptable anesthesia, followed by professional confirmation of death or a second method of euthanasia to ensure a humane death and no chance for recovery.

LITERATURE CITED

13. Goldston, Linda. 1999. The Animal Business, many zoos give away or sell surplus mammals, which often end up exploited or even hunted despite safe guards. San Jose Mercury News, San Jose, California, February 7-10.
**Table 1. Summary of unacceptable and conditionally acceptable methods of euthanasia.**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air embolism</td>
<td>Only in anesthetized animals. Causes convulsions, opisthotonos, vocalization.</td>
</tr>
<tr>
<td>Chloroform, ether</td>
<td>Unacceptable. Hazardous to personnel.</td>
</tr>
<tr>
<td>Cyanide</td>
<td>Unacceptable. Hazardous to personnel. Aesthetically objectionable</td>
</tr>
<tr>
<td>Decapitation</td>
<td>Can be performed in small mammals &lt; 1 kg by trained personnel only. Recommended to be performed on anesthetized animals. Not recommended as sole agent for ectotherms as they can withstand hypoxia for long periods.</td>
</tr>
<tr>
<td>Decompression</td>
<td>Unacceptable. Time to unconsciousness is not known</td>
</tr>
<tr>
<td>Drowning</td>
<td>Unacceptable, inhumane.</td>
</tr>
<tr>
<td>Electric stunning</td>
<td>Must be performed by trained personnel with specialized equipment. Must be exsanguinated immediately to ensure death.</td>
</tr>
<tr>
<td>Electrocution</td>
<td>Must be performed on unconscious animals by trained personnel with specialized equipment. Cardiac defibrillation occurs 10-30 sec before unconsciousness.</td>
</tr>
<tr>
<td>Exsanguination</td>
<td>Only in anesthetized animals. Hypovolemia induces anxiety.</td>
</tr>
<tr>
<td>Gunshot</td>
<td>Penetrating captive bolt is preferred. A free bullet shot must be performed in field conditions by trained personnel. Position of weapon differs with each species.</td>
</tr>
<tr>
<td>Hyperthermia</td>
<td>Unacceptable, inhumane. Animals should not be dropped into boiling water.</td>
</tr>
<tr>
<td>Nicotine, Mg Sulfate,</td>
<td>Conditionally acceptable. When used alone they cause respiratory arrest before unconsciousness, so the animal may perceive pain. May be used after approved method of anesthesia.</td>
</tr>
<tr>
<td>KCl, all neuromuscular blocking agents</td>
<td></td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>Alone does not induce anesthesia. Distressful hypoxemia develops before respiratory or cardiac arrest.</td>
</tr>
<tr>
<td>Rapid freezing</td>
<td>Only in anesthetized animals. Formation of ice crystal is considered painful in all species. Commonly used as an adjunctive means of euthanasia.</td>
</tr>
<tr>
<td>Strangulation</td>
<td>Unacceptable, inhumane. It is not known when unconsciousness will be reached.</td>
</tr>
<tr>
<td>Strychnine</td>
<td>Unacceptable. Causes violent convulsions, and painful muscle contractions.</td>
</tr>
<tr>
<td>Stunning</td>
<td>Must be followed by an approved method of euthanasia. Creates only unconsciousness.</td>
</tr>
</tbody>
</table>
ARTIFICIAL INSEMINATION IN THE SCIMITAR-HORNED ORYX AS A CONSERVATION MANAGEMENT TOOL

Steven L. Monfort, DVM, PhD,1* Barbara A. Wolfe, DVM, PhD,1,2 Evan S. Blumer, VMD,3 Mark W. Atkinson, BVSc,3 Rebecca E. Spindler, PhD,1 Budhan S. Pukazhenthi, BVSc, PhD,1 Mitchell Bush, DVM,1 David E. Wildt, PhD,1 Terri L. Roth, PhD1,5 and Catherine J. Morrow, PhD1,4

1Conservation & Research Center, National Zoological Park, Smithsonian Institution, Front Royal, VA 22630 USA; 2North Carolina Zoological Park, Asheboro, NC 27203 USA; 3The Wilds, Cumberland, OH 43732 USA; 4Animal Behavior and Welfare, Ruakura Agricultural Research Centre, Hamilton NZ; 5Center for Research in Endangered Wildlife, Cincinnati Zoo & Botanical Garden, Cincinnati, OH 45220 USA

Abstract

Despite the implementation of effective small population management strategies, insufficient funding and enclosure space can preclude breeding genetically valuable individuals, and this can adversely impact gene diversity. For species like the scimitar-horned oryx (Oryx dammah), which thrives in large numbers in captivity, artificial insemination (AI) has potential for eliminating the risks of animal transport, and for optimizing the use of limited enclosure space, while simultaneously preserving extant gene diversity. Maximizing the full potential of AI, however, requires both effective sperm cryopreservation techniques and the ability to reliably produce offspring using frozen-thawed sperm. Substantial progress has been made in the scimitar-horned oryx towards understanding the female’s reproductive cycle, manipulating estrus/ovulation, developing safe and reliable approaches for collecting and storing viable spermatozoa, and optimizing methods for proper deposition of sperm at the appropriate time and site within the reproductive tract. Recent results15 demonstrate that non-surgical AI in scimitar-horned oryx using frozen-thawed sperm results in pregnancy rates approaching 40% after a single fixed-time insemination. Summary data from 72 inseminations across three separate AI trials conducted at the Conservation & Research Center and the Wilds are presented here to illustrate how AI, combined with genome resource banking, can augment captive breeding management of the scimitar-horned oryx.

Introduction

Less than a century ago, hundreds of thousands of scimitar-horned oryx inhabited the Sahel, a semi-arid transition zone south of the Sahara from Senegal to Sudan, and the northern edge of the Sahara from Morocco to Egypt. Although well adapted to harsh, arid environments, poaching, habitat destruction, recurrent drought and civil unrest led to the extinction of scimitar-horned oryx across their historic range.

The managed global population of captive scimitar-horned oryx exceeds 1,500 individuals, including small populations that have been re-introduced into fenced habitats in Tunisia and Morocco.
Amazingly, scimitar-horned oryx now occupy > 7% of the 16,000 spaces set aside for hoofstock species within zoological institutions worldwide. Metapopulation management is problematic because all captive oryx (descended from 40-50 wild founders) exist in fragmented populations widely dispersed at dozens of locations worldwide. This scenario has mandated that global ex situ management tactics focus both upon moderately reducing captive numbers of scimitar-horned oryx while still maintaining adequate genetic diversity to avoid inbreeding depression.

Compared to most exotic ungulates, the reproductive database for scimitar-horned oryx is extensive. Previous studies have investigated the ovarian cycle\cite{10,13,14} and ovulation induction,\cite{2,4,7,13-16,20,21} as well as semen collection and cryopreservation.\cite{5,6,7,11,17-19} And while methods for synchronizing estrus and ovulation have been reported for the scimitar-horned oryx,\cite{2,7,12,13,15,16,20,21} there are few published reports describing fertility after estrus synchronization.\cite{7,15} Numerous factors affect AI success rates including sire selection (which impacts pre-freeze semen quality), efficacy of estrus synchronization, semen freezing methods (which impacts post-thaw sperm quality), sperm concentration and total number of sperm inseminated, and timing of insemination. Since our intention was to integrate our research with a semen cryobanking program and strictly managed breedings, there was little that could be done to affect sire selection. Of the remaining factors, the total number of motile sperm inseminated could be regulated if post-thaw sperm motility and concentration were known. Thus, our AI studies were designed to investigate (1) two ovulation synchronization protocols, (2) chilled vs. frozen-thawed sperm, and (3) timing of AI relative to synchronization of ovulation.

The present paper reviews results of three AI trials (28, 20, and 24 animals, respectively) conducted consecutively over 3 yr (1996-1999). Study animals for each trial were divided equally between the Conservation & Research Center (CRC, Front Royal, VA) and the Wilds (Cumberland, OH). Strategies for future development of semen banking to augment captive genetic management and re-introduction of scimitar-horned oryx also are discussed.

**Trial 1: Comparing Ovulation Synchronization Protocols for AI\cite{15}**

Fourteen females per group \((n = 28\) total) were inseminated with frozen-thawed sperm after receiving either two i.m. injections of prostaglandin-F2\(_\alpha\) (PGF2\(_\alpha\)-only, 500 \(\mu\)g) or the same treatment combined with a modified progesterone-containing intravaginal CIDR-B device (11 day insertion interval; CIDR + PGF2\(_\alpha\)) to synchronize ovulation. Females were transcervically inseminated 56.0 ± 1.1 hr \((x \pm SEM)\) after CIDR withdrawal and/or the second PGF2\(_\alpha\) injection using 27.9 ± 1.6\times10^6 motile, thawed sperm, divided equally between both uterine horns. Semen for all AI trials was diluted in EQ extender (20% egg yolk, 5.5% lactose, 1.5% glucose and 0.25% triethanolamine lauryl sulfate; 5% final glycerol concentration) and frozen in 0.5 ml straws directly on dry ice for 10 min before plunging and storage in liquid nitrogen.\cite{17} Post-thaw sperm motility averaged 46.4 ± 1.6\% (forward progressive motility, 3.0 ± 0.1, scale 0-5), and there were no differences \((P > 0.05)\) between pregnant \((23.7 ± 1.2\times10^6)\) and non-pregnant \((29.1 ± 1.8\times10^6)\) females in the number of motile sperm inseminated.

AI was conducted non-surgically in females that were anesthetized using a combination of...
medetomidine (0.03-0.09 mg/kg) and ketamine (2.5-3.0 mg/kg); atipamezole (0.15-0.20 mg/kg) was used as antagonist. A detailed description of the efficacy to this anesthetic combination will be published in a separate manuscript. At both the CRC and the Wilds, animals were anesthetized in, or near their home pens, transferred to a nearby location for AI, and then returned to their home pen to recover (see exception described in Trial 2). To ensure consistency between locations, animals were matched for parity and age among treatment groups, similar animal handling and restraint facilities were used, identical research and anesthesia protocols were followed, the same semen donors were used, and the same technical staff conducted veterinary and insemination procedures.

The increase in fecal progestogen excretion (indicative of corpus luteum [CL] development) was delayed ($P < 0.05$) in 5/10 of the CIDR + PGF2α females (16.8 ± 2.5 days) compared to the remaining CIDR+PGF2α individuals (7.6 ± 0.7 days) and all PGF2α-only (8.6 ± 0.8 days) females. The induced luteal cycle lengths (nadir to nadir fecal P) for non-conceptive PGF2α-only females was 25.3 ± 0.3 days, whereas CIDR+PGF2α females exhibited either normal ($n = 5$, 26.0 ± 0.7 days) or delayed ($P < 0.05$) luteal cycles ($n = 5$; 35.5 ± 3.8 days). The lack of between treatment differences ($P > 0.05$) in fecal estrogen excretion after CIDR withdrawal/PGF2α administration suggested that follicle development was not adversely affected by short-term progesterone treatment. However, the 9-23 day delay in the onset of the post-synchronization luteal phase (i.e., the approximate time required for a new ovulatory follicle to be recruited, ovulated, and luteinized) in several of the CIDR + PGF2α females suggested that estrogenic follicles either failed to ovulate, or that ovulated follicles failed to fully luteinize. In summary, more pregnancies ($P < 0.05$) resulted in PGF2α-only treated females (35.7%; 5/14 diagnosed pregnant; 4 live births) compared to CIDR + PGF2α counterparts (0/14).

**Trial 2: Chilled vs. Frozen-Thawed Sperm for Producing Offspring After Transcervical AI**

We hypothesized that chilled (vs. frozen-thawed) scimitar-horned oryx semen would provide superior conception rates since results from a preliminary trial showed that sperm longevity was prolonged compared to thawed sperm (i.e., fresh sperm diluted in EQ without glycerol at 4°C was motile for at least 88 hr). Thus, a second AI trial was designed to compare the efficacy of freshly collected, chilled semen to frozen-thawed sperm for producing offspring after fixed-time intrauterine AI. Nineteen females (one CRC female died during anesthetic induction before AI was conducted) synchronized with PGF2α-only were transcervically inseminated 54.2 ± 0.3 hr after the second PGF2α injection using ~40×10⁶ motile, thawed ($n = 12$) or chilled sperm ($n = 12$) into each uterine horn. One ejaculate from a single male at each location was used for all inseminations. Half the pooled semen batch (chilled slowly to 4°C) was frozen,¹⁷ and the remainder was stored (4°C, < 24 hr) until used for inseminations. Pre-insemination motility of chilled sperm was > 70%, and progressive status was > 3.0. Acrosome integrity of chilled sperm (82.3 ± 4.9% intact acrosomes) was superior ($P < 0.05$) to frozen-thawed sperm (69.3 ± 2.1% intact acrosomes). Semen extenders, semen freezing methods, anesthesia protocols and AI techniques were identical to Trial 1, but endocrine monitoring was not conducted.
Despite using a proven ovulation synchronization protocol and inseminating with greater numbers (Trial 2, 40x10^6/horn vs. Trial 1, 14x10^6/horn) of high quality sperm (Trial 2, ~70% motility @ 3.0 status vs. Trial 1, ~45% @ 3.0 status), only a single female became pregnant (the Wilds, frozen-thawed sperm).

There were three notable protocol differences in Trial 2 vs. Trial 1. First, the mean time to AI after the second PGF2α injection was shortened by 2 hr (Trial 2, 54.2 ± 0.3 hr vs. Trial 1, 56.1 ± 1.1 hr; P < 0.05); this unplanned result derived from the increased speed and experience of the AI team. Second, CRC females were taken by trailer to the veterinary hospital 2-3 days before AI procedures to ensure proximity to medical instrumentation and to achieve consistent ambient temperatures during the AI procedures (research protocols at the Wilds were unchanged from Trial 1). Third, half the research subjects were inseminated with chilled semen.

Previous research showed that onset (range, 29-44 hr) and duration (range, 3-41 hr) of behavioral estrus (in the presence of a vasectomized bull) in scimitar-horned oryx was highly variable following PGF2α administration, both among females, and between repeated experiments. Despite such high variability, pregnancy rates following AI at 54 hr (after a second PGF2α injection) were excellent in Trial 1. We speculated that the stress associated with moving animals to a new location before AI may have blocked or delayed ovulation in females during Trial 2. The mismatch between ovulation and insemination may have been further exacerbated by the advancement in the mean time to AI. Although fecal corticoid metabolites were not documented in the present study, recent results (C. Morrow, unpublished data) suggested that scimitar-horned oryx taken by trailer to the veterinary hospital for medical treatments excreted increased fecal corticoids over a prolonged interval.

Although subjective measures of sperm motility and progressive status, and objective measures of acrosomal integrity, suggested that chilling and storage for up to 24 hr had little impact on sperm viability, we could not rule out the possibility that prolonged storage at 4°C inhibited sperm capacitation.

**Trial 3: Impact of AI Timing on Fertility**

Trial 3 was designed to examine the impact of the timing of AI relative to ovulation synchronization on fertility. We tested the hypothesis that no fertility differences existed among scimitar-horned oryx cows inseminated 56, 64 or 72 hr after the second PGF2α injection. Twenty-four PGF2α-only synchronized females were transcervically inseminated 56 ± 0.3, 64.3 ± 0.3 or 72.0 ± 0.3 hr after the second PGF2α injection with ~50x10^6 motile, thawed sperm into each uterine horn. Post-thaw sperm motility ranged from 70-80%, and progressive status ranged from 3.0-3.5. Semen extenders, semen freezing methods, anesthesia protocols and AI techniques were identical to Trials 1 and 2.

Nine pregnancies—three in each treatment group—were achieved in Trial 3. Although numbers of study animals were relatively small, the null hypothesis that no fertility differences existed across an 18-hr interval (56-72 hr) after the second PGF2α injection was accepted. Six of 12 animals (50%) became pregnant at CRC, whereas 3/12 (25%) conceived at the Wilds. While treatment
groups were balanced within location for age ($P > 0.05$) and parity, females at the Wilds (10.4 ± 0.5 yr) were older ($P < 0.05$) than CRC females (8.2 ± 0.7 yr). There were no among-treatment differences in pregnancy outcome, but increased cervical dilation and reduced cervical tone in the 56- and 64-hr treatment groups facilitated passage of insemination pipettes for intrauterine insemination.

**Discussion**

Births resulting from AI have been reported in only four antelope species, including one Speke’s gazelle ($Gazella spekei$), one addax ($Addax nasomaculatus$), six blackbuck ($Antilope cervicapra$) and six scimitar-horned oryx. Despite these successes, semen cryopreservation and AI still has not been used for managing captive antelope populations. Inclusive of the three AI trials described in the present paper, we have produced 15 pregnancies in scimitar-horned oryx, and have achieved conception rates with frozen-thawed semen that range from 25-50% after a single insemination. These results provide strong incentive for the establishment of a genome resource bank for oryx semen.

While it was not surprising that environmental and/or management factors may have influenced pregnancy outcome, our results demonstrated that these AI techniques can be readily adapted to accommodate the diversity of management and husbandry schemes likely to be encountered within zoological institutions worldwide. This adaptability will be particularly important when regional sub-Saharan African re-introduction programs for scimitar-horned oryx are initiated. It is clear that integrated genetic management efforts will continue to be vital for protecting and managing animal populations within native habitats, as well as for captive breeding programs. We are confident that semen banking and routine offspring production after AI will become a useful tool for bridging ex situ and in situ conservation management programs for scimitar-horned oryx.

**ACKNOWLEDGMENTS**

Special thanks go to the animal management, veterinary support and keeper staff at the Conservation & Research Center and the Wilds. Research was funded by grants from the Scholarly Studies Program of the Smithsonian Institution, the Friends of the National Zoo, and the Morris Animal Foundation.

**LITERATURE CITED**

ARTIFICIAL INSEMINATION OF AFRICAN (*Loxodonta africana*) AND ASIAN (*Elephas maximus*) ELEPHANTS

Thomas B. Hildebrandt, DVM,1* Frank Göritz, DVM,1 Robert Hermes, DVM,1 Dennis Schmitt, DVM, PhD,2 Janine L. Brown, PhD,3 Harald Schwammer, PhD,4 Naida Loskutoff, PhD,5 Nancy C. Pratt, PhD,6 John L. Lehnhardt, BVSc,6 Richard J. Montali, DVM,3 and Debbie Olson, BVSc7

1Institute for Zoo Biology and Wildlife Research, Berlin, D-10315 Germany; 2Dickerson Park Zoo, Springfield, MO 65803 USA; 3Conservation and Research Center, National Zoological Park Smithsonian Institution, Front Royal, VA 22630 USA; 4Vienna Tiergarten Schönbrunn, Vienna, A-1139 Austria; 5Henry Doorly Zoo, Omaha, NB 68107-2200 USA; 6Disney’s Animal Kingdom, Lake Buena Vista, FL 32830-1000 USA; 7Indianapolis Zoo, Indianapolis, IN 46222-0309 USA

Abstract

Successful captive elephant management is a priority among zoo and wildlife organizations worldwide. Captive populations have been traditionally maintained by collecting from the wild, transport of captive females on long-term breeding loans, and to a lesser extent, by management of males on-site, which can be unrealistic for many zoos. Conservation and safety concerns, along with the growing acknowledgment among elephant caretakers that removing females from their familiar social groupings for breeding loans can cause distress, have all contributed to the need for development of assisted reproductive techniques. Demonstration of a successful artificial insemination (AI) would open up many possibilities for captive elephant management, including the collection of genetic material from the wild for integration into captive populations once semen cryopreservation techniques have been perfected. A new technique involving the application of ultrasonography for reproductive assessment and AI has been implemented in the elephant management program of several zoos. The technology for this project was developed at the Institute for Zoo Biology and Wildlife Research in Berlin in cooperation with the company A. Schnorrenberg.1

The AI component of this project involved simultaneous imaging by ultrasonography and endoscopy for verifiable semen placement. Both these components have never been accomplished together in an elephant AI. The insemination technique is non-invasive and has resulted in verifiable sperm deposition directly into the cervix.

Ultrasound-guided AI has been attempted, in four nulliparous African cows at the Indianapolis Zoo, “Ivory” (16-yr-old), “Tombi”, (22-yr-old), and “Kubwa” (22-yr-old), and the Vienna Tiergarten Schönbrunn “Sabi” (13-yr-old). Additionally, the uniparous Asian cow “Shanthi” (23-yr-old) was artificially inseminated at the National Zoo. All five females are wild-born.

The reproductive hormone levels (progesterone [P₄]; luteinizing hormone [LH]) have been monitored from blood samples taken from their ear veins on a routine basis. All five females were excellent candidates for this project for several reasons:

1. they are of prime breeding age,
2. they are extremely calm-mannered, tractable and well-trained,
3. they are in very good general and reproductive health,
4. they have been palpated both vestibularly and rectally on a routine basis, and have shown no signs of distress or injury during these procedures.

The technique for transrectal ultrasonography in elephants is performed in standing or laying position without the use of tranquilizers, anesthetics or restrictive devices. A real-time B-mode ultrasound scanning system was used. For visualizing the caudal component of the urogenital tract (vestibule, urethra, vagina, urinary bladder, cervix, caudal corpus uteri) a 3.5 MHz transducer was manually introduced into the rectum with ultrasound gel for coupling. To visualize the cranial component of the genital tract (cranial corpus uteri, uterine horns, ovaries, surrounding tissues) a 5.0-7.5 MHz transducer is attached to a specially adapted extension and guided manually into the rectum. Ultrasonography can provide valuable information on ovarian activity, uterine integrity and reproductive disease or dysfunction.\textsuperscript{2,3} It can be used to visualize structures of the entire reproductive tract.

Ultrasonographic examinations of the five AI candidates revealed that there were no indications of reproductive pathology in the urogenital tract or in the ovaries. Both endocrine data and ultrasonographic images were used to determine the timing of AI trials. In preparation for the AI attempts, the females were monitored daily for circulating levels of P\textsubscript{4} and LH. Two LH peaks,\textsuperscript{4} separated by 19-21 days, were detectable in their estrous cycle, with the second peak being the ovulatory LH surge. Detection of the first peak provided us with a 3-wk window to prepare for the inseminations. Transrectal ultrasonography was employed daily in this time period to identify morphologic changes in the vagina and endometrium and to characterize developing ovarian structures during the follicular phase. Ultrasonography allowed the visualization of follicle growth and maturation and the development of the Graafian follicle. The ruptured ovulatory follicle and corpus hemorrhagicum could also be visualized. The visualization of these ovulatory events have never been possible before.

The semen donor for the AIs in “Tombi” and “Kubwa” was a 20-yr-old African bull named “Dale”. He is owned by Jo-Don farms currently being housed at the Kansas City Zoo. The ejaculate for the insemination in “Ivory” was collected from a 16-yr-old African bull with the name “McLean” at Disney’s Animal Kingdom. The selected breeding partner for the African cow “Sabi” at the Vienna Tiergarten was a 16-yr-old bull named “Tembo” housed at the Colchester Zoo. The insemination in the Asian female “Shanthi” was performed with semen collected from the 13-yr-old bull “Calvin” (African Lion Safari) and the 35-yr-old bull “Onyx” (Dickerson Zoo). In general, the semen was collected by rectal palpation of the accessory glands sometimes combined with manual penile stimulation.\textsuperscript{6} Extended semen was transported by air at 4°C in a refrigerated vessel. The samples were warmed to 37°C prior to insemination. Samples used for AI trials ranged in motility status from 5-95%.

No sedation or physical restraint was used for the insemination protocol. Sterile technique was employed throughout the procedures. A balloon catheter was inserted in the vestibule (Canalis
urogenitalis) to slightly distend the reproductive tract for optimal visualization and placement of an endoscope, and insemination catheter. Both endoscopic and ultrasonographic visualization were used to guide the placement of semen deep into the cervix if possible. The actual introduction of semen was monitored ultrasonographically to verify its placement. The time needed for an AI procedure varied from 10 min to 2 hr at maximum. Olfactory cues, such as semen, urine, feces and temporal gland secretions, were presented to the AI candidates before and after the AI series. They showed strong reactions to these stimuli, often rumbling and pelvic thrusting. They have shown no adverse behavioral, physical or physiologic effects of this AI program.

Following the AI procedures the females were monitored endocrinologically for P4 on weekly basis. The first ultrasonographic examinations were performed about 8 wk after the insemination. The preliminary result of this project are two successful AI’s. The 16-yr-old female African elephant “Ivory” and the 22-yr-old “Kubwa” are pregnant. The AI in the 22-yr-old African female “Tombi” was not successful due to poor semen quality (low motility and concentration). There were no results regarding the AI’s in the cows “Sabi” (13-yr-old) and “Shanthi” (23-yr-old) before the abstract was submitted.

Artificial insemination (AI) is one of the most effective methods for improving the breeding success of domestic species. But for 35 yr, different AI methods have never produced a confirmed elephant pregnancy until the year 1998. The introduction of AI in captive elephant breeding programs is enormously important in terms of the ultimate viability of assisted reproduction for use in the entire captive population of elephants. Most of which may never have the opportunity for natural conception. The Dickerson Park Zoo, one of the collaborators in this project, announced the first successful insemination of a primiparous female Asian elephant in June of 1998. Last year a total of three elephants became pregnant by AI in North America. “Kubwa” and “Ivory” are the first virgin elephant cows to be successfully impregnated by artificial insemination. The combination of a reproductive assessment program with a newly developed insemination technology resulted in this groundbreaking success. This project has been a team effort, requiring the co-operation and expertise of many individuals: curators, keepers, researchers, pathologists, volunteers, educators and public relations specialists.

ACKNOWLEDGMENTS

The authors are grateful for the assistance from the elephant staff of the Indianapolis Zoo, Smithsonian, National Zoological Park, and Vienna Tiergarten Schönbrunn for training the female elephants to stand unrestrained for the AI procedure. The authors thank the elephant staff of the Kansas City Zoo, Disney’s Animal Kingdom, Dickerson Zoo, Colchester Zoo, and the African Lion Safari for the semen collections.

LITERATURE CITED

PRELIMINARY RESULTS OF NON-INVASIVE MONITORING OF THE ESTROUS CYCLE IN FEMALE ASIAN ELEPHANTS (*Elephas maximus*) THROUGH FECAL STEROID ANALYSIS

Fernando Gual-Sill, MVZ, MSc,1* Amanda R. Pickard, PhD,2 William V. Holt, PhD,2 and Daphne Green2

1 Zoológico de Chapultepec, 1era sección del Bosque de Chapultepec, C.P. 11850, México, DF, Mexico; 2 Institute of Zoology, Zoological Society of London, Regent’s Park, London NW1 4RY, UK

Abstract

For a number of years, estrous cycle monitoring and pregnancy detection in the Asian elephant has been performed using urinary steroid hormone metabolite analysis; this technique presents some practical problems. Monitoring the reproductive status through fecal steroid analysis is possible in this and many other species. The steroid metabolite profiles of female Asian elephants were monitored by enzyme-linked immunosorbent assay (ELISA), to provide detailed information about the estrous cycle and pregnancy in this species, and to investigate causes of reproductive failure. Fecal and matched urine samples were non-invasively collected regularly for 6 mo from captive female Asian elephants. (*n* = 4 cyclic; *n* = 1 acyclic). The samples were frozen at -20°C. Gas Chromatography and Mass Spectroscopy (GC-MS) procedures were used to investigate the steroid hormone metabolite profile and to identify the major excretory metabolites; no steroid metabolites were found in the concentrated extracted feces of this species using the currently available methodology. The fecal pregnanetriol profile observed in three of the cyclic females showed a clear relation with their matched urinary pregnanetriol profile and a cyclic pattern was demonstrated. Fecal pregnanetriol values increased from an overall mean of 94.67 ng/g of dry feces (± 13.24, range 31.5-219.12 ng/g) during the inter-luteal period to a luteal phase mean of 334.61 ng/g dry feces (± 43.48, range 34.35-1035.1 ng/g). All the data collected from the fecal and urinary analysis of pregnanetriol in all five individuals investigated demonstrated a significant relationship between urinary and fecal pregnanetriol. The acyclic individual showed a mean fecal pregnanetriol concentration of 84.91 ng/g (±13.06) and values ranged from 33.17 ng/g to 211.42 ng/g. Fecal steroid hormone metabolite analysis for monitoring estrous cycles in Asian elephants may be used in the future to monitor free-roaming, wild or semi-wild individuals as well as those in captivity to assist reproductive and conservation programs of this highly endangered species.

Introduction

Assessing the reproductive status of the individuals in captive breeding programs is essential, but invasive techniques are usually not feasible in wild species. Some techniques for non-invasive monitoring of reproductive events in wild animals that have been developed have advantages such as being non-stressful for the individuals that are being monitored as well as being relatively safe for the human beings involved in the collection of the samples required. They also allow long term monitoring of species that show long reproductive cycles and require constant sampling and give
the opportunity to monitor free-ranging wild animals as well as captive individuals. Most of these techniques are based on the evaluation of hormone levels including metabolites of progesterone and estrogens excreted in urine, feces and saliva. In the female Asian elephant, the estrous cycle and reproductive events have previously been assessed through the analysis of blood hormone concentrations and more recently by means of urinary steroid hormone metabolite analysis. Non-invasive monitoring techniques are necessary in this species due to the danger involved in its management in captivity and the impracticability of continuous blood sampling and other invasive procedures in free-ranging individuals.

The Estrous Cycle: Based primarily on measurements of progesterone (and its metabolites) and luteinizing hormone (LH) in plasma and/or urine, the estrous cycle in Asian elephants averages 13-16 wk. Ovulation occurs at the end of each follicular phase. It is still uncertain if elephants are normally poly- or mono-ovulatory.

Metabolism and Excretion of Steroid Hormones: The gonads and the placenta are the main sources of androgens, estrogens and progestogens. The inter-conversion of steroids may take place peripherally, and steroid hormone metabolism occurs in many tissues including the gut, skin, uterus and mammary gland but mainly in the liver. The biologically inactive metabolites are excreted in the urine, in the bile into feces and to a certain extent in milk and saliva. The formation of pregnanetriol follows a separate pathway from that involved in the formation of 20α-hydroxyprogesterone and pregnanediol, two of the major progesterone metabolites found in Asian elephant feces according to Hoppen, Diaz de Aguirre, Hagenbeck, Boer and Schwarzenberger. Five-beta-pregnanetriol is a major urinary progesterone metabolite in Asian elephant urine. Urine is the major route of steroid elimination in most species examined. The route of excretion and the metabolic endproducts of steroid hormone metabolism can vary considerably between species. Urinary analysis has been used extensively for the detection and quantification of steroids in nondomestic species, in different reproductive stages. Creatinine is usually excreted at a relatively constant rate and it is used to index the steroid hormone concentrations in each sample in order to adjust variations in urine output. Niemuller, et al. identified 5β-pregnanetriol as the major urinary progesterone metabolite in the Asian elephant.

Fecal Analysis: Fecal steroid metabolite analyses have been used to assess reproductive status in more than 30 mammalian species.

Methods

Fecal and matched urine samples were non-invasively collected regularly for 6 mo from five captive female Asian elephants. According to previous urinary pregnanetriol profiles, four females were known to be cyclic and one was acyclic. The samples were frozen at -20 °C.
GC-MS: GC-MS was performed to investigate the steroid hormone metabolite profile and to identify more accurately the major excretory metabolites, which reflect the reproductive status of the Asian elephant.

Creatinine Determination: After initial thawing, urine samples were analyzed for creatinine concentration by the method of Hodges and Green to correct for variations in fluid intake and urine output.8

Pregnanetriol Enzymeimmunoassay: Immunoreactive 5β-pregnane-3α,17α,20α-triol (pregnanetriol) was measured using a validated modification of the method described by Niemuller, et al.16

20α-hydroxyprogesterone Enzymeimmunoassay: Fecal 20α-hydroxyprogesterone enzymeimmunoassay was performed as described by Hindle, Möstl and Hodges.7

Urinary Analysis

Urinary immunoreactive pregnanetriol was measured using the pregnanetriol enzymeimmunoassay described above. Concentrations of urinary pregnanetriol were expressed in ng/mg creatinine. Complete profiles from the five individuals described above were obtained from weekly samples over a period of 6 mo.

Fecal Analysis

Sample Preparation and Extraction: Prior to extraction, all fecal samples were thawed and dried in an oven for 18 hr at 60°C. The entire sample was thoroughly mixed, pulverized and the fecal powder was then separated from the hay by sieving through a fine mesh. Five different procedures were used to test which would optimize the extraction of elephant fecal steroids. The extraction procedure which gave the best results was a variation of the extraction procedure described by Möstl, Lehmann and Wenzel: 0.1 g of dried fecal powder was combined with 0.2 g of aluminium oxide, 1.2 ml of methanol and 1 ml of distilled water.14 After being vortexed for 10 min, the suspensions were centrifuged for 30 min at 2,400 rpm, +4°C and the supernatant was assayed directly after being diluted as appropriate. Aluminium oxide was used to remove visible background pigment.5 This method has proven to be successful for fecal extraction in black rhinoceros for reproductive steroid monitoring.4 Fecal steroid concentrations were expressed in mg/g of dry feces.

Fecal Hormone Assay: The cross reactivity of fecal extracts was tested with the antisera raised against pregnanetriol and 20α-hydroxyprogesterone, using the enzymeimmunoassay methods outlined above. Weekly fecal samples from a complete estrous cycle of one individual were analyzed using both the pregnanetriol and 20α-hydroxyprogesterone assays, and compared with the hormone profile obtained from the analysis of pregnanetriol in matched urine samples. On the basis of the results obtained all fecal analyses were performed using the pregnanetriol enzymeimmunoassay, with samples diluted 1:25 in assay buffer.
Results

GC-MS: From the trials that were carried out, no steroid metabolites were found in the concentrated extracted feces of this species using the currently available methodology.

Fecal 20α-hydroxyprogesterone Profiles: While capable of discriminating changing concentrations of fecal 20α-hydroxyprogesterone (range 5.8 ng/g-1054.6 ng/g), the complete profile of one cyclic female did not reveal a cyclic pattern of steroid excretion. No relationship between the fecal profile and the urinary pregnanetriol profile was seen using any of the five extraction procedures.

Fecal Pregnanetriol Profiles: Fecal extracts from all five elephants demonstrated a pattern of pregnanetriol excretion which was temporally correlated with that observed in their matching urinary pregnanetriol profile.

Reproductive Assessment of Individual Asian Elephants: The fecal pregnanetriol profile observed in three of the cyclic females showed a clear relation with their matched urinary pregnanetriol profile and a cyclic pattern was demonstrated. The individual profiles of these three females were used to produce a composite profile of the mean and SEM values of the urinary and fecal immunoreactive pregnanetriol concentrations during one cycle (Fig. 1). All data were aligned to week 0 which corresponds to the week before the rise in urinary pregnanetriol (which reflects the start of the luteal phase). Fecal pregnanetriol values increased from an overall mean of 94.67 ng/g of dry feces (±13.24, range 31.5-219.12 ng/g) during the inter-luteal period to a luteal phase mean of 334.61 ng/g dry feces (±43.48, range 34.35-1035.1 ng/g). One of the cyclic females did not show a good relationship between the urinary and the fecal pregnanetriol profile. The acyclic individual showed a mean fecal pregnanetriol concentration of 84.91 ng/g (±13.06) and values ranged from 33.17 ng/g to 211.42 ng/g. No significant difference was found between the mean value of fecal pregnanetriol of an acyclic individual (84.91 ng/g) and the mean value of fecal pregnanetriol (94.67 ng/g) during the inter-luteal phase of the composite profile; however, the acyclic female showed no increase in fecal pregnanetriol excretion indicating the onset of a luteal phase throughout the study period.

Discussion

The lack of ability to use the GCMS technique for the elephant could be due to the fact that steroid metabolites are found in relatively low concentrations in their feces. Further studies are required. The fecal 20α-hydroxyprogesterone profile for one of the individuals did not show a cyclic pattern but was capable of discriminating changes in concentration. Comparison of the fecal pregnanetriol profile with the urinary pregnanetriol profile throughout a complete ovarian cycle demonstrated cyclic variations in concentration, and a good correlation between fecal and urine concentrations during all stages of the ovarian cycle. Niemuller, et al. found a significant correlation between immunoreactive pregnanetriol concentrations and plasma progesterone concentrations throughout the ovarian cycle.16 Therefore, we assume that fecal pregnanetriol concentrations are correlated with plasma progesterone concentrations and do reflect the reproductive status of the Asian elephant.
The findings reported in this study and the significant correlation of fecal and urinary pregnanetriol in cyclic and acyclic individuals provide evidence that pregnanetriol is a significant fecal progesterone metabolite in female Asian elephants, and that changes in fecal concentrations can be used to assess the reproductive status of female Asian elephants. The fecal pregnanetriol immunoassay should be further studied in order to evaluate the feasibility of this technique for routine non-invasive monitoring of the reproductive function of captive and wild Asian elephants.

ACKNOWLEDGMENTS

The main author wishes to thank The British Council in Mexico City, CONACYT and YUMKA Park, Tabasco, Mexico for their support while working on this project. All data reported on this paper was obtained from the MSc research project of the main author (MSc in Wild Animal Health: Royal Veterinary College – University College of London / The Institute of Zoology - Zoological Society of London, 1998).

LITERATURE CITED


**Figure 1.** Composite profile of the mean ± SEM of the (a) urinary and (b) fecal immunoreactive pregnanetriol concentrations during one cycle (*n* = 3).
ASSISTED REPRODUCTIVE TECHNOLOGIES IN A NEW WORLD PRIMATE, THE COMMON MARMOSET MONKEY (*Callithrix jacchus*)

V.S. Marshall,1* P. Tannenbaum,1 M.A. Browne,1 L. Knowles,1 J.K. Kalishman,2 and J.A. Thomson1

1Wisconsin Regional Primate Research Center, 1220 Capitol Court, Madison, WI 53715 USA; 2University of Washington School of Medicine, Box 357190, Seattle, WA 98195 USA

Abstract

The marmoset monkey (*Callithrix jacchus*) is a small New World primate which is used for biomedical research. It has a 28-day ovarian cycle, produces two or more embryos per cycle and is easily kept and bred in captivity. Its cycle can be synchronized using prostaglandin F2α, which resets the cycle to the beginning of the follicular phase,2 so that a number of individuals can be cycling synchronously. This affords the opportunity to perform embryo collections and transfers from a number of individuals on the same day. These characteristics make the marmoset a very favorable model for reproductive research.

Until recently, collection of embryos and gametes from marmoset monkeys has required laparotomy, an invasive procedure which can only be performed once in any animal used for research purposes. Additionally, collection of sperm has involved anesthesia of males for electroejaculation, or involvement of a female to wash ejaculated sperm from the vagina. Here we report the development of non- or minimally invasive assisted reproductive technologies for marmosets.

Non-surgical collection and transfer of embryos has been reported previously.1,3 Briefly, females are lightly anesthetized and placed in a restraining device which secures them in dorsal recumbency. Vaginal dilation is achieved by introduction of a glass speculum and the cervix is visualized using illumination provided by an otoscope. A 19-ga cannula and stylet are placed at the cervical os and gently guided into the uterus by transabdominal palpation. For embryo collection, the contents of the uterus are flushed with 3-5 ml of phosphate buffered saline, or, for embryo transfer, approximately 2 µl of collection medium containing one or two embryos is deposited in the uterine lumen.

Laparoscopic collection of marmoset oocytes has been developed. Traditional laparoscopes are large and awkward to use in an animal as small as a marmoset monkey. The procedure reported here uses an otoscope for the laparoscopic procedure. The otoscope is introduced through an incision in the abdominal wall, and the reproductive tract can be viewed directly through the plastic otoscope head. The device for follicular aspiration consists of a 20-ga needle attached to a short length of tubing and uses a 10 ml syringe to afford controlled, gentle suction of the follicular contents into a 5-ml tube. 158 follicles have been aspirated using this technique and 81 (51%) oocytes have been collected.
Sperm collection from marmosets is performed using a Ferticare™ vibrator (ILTS Inc.). A small piece of silicon tubing is inserted into the vibrator and used as an artificial vagina. Once males are habituated to the procedure, ejaculates can be reliably collected in an average of 9 sec. These non- or minimally invasive procedures decrease the stress involved for each procedure and increase the efficient use of marmosets for reproductive research. Additionally, these procedures may be effective in assisted reproduction programs for endangered marmosets and tamarins.

LITERATURE CITED

COMPARATIVE FOLLICULAR DYNAMICS BETWEEN THE BRAZILIAN RAINBOW BOA (Epicrates cenchria cenchria) AND THE BALL PYTHON (Python regius)

Jody E. Martin de Camilo, MS\textsuperscript{1,2,*} Cheryl S. Asa, PhD,\textsuperscript{2} Jeff A. Ettling, MS,\textsuperscript{2} Aaron M. Hampton,\textsuperscript{2} and Norman Haskell\textsuperscript{2}

\textsuperscript{1}Saint Louis University, Department of Biology, 3507 Laclede Ave. St. Louis, MO 63103 USA; \textsuperscript{2}Saint Louis Zoological Park, 1 Government Drive, St. Louis, MO 63110 USA

Abstract

Little is known about cyclic ovarian events that occur during the reproductive season for tropical reptiles. The ability to determine the timing of ovarian follicular events provides insight into the reproductive cycle, including seasonality, stages of follicular development, fertilization and determination of gravidity or pregnancy. Follicular dynamics were studied in two closely related species of snakes, the ball python and Brazilian rainbow boa, having different reproductive modes, oviparity and viviparity, respectively.

Ultrasound, a noninvasive diagnostic tool,\textsuperscript{2} was used to assess, classify and quantify follicular changes over a 2-yr period. Ultrasound imaging (ALOKA 500V, Corometrics Medical Systems, Inc., Wallingford, CT 06492) was conducted biweekly for eight adult females of both species. The females were anesthetized using cotton saturated with 3 ml isoflurane (IsoFlo, Abbott Laboratories, North Chicago, IL 60064) in a 60-cc syringe case that fits as a nose cone.\textsuperscript{1} The animal’s body was immersed in water to enhance ultrasound transmission. A short linear array transducer (7.5 MHz, Corometrics) was placed right lateral to midline on the ventral surface. The ovoid nonechogenic gall bladder was used as an anatomic landmark located approximately 2/3 the distance from the tip of the snout. The ovaries, just caudal to the gall bladder, are positioned asymmetrically so that the left ovary is distal to the right.

Ovarian cycles were observed in both species. Follicular stages included: 1) nonvitellogenic, 2) early vitellogenic, 3) vitellogenic, and 4) ovulatory. Ultrasonographically, the process of follicular maturation appeared similar in the oviparous and viviparous species. Mean follicle length and width were consistently larger in boas for all pairwise contrasts (Fisher’s protected LSD, $P < 0.0001$, Table 1), which could be attributed to differences in body size between pythons ($0.68 \pm 0.23$, $x \pm SE$) and boas ($2.57 \pm 0.34$; t-test, $P = 0.0414$). Differences were also detected in the number of follicles during each follicular stage (Table 1). The number of follicles ($y$) was an exponential decay function of mean follicle size ($x$). Regression analysis revealed that the relationship for pythons was: $y = 331.4x^{-0.6142}$, $R^2 = 0.9481$; and boas: $y = 1186x^{-0.6816}$, $R^2 = 0.9586$. This pattern indicates that there is a trade off between follicular size and number.
ACKNOWLEDGMENTS

We greatly appreciate Dale DeNardo D.V.M., Ph.D. University of California, Berkeley, for advice on the ultrasound technique.

LITERATURE CITED


<table>
<thead>
<tr>
<th>Follicular stage</th>
<th>Ball python</th>
<th>Rainbow boa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Length</td>
</tr>
<tr>
<td>Nonvitellogenic</td>
<td>917</td>
<td>5.5 ± 0.03</td>
</tr>
<tr>
<td>Early vitellogenic</td>
<td>310</td>
<td>8.5 ± 0.07</td>
</tr>
<tr>
<td>Vitellogenic</td>
<td>159</td>
<td>13.4 ± 0.16</td>
</tr>
<tr>
<td>Ovulated</td>
<td>100</td>
<td>22.6 ± 0.35</td>
</tr>
</tbody>
</table>
IS THERE ANY RHYME OR REASON TO RHINOCEROS REPRODUCTION? - A SUMMARY OF REPRODUCTIVE CHARACTERISTICS, SPECIES-SPECIFICITIES AND CHALLENGES FOR THE FUTURE

Terri L. Roth, MS, PhD1* and Janine L. Brown, PhD2

1Center for Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220 USA; 2Conservation & Research Center, Smithsonian Institution, 1800 Remount Road, Front Royal, VA 28007 USA

Abstract

Progress in characterizing the reproductive physiology of all four captive rhinoceros species has revealed interesting variation among species within this taxon and also has been useful in defining future challenges for ensuring the long-term stability of captive breeding programs. Our goal was to combine information from our large-scale study in which the reproductive cycles of Sumatran \( n = 2 \) and African black \( n = 15 \) and white \( n = 11 \) rhinoceroses were monitored, with previous reports from other investigators to produce a concise summary of what is known today about rhinoceros reproductive physiology. Noninvasive fecal hormone metabolite monitoring has been the primary method used for characterizing reproductive cycles, and resulting data have provided a foundation of basic knowledge upon which we now can build by employing additional research tools. For example, ultrasonography already has proven useful for identifying reproductive characteristics that otherwise might have remained undetected.

The African black rhinoceros \( (Diceros bicornis) \) has been the most prolific and best studied of the captive rhinos.1,2,9 Most female black rhinos are exhibiting reproductive activity. Their reproductive cycles average about 25 days, however, variable cycle lengths are common with approximately 50% of cycles <20 or >30 days. Although reproductive success has been relatively high, there are several animals that appear to be healthy and reproductively active but continue to breed without becoming pregnant. Identifying the cause of this apparent infertility is the primary challenge ahead for black rhinoceros reproductive research. However, the greatest threat to the captive black rhinoceros population is their unusual susceptibility to several uncommon diseases.

Similar fecal progesterone metabolite monitoring studies in the African southern white rhinoceros \( (Ceratotherium simum) \) have proven more difficult to interpret,5,6,10 and reproductive success in this species is inferior to that in its close relative, the black rhinoceros. Approximately 50% of captive female white rhinos appear acyclic. The remaining female white rhinos can be categorized as exhibiting one of three different types of reproductive cycles: 1) 60-70-day cycles; 2) 30-35-day cycles; or 3) a mixture of long (60-70-day) and short (30-35-day) cycles. Several females with 70-day cycles are breeding without producing calves. These long cycles are characterized by an extended luteal phase, and fertility is questionable since no successful pregnancies have been documented in animals exhibiting long cycles exclusively. Determining the causes of both

1999 PROCEEDINGS AMERICAN ASSOCIATION OF ZOO VETERINARIANS 97
Acyclicity and extended cycles are research priorities for the southern white rhinoceros. Additionally, early pregnancy loss and uterine pathology have been documented by ultrasound and further examinations of additional animals are necessary to determine their prevalence and potential association with reduced fertility.

The reproductive cycle of the Indian rhinoceros (Rhinoceros unicornis) has been characterized by both behavioral observations and urinary hormone metabolite monitoring. The reproductive cycle appears to vary, ranging from 39-64 days. In this rhinoceros species, increases in urinary estrone conjugates are associated with estrus behavior and breeding. Recent research using serial ultrasound examinations in a regularly cycling female has revealed the development of extremely large follicles (>10 cm diameter) several days before ovulation which might explain the high levels of estrone produced by this species during estrus. Captive breeding of the Indian rhinoceros has been relatively successful, however, aggressive interactions between some male-female pairings, even during the female’s estrus, have interfered with breeding success on several occasions. These behavioral incompatibilities between specific pairs limit our ability to genetically manage the captive population, and the development of artificial insemination may provide a useful method for overcoming this hurdle in the Indian rhinoceros captive breeding program.

The other Asian rhinoceros in captivity, the Sumatran rhinoceros (Dicerorhinus sumatrensis), has been studied intensively during the last few years. In the last century, captive breeding efforts with this species have been unsuccessful, in part, due to difficulties detecting estrus behavior and aggressive interactions between pairs when animals are introduced during the female’s nonreceptive period. Long-term, serial ultrasound examinations and serum hormone analyses have revealed that the Sumatran rhinoceros experiences a 21-day reproductive cycle and appears to be an induced ovulator, a characteristic not previously reported within the Perissodactyla family. Early pregnancy loss has been detected in one animal on three occasions and uterine pathology has been reported in several other animals. The reason for this uterine pathology is unknown and warrants investigation. Similarly, the cause of early pregnancy loss is a mystery and determining why it is occurring and how to overcome it will be research priorities as efforts to produce offspring in the captive Sumatran rhinoceros continue.

In summary, the reproductive cycle of each rhinoceros species differs, ranging from 21 days in the Sumatran rhinoceros to 70 days in some white rhinos. Similarly, ovarian activity differs among species. For example, preovulatory follicles in the Sumatran rhinoceros are ~25 mm in diameter, and breeding appears to induce ovulation. In contrast, preovulatory follicles in the Indian rhinoceros may grow to >10 cm in diameter and spontaneously ovulate. Challenges for the future include understanding the reasons for and overcoming the challenges of: 1) repeated copulations without pregnancy; 2) early pregnancy loss; 3) uterine pathology; 4) extended luteal phase cycles and acyclicity in the white rhinoceros; and 5) aggressive interactions between pairs of Indian and Sumatran rhinos introduced for breeding.
LITERATURE CITED


INTERSPECIES EMBRYO TRANSFER—A FEASIBLE STRATEGY FOR SAFEGUARDING ENDANGERED SPECIES?

Naida M. Loskutoff, PhD

Center for Conservation and Research, Henry Doorly Zoo, 3701 South 10th Street, Omaha, NE 68107 USA

Abstract

Traditional methods of captive breeding, (i.e., the pairing of genetically compatible individuals) have limitations that inherently prevent such programs from reaching their ultimate goals of safeguarding certain species. As discussed previously, these limitations include:
1) the amount and quality of physical space available to house adequate numbers of animals for maintaining genetic diversity;
2) the ability to adequately monitor and maintain the health and welfare of wildlife species in captive conditions when there is typically little or no information available on their life histories;
3) the ability to accommodate species-specific behavioral cues, which for some animals is vital for ensuring reproductive success;
4) meeting the nutritional requirements of wildlife in captive conditions with processed, artificial diets for cost-effectiveness;
5) the ability to properly identify and treat reproductive failure, which typically is not directly attributable to a physiologic condition, but rather to a lack of information regarding the unique requirements (e.g., environmental and social) for inducing breeding activity in a particular species; and
6) the ability to properly manage the genetics of small captive populations.

Reduced genetic variation and deleterious inbreeding effects are significant problems facing small populations, yet in the last decade it was estimated that 76% of the over 2700 mammals, reptiles and birds commonly housed in zoos are represented by fewer than 25 individuals. Considering the problems, limitations and inherent risks associated with traditional approaches to captive propagation, it should not be surprising that innovative methods such as assisted reproductive techniques be considered as essential supplements to future captive breeding strategies. Certainly, assisted reproductive techniques such as artificial insemination and embryo transfer have clearly benefitted livestock production for managing the genetics and expediting the generation of valuable stock. In the case of embryo production by in vitro fertilization, over 80,000 human babies have been produced to date using this technique.

However, despite the advances in assisted reproductive technology in livestock and humans, it must be acknowledged that its practical application with regard to the preservation of rare or endangered species is regrettably premature at this time. By definition, endangered species are of too limited numbers to acquire adequate material to conduct the appropriate research. Indeed, it may be that there is insufficient time (particularly so in the case of short-lived species) to conduct investigations.
to disclose features specifically unique to their reproductive biologies, which is paramount for the development of effective and reliable methods for assisting reproduction. This is a particular dilemma for those species that do not have suitable non-endangered or domestic animal counterparts to serve as developmental models or, perhaps, as surrogate recipients for interspecies embryo transfer.

Interspecies embryo transfer presents an additional challenge to assisted reproduction in that its success depends on the selection of appropriate donor/recipient combinations. There have been several reports of successful interspecies transfers of embryos from rare or endangered species, including gaur\(^2\) and banteng\(^25\) embryos transferred to domestic cattle, a bongo embryo transferred to an eland,\(^7\) mouflon\(^4\) and Armenian red sheep\(^9\) embryos into domestic sheep, Grant’s zebra\(^2,17,24\) and Przewalski’s horse embryos transferred to domestic horses,\(^17,24\) and an Indian desert cat embryo transferred to a domestic cat.\(^20\) However, it must be realized that despite these well-publicized successes, there have been many more failed attempts at producing viable offspring via interspecies embryo transfer. These failures have been manifested in a number of ways including early resorptions of the foreign embryos, or mid- to late-term abortions, prolonged and complicated pregnancies in equids,\(^17,24\) wild cattle,\(^23\) and buffalo,\(^6,8\) and inexplicable developmental abnormalities in aborted or stillborn fetuses in nondomestic sheep.\(^3\)

The first successful interspecies transfer of an embryo collected from a zoo animal (gaur) to a domestic cow was reported in 1981 by Stover, et al. However, these investigators reported that of three pregnancies established, two were lost between 5 wk gestation and near term. The placenta from the one live gaur calf born contained an abnormally low number of placentomes and abnormal histologic architecture.\(^13\)

At the Henry Doorly Zoo, we have incorporated genome resource banking and assisted reproduction in the captive breeding program for gaur. Several investigations have been performed to develop optimal methods for cryopreserving gaur epididymal sperm collected post-mortem\(^12\) as well as semen collected from live bulls by rectal probe electrostimulation.\(^10,15,22\) Live gaur calves have been produced by artificial insemination using thawed gaur semen\(^10\) and epididymal sperm collected and cryopreserved 27 hr after death.\(^12\) Currently, approximately 14,000 individual straws of cryopreserved gaur sperm from 48 different gaur bulls (18 of which are no longer living) are being stored at the genome resource bank at the Henry Doorly Zoo.

Live gaur calves have also been delivered from in-vitro-produced embryos from oocytes collected post-mortem\(^14\) as well as by ultrasound-guided, transvaginal oocyte retrieval.\(^1\) In fact, a total of seven gaur calves were produced by the transfer of fresh, in-vitro-generated embryos transferred singly into 17 domestic cattle (41% efficiency). Unfortunately, all seven of these calves died within 1 wk after parturition. The reasons for these losses were inconclusive; however, evidence of immunologic rejection of the gaur fetuses by the domestic cow dams was apparent.\(^11\) It is also highly likely that the interspecies pregnancies were further compromised by the fact that the embryos were in-vitro-derived, based on reports of intraspecies transfers of in-vitro-produced
embryos in domestic cattle which indicate higher calf mortality rates as compared to pregnancies produced from in-vivo-derived embryo transfers.\textsuperscript{16}

In conclusion, assisted reproductive technology is becoming an increasingly important management strategy for the conservation of nondomestic species.\textsuperscript{19} However, it is becoming evident that the greatest benefit from this technology will be in the genetic management of stable populations, rather than for the propagation of endangered species. Interspecies embryo transfer, although an attractive and exciting concept, has historically resulted in many more failures than successes. Perhaps with continued research and improved knowledge of the unique reproductive physiologies of novel species can we expect to increase the likelihood of utilizing this technique to safeguard rare or endangered species.

LITERATURE CITED

A DISSEMINATED ROUND CELL TUMOR IN A RING-TAILED LEMUR (Lemur catta)

Geoffrey W. Pye, BVSc, MSc, R. Avery Bennett, DVM, MS, Leo J. McSherry, DVM, and Scott P. Terrell, DVM

1Department of Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32609 USA; 2Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32609 USA; 3Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32609 USA

Abstract

A 13-yr-old intact male ring-tailed lemur (Lemur catta) with a 10-day history of depression, anorexia, polyuria, and polydipsia was referred to the Veterinary Medical Teaching Hospital, University of Florida for evaluation. The lemur was in poor body condition, but no other abnormalities were noted on the physical examination. A complete blood count and serum biochemistries revealed that the animal was anemic, hypoproteinemic (with hypoalbuminemia and normoglobulinemia), and hyponatremic. Whole body survey radiographs were unremarkable. An abdominal ultrasound revealed a homogenous increase in echogenicity of the liver, while the spleen appeared enlarged with mottled parenchyma. Ultrasound-guided aspirates were taken of the liver and spleen, and a bone marrow aspirate was also collected. Cytology of the bone marrow aspirate revealed that the myeloid and erythroid series was complete with orderly maturation. The myeloid:erythroid ratio was increased at 4:1. Small numbers of large, immature mononuclear cells suggestive of monocytes were seen. These cells had a variable nuclear:cytoplasmic ratio and large, prominent, irregular nucleoli. Erythrophagocytosis was also observed. The sample was interpreted as a possible malignant histiocytic infiltrate with relative myeloid hyperplasia and marked erythroid hypoplasia. Both the liver and spleen contained populations of large anaplastic mononuclear cells, similar to those seen in the bone marrow. Malignant histiocytosis was suggested as a possible diagnosis. Biopsies of the bone marrow and liver, on histopathologic examination, revealed infiltrates of anaplastic, round, neoplastic cells.

The lemur was euthanatized because the owner did not wish to pursue treatment. On necropsy, neoplastic cells were found in the spleen, liver, lung, kidney, multiple lymph nodes, small intestines, pancreas, and bone marrow. Further studies, including immunohistochemistry and electron microscopy, are being undertaken to further identify the neoplastic cell population.
DIAGNOSTICS AND TREATMENT OF SEVERE SWELLING OF THE PHARYNGEAL TISSUES OF AN AFRICAN ELEPHANT (Loxodonta africana)

Laurie J. Gage, DVM,1* David R. Blasko,1 and Larry D. Galuppo, DVM2

1Six Flags Marine World, 2001 Marine World Pky, Vallejo, CA 94589 USA; 2University of California, Davis School of Veterinary Medicine, Davis, CA 95616 USA

Abstract

A 10-yr-old, 1545-kg female African elephant (Loxodonta africana) presented with inspiratory dyspnea, stridor, and stertorous respiratory sounds. The problem had begun gradually 7 days after the elephant had started eating from a new shipment of orchard grass hay, and progressively worsened over the next 7 days. At that point the elephant was sedated with 100 mg xylazine i.v. and 10 mg butorphanol i.v. The sedation caused the clinical signs to worsen. While standing, a 3-m endoscope was passed through the left nostril of its trunk and advanced until the pharyngeal area could be visualized. The pharyngeal tissue was markedly swollen creating folds that completely obscured the epiglottis and arytenoid cartilages. There was an approximately 7-cm opening in the swollen tissue that vibrated during respiration, through which food and water had to pass. Blood was drawn for a complete blood count and blood chemistry analysis. The white blood count was moderately elevated to 19,500. (normal range of 12,000 to 16,000) The elephant treated with 6,000 units/kg penicillin G procaine and 6,000 units/kg penicillin G benzathine i.m., s.i.d. (Dual-Pen, TechAmerica Veterinary Products, Kansas City, MO 64190) and 0.02 mg/kg dexamethasone i.m., s.i.d. (Azium, Schering Animal Health, Union, NJ 07083) and its clinical signs improved. The dose of steroids was decreased systematically over the next 4 wk.

Four weeks later a new shipment of timothy hay was introduced to the diet. After 8 days on the new diet the elephant developed urticaria on its ventral abdomen and the medial aspects of its rear limbs. The urticaria resolved after 7 days. Ten days after receiving the new hay it demonstrated pharyngeal dysphagia when it started to drop masticated food from its mouth and had difficulty swallowing. Treatments of 750 mg diphenhydramine i.v. seemed to afford some relief. The diet was changed to orchard grass hay. The pharyngeal condition became progressively worse and 4 days later it could not swallow food or water for over 24 hr. This caused copious amounts of saliva to drip from its mouth. The condition included mild respiratory dyspnea. A complete blood count and blood chemistry analysis were performed. A white blood cell count of 16,500 was within normal range, and no other blood abnormalities were noted. The keepers noted that while being fed the new diet of orchard grass, the elephant had access to timothy grass hay, still offered to the other elephants, which it preferred over the orchard grass. This access was eliminated. The steroid dose was increased to 0.06 mg/kg dexamethasone i.m., s.i.d., 750 mg diphenhydramine i.m. was given s.i.d. × 10 days and another endoscopy was performed. The appearance of the pharyngeal tissues was similar to the first examination, and the opening of the esophagus could not be found with the endoscope. The pharyngeal tissue appeared to respond to the therapy as the elephant was able to swallow food normally over the next few weeks.
Two other African elephants, one of similar age, had endoscopy performed for comparison. The epiglottis and the corniculate processes of the arytenoid cartilages of these animals were easily viewed, as was the opening to the esophagus.

Serum was sent to Spectrum Laboratories for allergy testing. There was a strong positive reaction indicating allergy to all of the grass hays, including timothy, orchard, and rye, as well as a variety of other food items. The only types of hay it was not allergic to were alfalfa and oat. Serum from another elephant was used for comparison allergy testing and did not show similar positive reactions.

Antibiotic and steroid therapy were continued for 4 mo. Nutritional supplementation was directed at long term allergy relief. After 2 mo of therapy that included a strict hypoallergenic diet, antibiotics, steroids, and vitamins, endoscopy was performed. Moderate improvement was noted. Antibiotic therapy was continued however the steroid dose was decreased slowly. Six weeks later another endoscopy allowed us to see marked improvement. The epiglottis and arytenoid were now clearly visible. At this point the elephant could eat, drink and breathe normally.

It could not be determined conclusively if the swollen pharyngeal tissue was the result of an infection, an allergy or another etiology. Clinical response and comparative serum testing for allergies suggest a food-allergy etiology.

The use of the 3-m endoscope as a diagnostic aid was critical to the treatment of this elephant. The elephant had previously been trained to allow a stomach tube to be passed into the trunk which later allowed easy passage of the endoscope with only light tranquilization. All of the elephants used for comparison were well-trained for medical behaviors and tolerated the endoscopic procedure extremely well with minimal sedation. However if elephants are exhibiting clinical signs of pharyngeal dysfunction and respiratory distress, it is important to be cognizant of the potential exacerbation of the problem after sedation with alpha-2 agonists.
STANDING IMMOBILIZATION AND ANESTHESIA IN AN ASIAN ELEPHANT (Elephas maximus)

Murray E. Fowler, DVM,1* Eugene P. Steffey, DVM, PhD,2 Larry Galuppo, DVM,2 and John R. Pascoe, BVSc, PhD2

1Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616 USA; 2Department of Surgery and Radiology, School of Veterinary Medicine, University of California, Davis, CA 95616 USA

Abstract

Purpose

A 44-yr-old Asian elephant, the matriarch of a herd of breeding elephants at a zoo, developed a foot infection that progressed to osteomyelitis of the phalanges. Surgical removal of P-3 and part of P-2 was recommended. A complicating factor was that degenerative joint disease had developed over the years and the right elbow was nearly ankylosed. According to the keepers, the elephant had not lain down for 8 yr. The animal slept leaning against a wall or a fence. Although docile, well trained and managed under free contact, it would not or could not lie down on command.

Facility Modification

A decision was made to modify existing facilities to allow immobilization in the standing position and then gently lower the elephant to the floor. The patient weighed 2817 kg. The suspension system consisted of two steel “I’ beams braced on the top with right angle and diagonal steel strips bolted to the “I” beams. The beams spanned a distance of 9.8 m (32.2 ft) and were set on brackets anchored to solid concrete walls.

The hoist system was powered by hydraulic pump used to operate the door system in the elephant house. The hoist was designed to move horizontally on a “hammerhead” trolley, rolling on the suspension system’s I beams. A continuous loop of wire cable extended from one wall to the other, under the control of an electro/hydraulic diverter valve. Vertical movement was achieved by a swivel hook/load block (with two sheave) having a lifting capacity at the hook of 9072 kg (20,000 lbs).

The sling bed (heavy nylon fabric) was supported by 22 5.08 cm (2 inches) nylon straps, each having a breaking strength of over 5443.2 kg (12,000 lbs). The frame superstructure of the sling was constructed of square, metal-welded tubing with a wall thickness of 0.64 cm (1/4 inch) and outside dimension of 5 cm (2 inches). The frame was 1.12 m (44 inches) long and 0.74 m (29 inches) wide, with 22 eye rings to attach straps. Four choker cables were used to attach the metal frame to the hook on the hoist.
A special waterbed was constructed that was 4.27 m (14 ft) long by 3.66 m (12 ft) wide, and 38 cm (15 inches) deep when fully inflated. The bed was constructed of 30 mm polyvinyl geotextile fabric, with four hose ports installed on one end.

Preoperative Care

Food was withheld from the elephant for 36 hr. Water was withheld for 12 hr. The elephant was moved from its normal night quarters, into the room where the support beam and the hoist were in place. The animal had walked through this room previously and was generally acquainted with the apparatus. The only people in the room with the elephant were its regular keepers. The animal was moved to the center of the support beam, and maintained at 90° to its long axis.

Immobilization

The keepers moved the sling into position and began to attach the straps to the metal framework at the top of the sling. The elephant became agitated and did not respond to the calming efforts of the keepers. Etorphine hydrochloride (M99) was administered (1.75 mg) intramuscularly in the hind limb via a pole syringe. By 15 min after the initial etorphine injection it had quieted and the remaining straps were anchored to the metal frame. The breast band was positioned ventral to the point of the shoulders to limit tracheal compression. In an attempt to reduce abdominal compression which in turn inhibits excursion of the rib diaphragm, the butt strap was adjusted to support the tuber ischi and caudal thigh muscles. The belly band was positioned cranially to support the sternum. An indwelling i.v. catheter was inserted into an ear vein and anchored in place.

After the sling was in place, long ropes were attached to each leg. The ropes on the two left legs were attached by a bowline knot just above the widest area of the foot. Those ropes were then thrown over the back of the elephant and extended to block and tackles anchored to rings in the wall. The other ropes were attached to the right limbs in a similar fashion.

The right front limb was given special attention. Two ropes were applied to that limb, one to pull the leg under the body, and the other, attached to a block and tackle, to pull the leg forward at the same time. The intent was to keep the leg in a position that did not make a direct fulcrum from the foot upward, but which by pulling forward would gently roll the elephant into lateral recumbency. The rolled-up deflated waterbed was pulled into the room and positioned for unrolling after the elephant was immobilized and lifted off its feet.

When all positions were manned, and additional 0.75 mg of etorphine was slowly administered through the i.v. catheter, 40 min after the first dose of etorphine. The sling was raised by the hoist as the animal began to visibly become immobilized. Within 3 min, the elephant was hanging in the sling. The hoist operator was directed to lift the elephant off the floor so the waterbed could be unrolled and pulled beneath the elephant’s feet. Tension on the leg ropes was applied and slackened as required during the lowering process. When the feet solidly contacted the waterbed, the hoist operator began to move the hoist on the support beam toward the right side of the room while
continuing to lower the elephant. The elephant was completely immobilized, and offered no resistance. The sling was left in place throughout the surgery. Two water hoses were attached to the waterbed ports and filling begun.

**Anesthesia**

Following positioning of the elephant in right lateral recumbency, the trachea was manually intubated with a 30-mm equine style, cuffed endotracheal tube and the tube cuff inflated. The orotracheal tube was connected to a standard large animal anesthesia circle system. A latex weather balloon replaced the traditional 20-30 L rebreathing bag used for equine anesthesia. The isoflurane vaporizer dial setting was maintained at settings of 1.5-2.0% for most of the anesthetic period to supplement periodic i.v. bolus doses of etorphine (total additional etorphine was 1.4 mg).

**Recovery**

Thirty minutes before the completion of the surgery, the isoflurane vaporizer was turned off, but oxygen continued to flow. As the inhalation anesthesia began to wane, additional etorphine was intermittently administered intravenously (total dose over the remaining 45 min of recumbency was 0.4 mg).

The waterbed was deflated by opening the four ports, before the completion of the surgery, so that when the foot had been wrapped and the elephant was under the effects of etorphine, the lifting process could begin.

The direction of pull on the leg ropes was reversed. As the lift progressed, the body was rolled into an upright position with the feet 10-15 cm (4-6 inches) above the waterbed, which was pulled from beneath the animal. The animal was then lowered so the feet contacted the floor, and the immobilization was reversed with naltrexone (250 mg), administered intravenously. In 3 min it was standing on its feet, with little or no support from the sling. The animal was allowed to stand and move about for another 2-3 min while we observed, watching for signs of complete reversal.

When the keepers began to release the sling straps from the metal frame, the elephant became agitated and it was not safe for anyone to be near its head. The animal began vocalizing and trying to lunge away from the slinging site. It was impossible to administer additional drugs intravenously, so 1.25 mg of etorphine were administered intramuscularly. In approximately 5 min it quieted down, and the keepers could release the rest of the straps and remove the sling from its body. No additional etorphine reversal agent was given. Within 1 hr of the last etorphine injection, it was possible for the keepers to approach and treat it as they had before the immobilization episode.

**Conclusions**
The keys to this successful standing immobilization were, (1) a trained elephant that responded to direct control by its keepers, (2) a well-designed and installed suspension system, (3) an excellently-designed and constructed hoist system that was able to track along the suspension system, and (4) an adjustable, sturdy sling. It was also necessary to use both an injectable narcotic and isoflurane inhalation anesthesia.
MANAGEMENT OF A MELTING CORNEAL ULCER IN A GREATER ONE-HORNED RHINOCEROS (Rhinoceros unicornis)

Amy Rae Gandolf, DVM,1* A. Michelle Willis, DVM,2 Evan S. Blumer, VMD1 and Mark W. Atkinson, BVSc1

1The Wilds, 14000 International Road, Cumberland, OH 43732 USA; 2College of Veterinary Medicine, The Ohio State University, 601 Vernon L. Tharp Street, Columbus, OH 43210 USA

Abstract

Keratomalacia has been reported in several companion animal species, often as a sequela to corneal trauma. Corneal insult can result in a significant host inflammatory response and rapidly progressive stromal collagenolysis with or without presence of bacterial infection. Despite aggressive treatment, keratomalacia can result in a loss of stromal architecture leading to perforation, iris prolapse, endophthalmitis, and phthisis bulbi in 24-48 hr. A paucity of information exists on corneal disease in captive nondomestic mammals. This report reviews the application of ophthalmic techniques established in equine and small animal practice to acute keratomalacia in a large captive nondomestic animal, the greater one-horned rhinoceros (Rhinoceros unicornis).

Acute unilateral keratomalacia occurred in a 19-mo-old greater one-horned rhinoceros born and raised at the National Zoo in Washington, D.C. and relocated to The Wilds in Cumberland, Ohio on recommendation of the Species Survival Plan and the Rhinoceros Taxon Advisory Group. The corneal disease was presumed secondary to transport-related trauma. An opacity involving 60% of the cornea of the left eye (OS) was identified approximately 48 hr post-shipping. Twenty-four hours after initial observation of the lesion, the opacity had progressed to approximately 80% corneal involvement with significant epithelial and stromal degeneration evident as a “dripping” appearance to the cornea. An ophthalmic examination was performed under sedation following remote i.m. delivery (Pneu Dart Inc., Williamsport, PA 17703) of 0.5mg etorphine hydrochloride (M99-Ten™, Wildlife Pharmaceuticals Inc., Ft. Collins, CO 80524) and 8 mg detomidine HCl (Dormosedan®, Pfizer Inc, West Chester, PA 19380). Medical treatment consisted of 0.2 ml topical ciprofloxacin HCl (Ciloxan®, Alcon laboratories Inc, Fort Worth, TX 76134) every 2 hr as a broad spectrum antibiotic with ability to penetrate the corneal epithelium; 0.2ml topical autogenous serum every 2 hr as an anticollagenase treatment; 0.2ml topical atropine (atropine sulfate ophthalmic solution USP 1%, E. Fougera & Co., Melville, NY 11747) every 8 hr for pain associated with ciliary contraction resulting from anterior uveitis; 1 g flunixin meglumine granules (Banamine®, Schering Corp, Kenilworth, NJ 07033) p.o. every 2 hr to reduce inflammation associated with the keratomalacia process and 30mg/kg sulfamethoxazole and trimethoprim tablets (960 mg USP, Mutual Pharmaceutical Co., Inc. Philadelphia, PA 19124) p.o. every 24 hr as a systemic antibiotic because of the risk of corneal rupture.
A follow up exam was performed by an ophthalmic specialist, 3 days after initial examination, and revealed little improvement. Slit lamp examination findings included the following: keratomalacia involving 80% of the central cornea, 2mm of clear peripheral cornea, 360° 2-mm limbus neovascularization, mild anterior uveitis, and bulbar conjunctival hyperemia OS. In addition, 2-mm temporolateral corneal erosion was present OD, as demonstrated by positive flourescein uptake and associated 1-mm corneal neovascularization extending from the dorsal limbus. This examination yielded a guarded prognosis for sight and appearance OS due to anticipation of a large corneal scar associated with stromal healing. Because response to medical therapy was inadequate, surgery was indicated as the most efficient method for rapid resolution of the keratomalacia process and return of ocular comfort.

The surgical procedure consisted of corneal debridement, which is essential in halting collagenolysis, and advancement of a 360° conjunctival graft. Post-operative treatment consisted of topical atropine every 6 hr for 2 days; flunixin meglumine 1 g, p.o. every 24 hr for 2 wk; topical ciprofloxacin HCl every 6 hr for 2 wk; a 2-wk course of every 8 hr 1% miconazole (Monistat I.V.®, Janssen Pharmaceuticals, St. Joseph, MO 64504) and stall confinement for 3 wk to reduce potential for trauma to the eye. The rhinoceros tolerated treatment and exhibited no discomfort other than occasional rubbing. Surgical therapy resulted in rapid healing and a minimal midcorneal scar with peripheral corneal clarity. Although medical therapy appeared to slow the progression of the lesion in this case, surgical intervention halted progression and brought rapid resolution. Following conjunctival graft trimming 6 wk post-operatively, the globe was intact and visual function was evident. Six months post-operatively, visual function, comfort, and appearance of the eye were dramatically improved.

Although captive wildlife species present their own challenges to therapeutic management, this case report demonstrates that ophthalmic techniques used in domestic species may be applied to nondomestic species such as the rhinoceros. The incorporation of surgical therapy salvaged the eye in this case and is the primary choice for resolution of any case of keratomalacia. Successful resolution of this case was also associated in part with a high degree of patient compliance with medical therapy. The challenges of following the demanding therapeutic regimen for ocular disease in captive nondomestic species is compounded by handling limitations and facilities therefore must be equipped to provide necessary veterinary management. Three immobilizations were necessary for resolution of this case. Each immobilization added risk and expense to therapy. The stress of capture and handling is likely to play a significant role in the results of therapy that must be weighed against the benefits of each treatment.
FOREIGN BODY REMOVAL IN AN OKAPI (*Okapia johnstoni*)

*Felicia Knightly, DVM* and *David E. Kenny, VMD*

*Animal Health Center, Denver Zoological Gardens, City Park, E. 2300 Steele St., Denver, CO 80205-4899 USA*

Abstract

The okapi is one of only two species in the family of Giraffidae. Unlike giraffe which inhabit a wide area on the African continent, okapi are confined to the dense tropical rainforests of the Democratic Republic of the Congo (formerly Zaire). Like the giraffe, okapi are browsers that select high protein forages and utilize a ruminant gastrointestinal tract to process and absorb nutrients. The proximal portion of the ruminant gastrointestinal tract is composed of a compartmentalized forestomach, a glandular abomasum and several associated orifices through which ingesta flows via intermittent, synchronized contractions. Although over 190 okapi have been bred in captivity and much has been learned about reproduction in this species there is little published material concerning invasive surgical procedures required to correct gastrointestinal abnormalities. Typically, a diagnosis of ruminal or abomasal impaction in domestic ruminants such as cattle carries an unfavorable prognosis. Impaction of the rumen and abomasum are described as causes of death in okapi but no accounts of successful correction, surgical technique, post-operative care and the ability of this species to tolerate invasive surgical manipulation have been reported.

In November 1998 a 22-mo-old male okapi which was born at the Denver Zoo was observed consuming a keeper’s work glove. The work glove was composed of woven cotton fiber and a rubberized, water-proof coating. Unfortunately, the keeper was unable to retrieve the glove before it was swallowed. Over the ensuing 72 hr the okapi became depressed as well as anorectic and demonstrated signs of abdominal discomfort. No fecal output was detected and the animal eventually became reluctant to rise from sternal recumbency. The okapi was initially sedated with 30 mg of xylazine (Xyla-ject, Phoenix Pharmaceutical, Inc, St. Joseph, MO 64506) intramuscularly via a pole syringe. Twenty minutes later, 0.7 mg of carfentanil citrate (Wildlife Laboratories, Fort Collins, Colorado 80524 USA) was administered intramuscularly via a pole syringe to achieve immobilization. As reported by Bush with regard to giraffe, regurgitation in the okapi also occurs within seconds of immobilization. Despite being maintained in sternal recumbency, this individual regurgitated enumerable times producing gallons of rumen contents before, during and after transport to the hospital. Decompression with a stomach tube and intubation with a 16-mm endotracheal tube facilitated safe rotation of the okapi into left lateral recumbency. Although a standard rumenotomy in domestic species is generally approached from the left flank, the necessity of exploring all of the chambers of the proximal gastrointestinal tract required an approach through the right flank. Initially the caudodorsal aspect of the rumen was exteriorized through an approximately 37-cm curvilinear incision caudal to the last rib. The rumen contents were predominantly liquid with very little fibrous material and complete evacuation facilitated further
A portion of the glove’s cotton lining was retrieved from the ruminoreticular orifice. The material appeared to be of adequate size to cause blockage of the orifice. The rumenotomy incision was closed with two inverting suture patterns. An abomasotomy was also performed in order to complete a thorough digital evaluation of the remaining compartments of the forestomach. Evacuation of the abomasum’s firmer fibrous content revealed a diffusely erythematous mucosal lining. Several smaller pieces of glove material were retrieved however the amount of material recovered could not account for the entire glove. Although evacuation of gastric contents enabled adequate exposure and visualization contamination of the peritoneal cavity was very difficult to prevent. Contamination was minimized by packing the margins of the incision with sterile laparotomy sponges and vigorously lavaging the exposed serosal surfaces. The abomasum was also closed with two inverting suture patterns. The body wall was closed in five layers using various sizes and appositional patterns of absorbable suture material. One gram of ceftazidime (Fortaz, Glaxo Pharmaceuticals, Research Triangle Park, NC 27709) was administered intravenously and 12,000,000 iu of penicillin G procaine and penicillin G benzathine (Aspen Veterinary Resources, Ltd., Kansas City, MO 64120) was administered subcutaneously peri-operatively. In addition, 400 g of flunixin meglumine (Banamine, Schering-Plough Animal Health Corp., Kenilworth, NJ 07033) was divided intravenously as well as subcutaneously for pain relief.

In this case post-operative concerns included: incomplete removal of the foreign material, aspiration pneumonia, peritonitis, gastrointestinal atony, elimination of essential gastrointestinal microflora and dehiscence of the suture line. Within 12 hr of anesthetic recovery the okapi appeared alert and reactive. Post-operative care consisted of gradual reintroduction to water, forages and produce over 72 hr following surgery. The okapi began defecating normally 6 days after the procedure. Produc was supplemented with maternal fecal material in an attempt to repopulate the gastrointestinal tract with essential microflora. Despite the absence of post-operative antibiotic therapy and gastrointestinal motility enhancers the only complications noted throughout recovery were compulsive licking along the suture line and several areas of pressure necrosis on bony prominences as well as the left side of the animal’s neck. Based on this case, it appears that okapi are able to tolerate surgical manipulation of the gastrointestinal tract for the purpose of foreign body removal however careful consideration of okapi physiology and behavior are essential for success.

LITERATURE CITED


MORTALITY OF COMMON CUTTLEFISH (*Sepia officinalis*) AT THE NATIONAL ZOOLOGICAL PARK: IMPLICATIONS FOR CLINICAL MANAGEMENT

Johanna Sherrill, DVM, MS, Lucy H. Spelman, DVM, Dipl ACZM, Carrie L. Reidel, BS and Richard J. Montali, DVM, Dipl ACVP and ACZM

1Department of Animal Health, 2Department of Invertebrates, and 3Department of Pathology, Smithsonian National Zoological Park, 3001 Connecticut Avenue, Washington, DC 20008 USA

Abstract

Common cuttlefish (*Sepia officinalis*), along with other cephalopods (squid, octopus), are frequently kept and bred in captivity. Cephalopods are commonly used in research, and information regarding their basic biology and husbandry can be found in references intended for the aquaculture industry.1,2,4,7,9,11,14,16,18 Published information addressing the diseases and treatment of cuttlefish is limited, however, and no reports currently exist on the mortality of this species in zoos and aquaria.6,7,10,15-18 Unlike a laboratory or propagation facility, zoos typically display a group of fewer than a dozen cuttlefish in tanks designed for exhibition and education.12 It is important to recognize potential disadvantages of a small-scale, captive environment for cuttlefish, particularly with respect to their health and longevity.

Seven hatchling cuttlefish, approximately 1 mo of age, were acquired in early July 1998 from the National Resource Center for Cephalopods of the Marine Biomedical Institute (Galveston, TX, USA). At various times between August 1998 and January 1999, these cuttlefish were presented to the Department of Animal Health at the National Zoological Park (NZP) with different degrees of mantle ulceration. Three of the cuttlefish seemed to respond to changes in husbandry and oral chloramphenicol therapy (40 mg/kg p.o. daily) injected into bits of food, but all seven ultimately died by the end of January 1999. Prior attempts to manage clinically abnormal cuttlefish at NZP using a variety of parenteral antibiotics have had limited success, with anorexia and mantle ulceration commonly reported as problems preceding death. Based upon clinical and pathologic information from NZP, literature review, and communication with experts in cuttlefish husbandry, recommendations for future clinical management of zoo-captive cuttlefish were formulated.

The NZP pathology database was utilized to obtain the causes of mortality determined over a 12-yr period, during which 160 of 180 common cuttlefish were completely necropsied. Recorded lifespans ranged from 1-14 mo of age. In cases of known sex, age, and weight at postmortem (*n* = 133), 33 cuttlefish were between 7-9-mo old and weighed an average of 376.2 g (males, *n* = 18) and 299.0 g (females, *n* = 15). Six carcasses were not retrieved and 14 were in an advanced state of autolysis. Many cuttlefish had multiple pathologic diagnoses. The most frequent conditions identified were: inanition (*n* = 38); mantle lesions (erosion/abrasion, ulceration, abscess, granuloma, necrosis, dermatitis, cellulitis) (*n* = 97); cuttlebone lesions (fracture, erosion, abscess, deformity) (*n* = 28); septicemia due to *Vibrio* spp. (*n* = 11), or other bacteria (*n* = 28); and secondary bacterial...
infections, especially of the cardiovascular \(\text{n} = 51\), respiratory \(\text{n} = 52\), renal \(\text{n} = 14\), ophthalmic \(\text{n} = 38\), gastrointestinal \(\text{n} = 60\), and reproductive \(\text{n} = 36\) systems.

As reported by their keepers, most of these cuttlefish developed anorexia \(\text{n} = 58\) and exhibited signs of secondary infection such as cloudy eyes \(\text{n} = 25\) prior to death. Other recurrent clinical signs were slow growth or failure to thrive \(\text{n} = 13\), depression and lethargy \(\text{n} = 15\), and agitation \(\text{n} = 13\), as indicated by dark coloration, erratic swim patterns, and/or swift propulsion against tank walls. Trauma to self or to others was commonly mentioned in keeper reports. In some cases cuttlebone fractures were directly associated with a history of self-trauma \(\text{n} = 4\). Sexually developing male cuttlefish were observed to sustain mantle injuries during intraspecific aggression \(\text{n} = 5\) which quickly progressed to ulcerative and granulomatous mantle lesions, ultimately leading to sepsis associated with gram-negative bacteria. Postmortem findings correlated with clinical signs such that most cuttlefish had mantle lesions, evidence of sepsis or secondary bacterial infections, and no body stores of fat.

Early detection of illness in this species is critical. Once anorexia develops, medical intervention is unlikely to be successful. Whenever feasible, mantle lesions should be prevented. If cuttlefish are exhibiting behaviors that put them at risk for development of mantle lesions, prophylactic, long-term antibiotics effective against gram-negative bacteria such as *Vibrio* spp. may delay disease progression. Strategies for preventing mantle ulcers include strict attention to tank design (smooth-sided walls), substrate (non-abrasive), population density (one male per small tank), nutrition, water quality, and temperature.\(^\text{16}\) In addition, environmental stressors, especially lack of hiding places, excessive activity near the tank, overcrowding, and poor water quality should be avoided.\(^\text{11,16}\) Monitoring cuttlefish sizes and rates of growth can be helpful in assessing their health and nutritional status.\(^\text{3,13,16}\) The growth rate of cuttlefish is rapid, resulting in a lifespan that rarely exceeds 1 yr in a captive laboratory setting.\(^\text{16}\)

The successful display of these unique aquatic invertebrates requires a substantial investment in proper exhibit design, husbandry, and staffing that must be considered by zoos wishing to acquire common cuttlefish. It is hoped that a description of previous NZP cases will help improve clinical management of cuttlefish in zoos and emphasize that husbandry limitations directly affect occurrence of disease conditions and mortality events in this species.

**ACKNOWLEDGMENTS**

The authors are grateful to Dr. J.M. Scimeca for advice and consultations. Special thanks to Ms. Nancy Spangler and Mr. B. Roffey for assistance in compiling data from necropsy reports. We thank the staff of the Invertebrate Department of the National Zoological Park devoted to the husbandry of cuttlefish included in this report, and the Friends of the National Zoo (FONZ) for financial support.

**LITERATURE CITED**
HEMIMANDIBULECTOMY TO RESOLVE *Actinomyces* sp. “LUMPY JAW” IN A BENNETT’S WALLABY (*Macropus rufogriseus fruticus*)

Wm. Kirk Suedmeyer, DVM,1* Candace Layton, DVM, ACVS,2 and Steven M. Riley, DVM, ACVS2

1Kansas City Zoological Gardens, 6700 Zoo Drive, Kansas City, MO 64132 USA; 2Veterinary Specialists of Kansas City, 10333 Metcalf Avenue, Overland Park, KS 66212 USA

Abstract

“Lumpy jaw” or necrobacillosis is a term used to describe the infectious inflammation of the mandibular bone in mammals.1-3,6,7 It is a chronic bacterial infection most often involving *Nocardia asteroides*, *Actinomyces bovis*, *Fusobacterium necrophorum*, and *Nocardia macropodidarum*, but additional bacterial organisms may be involved.1-3,6,7 Although it is most often described as occurring in the mandible, cases have been observed in the maxilla.7 Metastatic abscesses have been observed in numerous cases,1,3,7 and must be taken into consideration when initially evaluating a case of necrobacillosis.

“Lumpy jaw” occurs in numerous species of mammals, including marsupials, domestic and exotic ruminants and exotic porcines.1-3,6,7 It is thought to arise from a break in the integrity of the periodontal ligament. Reasons for this are unclear, although hypovitaminosis A, coarse stemmed plant material, and dental disease, combined with overcrowding, stress, and poor husbandry practices probably predispose to this condition.3-7

Treatment involves long term administration of antibiotics, curettage of the affected bone with local administration of iodine solutions, nitrofurazone, or sulfanilamide, and affected tooth extraction. Many cases are refractory to treatment and permanent extensive bone remodeling can occur.3,7

A 6-yr-old intact female Bennett’s wallaby, weighing 14 kg, was presented for a 1-day history of having a small amount of swelling over the ventral aspect of the body of the left mandible. During the day, the wallaby was maintained with six other conspecifics in a fenced, natural outdoor exhibit measuring 15 m × 10 m. During the evening, the animals were housed in an outdoor 25 m × 20 m fenced, gravel and sand enclosure with access to multiple wooden stalls with concrete floors. The diet consisted of an alfalfa based pelleted ration (Mazuri ADF, PMI Feeds, St. Louis, Missouri 63166-6812 USA), alfalfa hay, fresh daily browse in the form of bamboo (*Phyllostachys* sp.), and willow (*Salix* sp.), and moderate amounts of fresh fruits and vegetables as enrichment items.

The animal was manually restrained, and a physical examination revealed a firm mass involving the bone of the mandible. Treatment was initiated with 350 × 103 IU of penicillin G benzathine combined with penicillin G procaine (Pen BP-48, Pfizer Animal Health, Lee’s Summit, Missouri 64081 USA) i.m. via blowdart every 48 hr for 21 days. No improvement was noted, and the wallaby was immobilized with 140mg of ketamine hydrochloride (Ketaset, Fort Dodge Laboratories,
Overland Park, Kansas 66210 USA), and 25 mg xylazine hydrochloride (Rompun, Bayer Corp., Agriculture Division, Animal Health, Shawnee Mission, Kansas 66201 USA) i.m. via blowdart. The wallaby was maintained on a heated surgery table, and i.v. buffered fluids (Lactated Ringers Solution, Abbott Laboratories, North Chicago, Illinois 60064 USA) were administered at 20mL/kg/hr. Radiographs at that time revealed osteolysis and osteomyelitis of the left mandibular body. Oral examination revealed mild dental calculus, but no apparent gingival disease. The skin over the lesion was surgically prepped and incised. The bone was surgically curetted and flushed with 1% iodine solution. Characteristic “sulfur granules” were observed, and staining of the necrotic material revealed gram-positive filamentous rods and rosettes of presumed sulfur granules. Aerobic and anaerobic culture failed to demonstrate any growth. Antibiotic treatment was continued for an additional 45 days.

Minimal improvement was noted upon survey radiographs, and the wallaby was immobilized as before for reevaluation, additional bone curettage, and packing the resulting opening with iodine soaked sterile gelatin (Gelfoam, Upjohn Co., Kalamazoo, Michigan 49001 USA). Aerobic and anaerobic culture at that time revealed scant growth of Actinomyces sp. Histopathology of a bone biopsy revealed an osteomyelitis with filamentous, branching bacteria, presumably Actinomyces or Nocardia sp. Over the course of the following 3 mo, continued antibiotic treatment and two additional surgical curettages were performed as before. After the last immobilization, daily flushing of the resultant opening with iodine solution was performed. Minimal improvement was noted after an additional 10 days of treatment, and antibiotic therapy was changed to 100 mg clindamycin (Antirobe Aquadrops, The Upjohn Co., Kalamazoo, Michigan 49001 USA) p.o., b.i.d. for 90 days, at which time radiographs and physical palpation under anesthesia revealed progression of the osteomyelitis.

Hemimandibulectomy was proposed as a final alternative due to the chronicity and unresponsiveness of the lesion. The wallaby was anesthetized as before, intubated via a Cole technique with a 4.0 mm cuffed endotracheal tube (Baxter Healthcare Corp., Pharmaseal Division, Valencia, California 91355 USA), and maintained on 2.5% isoflurane (Aerrane, Anaquest, Madison, WI 53713 USA). The wallaby was placed in dorsal recumbency, the area over the ventral mandible was clipped, surgically prepped, and a standard hemimandibulectomy was performed by making an 8-cm incision which extended from the mandibular symphysis, along the left mandibular body past the angular process, then curved dorsally parallel to the mandibular ramus. Incision of the platysma muscle with the skin allowed elevation of the masseter and digastricus muscle muscles from the affected bone with a Freer periosteal elevator, taking care to avoid the facial artery and vein. Hemorrhage was controlled with monopolar electrocautery and suction. The mandibular symphysis was separated with an oscillating saw, followed by subperiosteal dissection along the more normal architecture of the mandibular ramus. The temporomandibular joint was sharply incised, and the left hemimandible was manipulated to allow elevation of the remainder of the masseter from the coronoid process. The surgical site was copiously lavaged with sterile saline prior to closure of the fascia of the masseter and digastricus muscles with 3-0 polydioxanone (PDS II, Ethicon, Inc., Somerville, New Jersey 08876-0151 USA) in a simple continuous pattern. The subcutaneous tissue was closed similarly, followed by intradermal skin closure with 4-0 polyglactin (Vicryl, Ethicon, Inc.). One injection of
penicillin (Pen BP) and 5 mg butorphanol tartrate (Torbutrol, Fort Dodge Laboratories) were administered i.m. post-operatively. The bone specimen was cultured aerobically and anaerobically, then fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 5 μm. The remaining dental arcades were filed to lessen the chance of buccal or gingival trauma. The wallaby recovered uneventfully, and was placed in a holding stall at the Animal Health Center of the zoo. Butorphanol was continued for 10 days, and antibiotics were changed to trimethoprim/sulfadiazine (Tribrissen 48%, Mortar and Pestle Pharmacy, Des Moines, Iowa 50310 USA) at 30 mg/kg s.i.d. via blowdart.

Cultures demonstrated scant growth of *Actinomyces* sp. Histopathologic interpretation of the bone revealed necrosis and osteomyelitis with occasional non-acid fast, filamentous bacteria, presumably *Actinomyces* sp.

The post-operative appearance was aesthetically pleasing. Minimal differences in physical appearance were observed; the wallaby was able to maintain the tongue in a normal position, although frequent extension/protrusion was observed for several days after surgery. A moderate median shift in the right mandible occurred secondary to the loss of supporting bone. This did not appear to interfere with mastication. Minimal eating was noted for the first 36 hr, but marked improvement was noted over the next several days. The wallaby would use the front feet to grasp food items and bring it to the right side of the jaw for mastication. The animal appeared to gain weight and was eating normally with minimal problems. Antibiotics were discontinued 20 days after surgery.

However, 28 days after surgery, the surgical site became infected. Additional therapy was initiated with daily flushing of the site with hydrogen peroxide, combined with 5 mg/kg enrofloxacin i.m. b.i.d. via blowdart. The infection spread through fascial planes to the tracheotomy incision, and the animal began to lose weight. Due to an overall poor prognosis, the wallaby was euthanatized.

Necropsy revealed abscess of the deeper mandibular tissues and ventral cervical musculature. *Acinetobacter calcoaceticus*, *Klebsiella pneumoniae*, and *Streptococcus* sp. were identified from aerobic cultures. All organisms were sensitive to enrofloxacin and trimethoprim/sulfadiazine. *Bacteroides fragilis* was identified from anaerobic culture, which was sensitive to penicillin. Tissues of all organs were sectioned, placed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 5 μm. Abscess of the fascial tissues with necrotic material surrounded by a wide zone of neutrophils and an outer zone of macrophages and neutrophils was observed.

**Discussion**

Despite secondary bacterial infection, which is a potential with any surgery, we considered this procedure a success. The results were aesthetically pleasing, the animal returned to normal eating habits, and initial weight gain was noted. The Cole technique for intubation worked well. Basically, a small incision is made over the caudal larynx after sterile preparation of the skin. A thin, sterile stylet is inserted in a retrograde direction through the skin and into the trachea. The stylet is
advanced along the tracheal, past the glottis, and out of the oral cavity. The endotracheal tube is placed over the stylet and advanced in a normograde fashion into the trachea until the juncture of the stylet and endotracheal tube is encountered. The stylet is then removed, the endotracheal tube is advanced and insufflated in a standard manner.

This radical surgical option may be successful in macropods affected with refractory cases of necrobacillosis, oral neoplasms, or non-repairable trauma. The median shift of the right mandible caused no initial concerns, as the wallaby appeared to adapt well to the loss of the left mandible. Long term concerns with proper mastication and dental diseases were discussed as potential future complications. Intravenous sodium iodine therapy was not elected in this case, but has been utilized without success in an additional case of necrobacillosis in a Bennett’s wallaby, and a greater kudu (Tragelaphus strepsiceros) (unpublished data). Secondary bacterial infections may be difficult to control, and appropriate antibiotic therapy is warranted. Pain relief should be addressed with such an extensive surgery. In this case, administration of butorphanol appeared to accelerate the return to normal behavior and eating habits.

A comprehensive review of management practices and diet are currently being conducted as an aid in preventing future cases of necrobacillosis in the macropod collection at the Kansas City Zoological Gardens.

ACKNOWLEDGMENTS

The authors would like to thank the animal care staff at the Kansas City Zoological Gardens for their help in resolving this case.

LITERATURE CITED

CUTANEOUS PYTHIOSIS (*Pythium insidiosum*) IN A SPECTACLED BEAR (*Tremarctos ornatus*)

Terry M. Norton, DVM, Dipl ACZM,1,2* Kenneth Latimer, DVM, PhD,3 Elizabeth Howerth, DVM, PhD,3 Teresa Bradley, DVM,2,4 Randy Basinger, DVM, Dipl ACVS,5 Arvind A. Padhye, PhD,6 Joe Newton, DVM, PhD,7 and Nadine Lamberski, DVM2

1St. Catherines Island Wildlife Survival Center, Wildlife Conservation Society, Midway, GA 31320 USA, 2Riverbanks Zoological Park and Botanical Garden, Columbia, SC 29210 USA (previous address of TMN and TB); 3University of Georgia, College of Veterinary Medicine, Department of Pathology, Athens, GA 30602 USA; 4Belton Animal Clinic, 511 Main St., Belton, MN 64012; 5Veterinary Referral Services, Columbia, SC 29210 USA; 6Division of Mycotic Diseases, Center for Infectious Diseases, Centers of Disease Control, Atlanta, GA 30333 USA; 7Department of Veterinary Pathobiology, Auburn University, College of Veterinary Medicine, Auburn, AL 36849 USA

Abstract

Case Report

On 14 September 1992, a 3.3-yr-old 115-kg male spectacled bear (*Tremarctos ornatus*) presented with a 3-cm circular excoriation with alopecia on the fifth digit of both the right front and right hind feet. The bear had been observed licking the wounds which occasionally caused them to bleed. The exhibit consisted of an indoor cement floor with multiple stainless steel metal barred enclosures with guillotine shift doors. The outdoor exhibit consisted of gunnite, soil, vegetation and a pool. The pool was drained and cleaned once per week. This bear was housed with his sibling and parents. The siblings and adult female were placed on exhibit together but kept separate from the adult male. The bear was started on 2880 mg trimethoprim sulfadiazine (Tribrissen, Coopers, 321 East Hawley Street, Mundelein, IL 60060 USA) s.i.d. for 10 days in a preferred food item. The wounds were sprayed with a dilute chlorhexidine solution (Nolvasan solution, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa USA).

Ten days later the wounds were larger in diameter, moist and appeared more invasive. The bear was immobilized with tiletamine and zolazepam (Telazol, Fort Dodge Laboratories, Incorporated, 800 Fifth Street Northwest, Fort Dodge, IA, 50501 USA) 750 mg i.m. by dart. Physical examination revealed a 4-cm circular, moist foul smelling wound on the fifth digit of the front foot and a similar lesion on the right hind foot which also had a missing toenail. Blood was collected from the femoral vein for a complete blood count (CBC), serum biochemical panel, and serum banking. Multiple skin biopsies were taken from each wound and submitted for histopathology and aerobic bacterial and fungal cultures. The animal recovered uneventfully. Histopathology revealed a deep fungal dermatitis with bacterial involvement. Aerobic bacterial culture revealed heavy growth of *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and an unidentified yeast. All the bacterial organisms were resistant to trimethoprim sulfadiazine but sensitive to enrofloxacin. All other diagnostic work was normal. Enrofloxacin (Baytril, Bayer Corporation, Shawnee Mission,

1999 PROCEEDINGS AMERICAN ASSOCIATION OF ZOO VETERINARIANS 123
Kansas 66201 USA) 272 mg b.i.d. and fluconazole (Diflucan, Roerig, A Division of Pfizer Inc., NY, NY10017) 600 mg s.i.d. were started in a preferred food item and the trimethoprim sulfadiazine was discontinued. Daily spraying with dilute povidone-iodine solution (Vetadine solution, Vedco, Inc, St. Joseph, MO 64504 USA) and topical ketoconazole cream (Janssen Pharmaceuticals, 6705 Millcreek Drive, Mississauga, Ontario L5N 5R9 Canada) was initiated. This regimen was continued for 6 wk. On some days the wounds seemed to be improving, however, if the bear licked the wounds they looked worse. The wounds appeared to be very pruritic.

A second workup was performed 6 wk after the first. The bear was anesthetized with tiletamine and zolazepam at the dosage used previously and maintained on isoflurane (Aerrane, Ohmeda Pharmaceutical Products Division Inc. Liberty Corner, NJ 07938-0804 USA). Radiographs revealed an osteomyelitis of phalanx 3 (P3) on the 5th digit of both the right front and right hind feet. Deep pockets of purulent material were found on both wounds. Surgical amputation of P3 on both feet was performed. The exposed joint was flushed with amphotericin B prior to closing. Blood was drawn for CBC, serum biochemical panel, and blastomycosis serology. Tissue was submitted for fungal (2 laboratories were used), mycobacterial, and aerobic and anaerobic bacterial cultures, and histopathology. The next day the bear had licked out all of the sutures from both amputation sites.

Histopathology revealed a multifocal nonsuppurative to granulomatous osteomyelitis with intralesional fungal hyphae. Aerobic bacterial culture revealed heavy growth of gram-positive and gram-negative bacteria, which were all sensitive to chloramphenicol and enrofloxacin. Neither fungal nor mycobacterial organisms were cultured. The bear was started on itraconazole (Sporanox, Janssen Pharmaceuticals, 6705 Millcreek Drive, Mississauga, Ontario L5N 5R9 Canada) 500 mg s.i.d., p.o. and chloramphenicol 2000 mg b.i.d., p.o. The open incisions were sprayed with dilute chlorhexidine. The bear was housed on cement due to the open wounds.

Three weeks after the second workup the bear presented with watery bloody diarrhea, vomiting and severe depression. Fecal examination for parasites was negative, however numerous white blood cells were found on a Diff-Quik (Dade International Inc., Miami, FL 33252-0672 USA) fecal smear. Feces were submitted for enteric bacterial pathogen culture and a piece of sloughed mucosa was submitted for histopathology. Itraconazole and chloramphenicol were discontinued, an electrolyte/glucose solution was placed in the bear’s water. The bear did not eat and would not take any medications orally for 3 days. The next day the bear started eating small amounts of yogurt, sweet potato baby food, and primate chow. Clostridium perfringens was cultured from the feces. Histopathology on the mucosa submitted revealed an intestinal cast composed of mucous, necrotic debris with eosinophils and ingesta. A homogenous population of large gram-positive bacterial rods was also observed. The bear was started on 1000 mg metronidazole (250 mg tablets, USP, Sidmark Laboratories Inc, East Hanover, NJ 07936 USA) b.i.d., p.o. for 10 days. The wounds on the feet had improved at this point, presumably because the bear had not been licking them. The feces were back to normal after 5 days of metronidazole treatment.

Three weeks later, the right hind foot wound had a purulent discharge. The bear was immobilized with the same dose of tiletamine and zolazepam as previously used. The right front foot appeared
to be almost completely healed. The right hind foot had an abscess that dissected between the lateral aspect of the third digit and involved most of the fourth digit. The wound was draining well. Radiographs of the right front foot were normal, while the right hind foot had a periosteal proliferation involving P1 and P2 of the fourth digit. Biopsy samples were taken for aerobic bacterial and fungal cultures and histopathology. Blood was taken for CBC, serum biochemical profile, and serum bank. The wounds were flushed with a dilute betadine solution. The animal was started back on itraconazole 500 mg s.i.d. orally. A lactobacillus product and yogurt were also given to the bear. Histopathology revealed a chronic purulent dermatitis/panniculitis with intralesional gram-positive cocci and a nodular eosinophilic dermatitis/panniculitis with intralesional fungal hyphae. Tissues section were stained with an anti-

Pythium

antibody using an immunoperoxidase technique and were positive. At this point the most likely organism producing the lesions was thought to be

Pythium insidiosum.

Fungal organisms were not isolated by culture. A diverse population of bacteria were cultured, as was the case previously. All other diagnostic work was normal.

Two weeks later the nail on the fourth digit of the right hind foot was lost and the third digit continued to have a purulent discharge. The bear was immobilized using telazol and isoflurane, as previously described. Both the third and fourth digits of the right hind foot were ulcerated and draining purulent material. The right front foot appeared to be healed, but had a fluctuant area at the amputation site. The right popliteal lymph node was enlarged. Radiographs revealed an osteomyelitis of the third and fourth digits of the right hind foot; the right front foot appeared radiographically normal. Aspiration of the fluctuant area on the right front foot revealed purulent material. The fourth and fifth digits on the right hind foot were surgically removed. The distal metatarsal joints of both digits looked normal. The right front foot abscess was lanced and flushed. Blood was taken for CBC, a serum biochemical panel, and serum itraconazole levels (19 hr post administration). Tissue was submitted for fungal culture, aerobic and anaerobic bacterial culture, and histopathology. A biopsy of the enlarged lymph node was submitted for histopathology. Histopathology revealed a chronic eosinophilic inflammation with multiple to coalescing foci of degranulating eosinophils, collagen degeneration and intralesional hyphae. Itraconazole levels were 1.4 µg/ml (therapeutic range in other species 2-8 µg/ml). Bacterial culture produced heavy growth of

Proteus vulgaris

which was sensitive to enrofloxacin. Enrofloxacin was started at 250 mg b.i.d., p.o. itraconazole, yogurt, and local flushing of the wound were continued.

Over the next 2 mo, the wounds appeared to improve some days and looked worse on other days. A final immobilization, 7 mo after the initial presentation, was performed to evaluate the wounds. The wounds had improved on the right hind foot, while the right front foot still had a fluctuant area which was filled with purulent material. Radiographs revealed progression of the osteomyelitis in both feet. Due to chronicity of the problem and the lack of response to treatment, the bear was euthanatized by i.v. barbiturate overdose (Beuthanasia-D Special, Schering-Plough Animal Health, Union, NJ, 07083-182 USA). Gross necropsy revealed an extremely enlarged right popliteal lymph node with purulent material noted on cut section. The right axillary lymph node was also enlarged, but looked fairly normal on cut section. Histopathology revealed similar skin and bone changes as previously described. Lymphoid hyperplasia with sinusoidal eosinophilia was found on the enlarged
lymph nodes. There was no evidence that the *Pythium* had disseminated to internal organs. A mold was reported to have been recovered and was forwarded to the Centers for Disease Control and Prevention for identification. *Pythium insidiosum* was cultured.

Medical problems were not noted in the other bears housed with this animal for a 5-yr period. Recently, both parents of the bear in this case report developed obstructive lesions in the colon caused by *Pythium insidiosum*. Surgical resection of the affected gastrointestinal tract and anastomosis appear to have been curative in these two cases.

**Discussion**

Pythiosis is an invasive, eosinophilic to pyogranulomatous disease caused by *Pythium insidiosum*. It is an aquatic organism belonging in the order *Peronosporales*, phylum *Oomycota*, kingdom *Protista*. There are many different species of *Pythium* which are commonly found in soil and aquatic environments. They are considered important plant pathogens. *Pythium insidiosum* is the only member of this genus that causes disease in mammals. Pythiosis has been documented to cause disease in dogs, cats, horses, cattle and humans, although it is most common in dogs and horses. This is the first report of pythiosis in an ursid species. The disease occurs most commonly in tropical and subtropical areas worldwide. Most reports in the US have originated from states bordering the Gulf of Mexico; however, there have been cases from Missouri, Georgia, North Carolina, South Carolina, and Tennessee. *Pythium insidiosum* is often found in stagnant water producing biflagellated zoospores that penetrate into the animal through open skin. The environment of this spectacle bear was not excessively wet, however, there was a pool in the exhibit.

Three forms of the disease occur in domestic animal species; cutaneous disease, gastrointestinal disease, and rarely a systemic form. The cutaneous form is most commonly seen in the horse, although also occurs in other species. It most commonly affect the extremities, tailhead, perineum, and face. The lesions are initially an ulcerated plaque or erosion. These lesions enlarge rapidly into large masses with ulceration and draining tracts. Eventually, a hemorrhagic to purulent exudate is observed coming from the draining tract. The lesions noted in this bear followed a very similar course. There have been occasional reports of osteomyelitis developing in chronic cases of cutaneous pythiosis. Animals with cutaneous pythiosis are often extremely pruritic and may self multilate. The bear in this report seemed to continually aggravated his wounds by licking and rubbing.

The gastrointestinal form of pythiosis affects the stomach, proximal small intestine, distal small intestine, and colon most commonly, however, any area can potentially be involved. Vomiting, weight loss, and a palpable abdominal mass are common clinical findings.

This organism does cause disease in humans, however, the zoonotic potential is low. The motile zoospore is considered to be the major infective form of the organism. The zoospores do not form in tissue. There have been no reported cases of animal-to-animal, animal-to-human or human-to-animal transmission.
Histopathology and culture on biopsy specimens are the preferred method for confirmation of pythiosis. A nodular to pyogranulomatous dermatitis and panniculitis with foci of necrosis and large numbers of eosinophils is typical for tissue infected with *P. insidiosis*. The organism is difficult to see with routine hematoxylin-eosin (H & E), thus a Gomori methenamine silver stain is used. Hyphal structures are wide, occasionally septate, and irregularly branching and are often found in the center of granulomatous lesion. Gastrointestinal lesions are histologically similar to cutaneous lesions. The submucosa and muscularis layers are most severely affected. An immunoperoxidase assay was originally developed and validated on formalin-fixed tissues from horses with *P. insidiosum*. It has been used to confirm the diagnosis of pythiosis in dogs, horses, cats and humans. It was helpful in developing a diagnosis sooner in this case. An immunofluorescence test is also available for detecting *Pythium* hyphal structures in cat, dog, and human tissue.

*Pythium insidiosis* grows readily on several media, including sabouraud dextrose agar, brain-heart infusion medium, corn meal agar and vegetable extract agar. It is helpful to wash the sample in sterile saline with or without ampicillin to inhibit secondary bacterial growth. The organism usually grows within 24 hr.

Wide surgical excision of diseased tissue is the therapy of choice. When a limb is involved, amputation is recommended. Amputation was not possible in this case because two limbs were involved. Histopathologic evaluation of arteries from the proximal end of amputation site and regional lymph nodes should be performed. Ascending arteritis is the most common form of the disease in humans. Prognosis is poor in all cases.

*Pythium insidiosis* is sensitive, in-vitro, to miconazole, ketoconazole, and fluconazole, but not to amphotericin B. The azoles should not be effective in treating pythiosis because this organism’s cell membrane does not contain ergosterol. Itraconazole has been used with limited success with the gastrointestinal form of the disease and has not been successful in treatment of the cutaneous form.

Immunotherapy using a vaccine has met with good success in horses with early lesions, but has not been very successful in chronic cases (longer than 2 mo).

The bloody diarrhea and vomiting in this case were most likely due to *Clostridium perfringens* enterotoxemia secondary to long term use of antibiotic and antifungal drugs. This diagnosis was based on histopathology and culture results. A *Clostridium perfringens* enterotoxin assay was not performed.

ACKNOWLEDGMENTS

We thank the animal husbandry and veterinary technical staffs at the Riverbanks Zoological Park for their care and technical assistance with the bear in this case report.
TREATMENT OF ALLERGIC DERMATITIS (ATOPY) IN A SUB-ANTARCTIC FUR SEAL (Arctocephalus tropicalis) USING IMMUNOTHERAPY

Kate Bodley, BSc(Vet), BVSc,1* Cree Monaghan, BSc, BVMS, MVS,2 and Ralf S. Mueller, Dr med vet, FACVSc, Dipl ACVD3

1Veterinary resident, Melbourne Zoo, PO Box 74, Parkville, Victoria 3052, Australia; 2Veterinarian, Perth Zoological Gardens, PO Box 489, South Perth, Western Australia 6151, Australia; 3Animal Skin and Allergy Clinic, 70 Blackburn Rd, Mt Waverley, Victoria 3149 Australia

Abstract

Atopy is a well-recognized clinical entity in dogs, and is defined as an inherited tendency to develop IgE antibodies and clinical allergy to environmental allergens.3 The most common presenting sign is pruritus, which may be accompanied by dermatologic lesions. There are three management options available: allergen avoidance, symptomatic therapy and immunotherapy.5 The currently preferred therapy is specific allergen immunotherapy, based on the results of intradermal skin testing and the identification of clinically relevant allergens.4 Successful treatment of inhalant allergic dermatitis using an injectable hyposensitization schedule has been reported in the polar bear (Thalarctos maritimus).1

A 13 yr-old female sub-Antarctic fur seal (Arctocephalus tropicalis) has been housed at Melbourne Zoo since 1991. In August 1993 the seal presented with evidence of a dermatopathy. Clinical signs included matting of the hair coat with loss of the protective guard hairs over the dorsum. Initially it was felt that poor grooming behavior may have been the cause of the coat abnormalities, as keepers believed that the animal did not use the grooming claws on the hind flippers effectively. Between 1993 and 1996 it became clear that the clinical signs were markedly seasonal (typically, signs first appeared during late autumn and resolved during mid-summer) and that pruritus (seen as rolling while swimming, scratching and rubbing) was the predominant clinical sign.

Intradermal skin testing was carried out in September 1996, using a panel of 61 aeroallergens. A diagnosis of allergic dermatitis was made based on positive reactions to allergens prepared from weed, grass and tree pollens and some insects. Through 1996-1998 the seal (body wt approximately 32 kg) was given non-steroidal symptomatic treatment, consisting of oral antihistamines (including terfenadine (Teldane® Hoechst Marion Roussel Australia) at 15-60 mg s.i.d.-b.i.d., and cetirizine hydrochloride (Zyrtec® Faulding Pharmaceuticals) at 5-10 mg s.i.d.) and essential fatty acid supplements (562.5 mg eicosapentaenoic acid, 375 mg docosahexaenoic acid, 350 mg cis-linoleic acid, and 5 mg dl-α-tocopheryl acetate orally every other day as Omega 3® Biochemical Veterinary Research Pty Ltd).

The symptomatic treatment was only temporarily successful. Therefore, over a 6-mo period during 1998, keepers habituated the seal to receiving small volumes of sterile saline by s.c. injection, using a tuberculin syringe with a 30-ga needle. Specific allergen immunotherapy was begun in July 1998,
using a vaccine containing ten allergens. These were chosen with reference to the results of the intradermal skin testing and the likelihood of exposure to particular allergens in the surrounding environment. The treatment protocol used was similar to those used in canine immunotherapeutic regimens: immunotherapy was administered in increasing doses once weekly (beginning at 2 protein nitrogen units [PNU]/allergen) for 15 wk, until the maintenance dose (2000 PNU/allergen) was reached. This was then given every 3 wk as a maintenance therapy.²

Following the two initial doses of vaccine, the seal presented with transient non-painful, fluctuant subcutaneous swellings. On examination these appeared to be areas of localized subcutaneous edema. They developed 3-5 days after each vaccination, and were therefore assumed to be vaccine-associated. Subcutaneous edema has not been reported as an adverse effect of immunotherapy in other species (Mueller, personal communication). On several subsequent occasions during the loading phase the seal showed evidence of a mild adverse reaction to the vaccine, being extremely pruritic for 2-3 days following the injection. Vaccine dosage adjustments and treatment with oral prednisolone (0.5 mg/kg s.i.d.) for 24 hr prior to and following the vaccination appear to have prevented any further reaction.

At present the seal is receiving the maintenance dose of vaccine subcutaneously every 3 wk. Keepers give the injection during a routine feeding session. To date, clinical response indicates that the treatment has been successful, with a marked decrease in the level of pruritus and an improvement in the quality of the hair coat.

ACKNOWLEDGMENTS

The authors would like to thank Drs Sonya Bettenay and Linda Vogelnest (Animal Skin and Allergy Clinic, Mount Waverley, Victoria) for providing advice on all aspects of the treatment program, and seal keepers Kim Beasley and Karen Svalesen for habituating “Lucy” to s.c. injections.

LITERATURE CITED

CHRONIC MYELOGENOUS LEUKEMIA IN A RED PANDA (*Ailurus fulgens*)

Jonathan Sleeman, VetMB, MRCVS,1* Wendy S. Sprague, DVM,2 J. Todd Painter, DVM,2 and Terry Campbell, DVM, PhD1

1Department of Clinical Sciences and 2Pathology, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO 80523-1620 USA

Abstract

A 12-yr-old female red panda (*Ailurus fulgens*) from the Riverside Zoo, Nebraska presented to the Zoological Medicine Service, Colorado State University in October, 1997 with a 1-yr history of partial anorexia and a recent abdominal enlargement. Physical examination under anesthesia was unremarkable. However, a complete blood cell count revealed a marked leukocytosis with predominantly toxic appearing mature neutrophils, and a moderate left shift (total white blood cell count = 164.0 × 10^3/µl, with 124.6 × 10^3/µl segmented neutrophils, and 14.8 × 10^3/µl band neutrophils). A mild anemia (PCV = 29%) was also detected.

The animal’s condition remained unchanged despite a 2-wk course of 2.5 mg/kg enrofloxacin (Baytril, Bayer Animal Health, Shawnee Mission, KS 66201 USA), and 15 mg/kg amoxicillin (Amoxi-inject, SmithKline Beecham, Exton, PA 19341 USA) given intramuscularly twice daily. A physical examination under anesthesia was repeated 3 wk after initial presentation and revealed marked generalized icterus with hepatomegaly and splenomegaly. Abdominal ultrasound confirmed the splenic and hepatic enlargement and revealed a mild ascites. Fine needle aspirates of the liver were performed for cytology and anaerobic, aerobic and fungal culture. No growth was obtained from the cultures and the cytology indicated hepatocellular degeneration. Thoracic radiographs were unremarkable except for mild peribronchial opacity. At this time, the hemogram indicated a marked leukocytosis (257.0 × 10^3/µl) characterized by a neutrophilia with a shift towards immaturity (i.e., mature neutrophils = 67.4% [173.1 × 10^3/µl], band neutrophils = 4.1% [10.5 × 10^3/µl], and metamyelocytes = 2% [5.2 × 10^3/µl]). There was also another cell type present which comprised 19.4% (49.8 × 10^3/µl) of the total white blood cells. These cells were slightly larger than the neutrophils and had light basophilic cytoplasm with fine, uniform deep basophilic granules and a lobated nucleus with a clumped chromatin pattern. Upon comparison with blood from a healthy red panda, it was determined that these cells were basophils. The rest of the white blood cell count consisted of 5.1% small lymphocytes (13.2 × 10^3/µl) and 2% monocytes (5.2 × 10^3/µl). In addition, there was a marked thrombocytosis (1.276 × 10^9/µl) and a mild anemia with a nucleated red blood cell count of 5.2 × 10^9/µl. A bone marrow aspirate of the proximal left humerus was performed and revealed marked myeloid and megakaryocytic hyperplasia. There were increased numbers of blasts present with some particles containing up to 17% blasts. Many basophils were also present in the marrow. The chemistry panel revealed evidence of liver disease with hyperbilirubinemia (5.1 mg/dl), mildly increased ALP (118 IU/L), GGT (49 IU/L), ALT (176 IU/L), and AST (310 IU/L).
and hypoalbuminemia (1.8 g/dl). No growth was obtained from two consecutive blood cultures and routine fecal examination for endoparasites was negative.

The antibiotics were changed to 100 mg/kg ticarcillin/clavulanic acid (Timentin, SmithKline Beecham, Exton, PA 19341 USA) intramuscularly twice daily, and it was additionally treated with 250 ml lactated ringers solution subcutaneously once daily. Despite the therapy its condition deteriorated. Abdominal laparoscopy was performed and the liver was diffusely swollen and tan in color, with a mottled appearance. When biopsied the liver was extremely friable and did not bleed. Some hemorrhage of unknown source was encountered and the procedure was terminated, however, it died shortly thereafter.

A full necropsy revealed florid myeloproliferation within the liver, spleen, and lymph nodes and a severe secondary hepatic lipidosis. In addition, the bone marrow was markedly hypercellular and was dominated by immature myeloid cells. The clinical and pathologic findings were consistent with a diagnosis of chronic myelogenous leukemia (CML). Unusual findings in this case include the basophilia and thrombocytosis. In humans, both can occur in association with CML and are considered paraneoplastic syndromes.

CML has been well characterized in humans, and 90% are associated with a specific chromosome abnormality. CML is rare in domestic animals and can be difficult to differentiate from a leukemoid reaction caused by inflammation, immune-mediated disease, or as a paraneoplastic syndrome. In domestic cats, feline leukemia virus infection has been associated with some cases of myeloproliferative disease. Myeloproliferative disorders of the granulocytes and/or monocytes are also rare in nondomesticated species but have been reported in a Texas tortoise (Gopherus berlandieri), snakes, a marine toad (Bufo marinus), a Byrne’s marsupial mouse (Dasyuroides byrnei), an African hedgehog (Atelerix albiventris), rabbits and rodents, and primates including an orangutan (Pongo pygmaeus). Megakaryocytic leukemia is particularly rare with only one case report in a nondomesticated species. Only a few neoplastic conditions have been reported in red pandas. To the authors’ knowledge this is the first report of CML in a procyonid, although interestingly, one case of chronic lymphocytic leukemia has been reported in a 7-yr-old female red panda.

LITERATURE CITED

AORTIC ANEURYSM AND SUBSEQUENT CARDIOPULMONARY ARREST IN A BURMESE PYTHON (Python molurus bivittatus)

Elizabeth Marie Rush, DVM,1* Thomas M. Donnelly, BVSc, DACLAM,1and James Walberg, DVM, DACVP1

1The E. & M. Bobst Hospital of The Animal Medical Center, 510 E. 62nd St., New York, NY 10021 USA

Abstract

An approximately 27-kg, 5-m-long, 8-yr-old, female Burmese python (Python molurus bivittatus) was presented with acute onset of respiratory arrest after prey strangulation. The owner had observed normal approach, attack and constriction of a live rabbit (Oryctolagus cuniculus) recently obtained from a local pet store. The rabbit was smaller than other rabbits previously fed to the python. The snake began to swallow the carcass, but after drawing back half of the rabbit, it suddenly backed away from its prey, open-mouthed. The snake gasped, then collapsed in its cage. The owner brought the snake immediately to the Animal Medical Center, which took approximately 35 min. During the commute, the owner witnessed 3-4 exaggerated inspirations by the snake.

Upon arrival, a brief physical exam was conducted. The snake was comatose, pale and hydrated. Respiration was absent and heart activity was not detected on palpation, observation, auscultation or using a Doppler probe. The initial oral exam was unremarkable, eyes and nasal chambers were clean and the abdomen was benign. There was no history of egg laying or binding. The snake was in good external physical condition, with clear and smooth scales, and no obvious scars. There were no ventral petechiae or other evidence of sepsis.

The snake was intubated with a 3.5-mm endotracheal tube, attached to a medium size Ambu bag, and provided manual intermittent positive pressure ventilation (IPPV) with 100% oxygen. Estimating the requirements for full lung field inflation, approximately 15 breaths/min were delivered. Simultaneous manual cardiac compression was performed. Epinephrine (0.4 ml of a 1:1000 dilution) was administered by intracardiac injection, followed in 5 min by 1.0 ml (0.54 mg/ml) atropine sulfate intracardiac. Exploration of the upper airway and esophagus for injuries or possible foreign bodies was unremarkable. A 20-ga Teflon catheter was placed in the right palatine vein and secured. A blood sample was taken from the catheter, collected with a blood gas syringe (Marquest™, Inglewood, CO). Electrolytes, glucose, hematocrit and total solids were determined from the blood sample. Blood gas parameters and electrolytes were determined using a Ciba-Corning 850 Blood Gas Analyzer. Results are as follows (with normal values in parentheses).7 There was a metabolic acidosis: pH was 6.855 and actual bicarbonate was 6.9 mEq/L. The chloride level was 120 mEq/L (100-150), potassium was 3.71 mEq/L (2-8), sodium was 147.4 mEq/L (120-170) and ionized calcium was 5.6 mg/dl (2.5-8.0). Anion gap was 24.2 mEq/L (10-27). The blood glucose level was measured using a blood glucose monitor (Accu-Check III, Boehringer Mannheim, Indianapolis, IN) and was 123 mg/dl (60-100). The PCV was measured in a
microhematocrit tube and was 25% (20-40); total plasma solids (using a refractometer) were 6.1 g/dl. Intravenous Lactated Ringer’s solution (LRS) was started as a 500 ml bolus and 250 mg prednisolone sodium succinate (Solu-Delta-Cortef, Pharmacia & Upjohn, Kalamazoo, MI) was added to an additional 250 ml LRS as a slow drip for shock. Heating pads were placed lengthwise on the snake to increase metabolic activity by elevating core body temperature. The ambient temperature was approximately 75°F. Three minutes after administration of the i.v. LRS bolus, a heart rate of 12 beats/min (BPM) was detected. A dose of 0.5 ml (50 mg/ml) diphenhydramine hydrochloride was given intramuscularly considering that the cardiopulmonary arrest may have been an anaphylactic response to a topical substance on the rabbit. Manual ventilations were stopped intermittently, for 1-min periods, to allow for spontaneous breathing by the snake. After approximately 40 min the snake attempted to breathe. The total respiratory rate was 10 breaths/hr. The respiratory attempts appeared exaggerated and were accompanied by cranio-caudal peristaltic movements of the thoracic and abdominal muscles.

One and one half hours post resuscitation, the snake’s heart rate fell to 2-3 BPM, at which time a second dose of 1.0 ml (0.54 mg/ml) atropine sulfate was given intravenously. Heart rate again increased to 12 BPM. Petechiation became noticeable on the ventral scales. Manual IPPV was continued for an additional 30 min, replacing the 100% oxygen with room air. Two hours after beginning cardiopulmonary resuscitation the manual IPPV was stopped. The snake did not breathe spontaneously and after 5 min of apnea, 1.0 ml (20 mg/ml) doxapram hydrochloride (Dopram-V, Fort Dodge, Cherry Hill, NJ) was given intravenously. This was followed by two respiratory efforts. The ventral petechiations began to resolve at this time. The snake was given 150 mg (22.7 mg/ml) enrofloxacin (Baytril, Bayer, Shawnee Mission, KS) by i.m. injection prophylactically, then re-dosed with 1.0 ml (20 mg/ml) doxapram hydrochloride before being placed in a heated oxygen cage overnight. The palatine catheter was removed at this time. The snake was not observed to breathe overnight and was pronounced dead the following morning, 12 hr after placement in the oxygen cage.

Complete necropsy was performed at the Animal Medical Center. Gross findings were bruising of the heart muscle and skeletal muscle secondary to injection administration and increased fat deposits. Incidental findings were abdominal adipose tissue cysts and small ovarian follicles. There were no other significant gross findings. Microscopic evaluation of the tissues revealed a dissecting organizing aortic aneurysm with separation of the intima and media. This was located in one aortic arch proximal to the first aortic branching, and was approximately 2 cm in length. Other microscopic lesions included steatitis of abdominal fat deposits, and a focal ulcerative enteritis. No cholesterol deposits or areas of mineralization were seen. Necropsy of the constricted rabbit revealed no gross abnormalities.

Cause of death was assumed to be due to cardiopulmonary arrest and shock secondary to sudden rupture of the aortic aneurysm. It is not certain what caused the initial insult to the cardiovascular system.

Discussion
To the best of the authors’ knowledge, aortic aneurysm and subsequent shock and death have not been reported in snakes. It is likely that this aneurysm occurred at a pre-existing weakened focus in the aortic intima secondary to a hypertensive episode at the time of prey constriction and swallowing. While reptiles are considered to have relatively low resting blood pressures, hypertension must be considered. Although it is not clear why the aneurysm presented as a dissection and not as a complete rupture, hypovolemic shock likely occurred at this time resulting in clinical signs. Dissecting organizing aneurysm of the aorta allows blood loss into the defect formed between the intima and media of the aorta. The blood pooling in the defect causes increased vascular resistance by impinging on the normal lumen. This results in increased stasis of blood, leading to hypercoagulability and impedance of blood flow to systemic tissues. The combination of prolonged cardiopulmonary compromise leads to shock and volume depletion, all of which contributed to the snake’s death.

In mammals, cardiovascular dysfunction is due to one of the following:

1. Disruption of the continuity of the circulatory system permitting blood escape. This prevents cardiac filling and resistance that the normal heart pumps against.
2. Cardiac conduction disorders or arrhythmias.
3. Any lesion preventing valvular function or obstruction leading to pressure abnormalities and/or regurgitant flow of blood.
4. Generalized failure of the cardiac pump.

In general, poikilotherms have few primary cardiovascular disorders, these few including heart enlargement and congenital cardiovascular abnormalities. Heart failure in conjunction with liver abnormalities has been reported. Congenital abnormalities are most often associated with juvenile death. Most of the cardiovascular disorders reported in reptiles are secondary, and are associated with infection, parasitism, nutritional imbalances (calcification of vessels, obesity, cholesterol deposits) and poor husbandry practices. Arteriosclerosis has been documented in iguanas (Iguana iguana), although no evidence of this condition existed in the snake presented in this paper. Thrombogenesis is reported to be of lower incidence in reptiles than mammals, but could occur in the presence of endothelial damage or altered blood flow. One human study indicates that obesity may decrease the clearance of procoagulants and cause fatty necrosis. If plausible in reptiles, this could predispose an animal to vascular stasis, endothelial damage, hypercoagulability and intravascular coagulation, as it does in humans. Hypertension associated with increased activity (15 min) has been found to decrease the circulating volume approximately 21% in some snakes, therefore increasing heart rate and PCV. This could also contribute to hypercoagulability and turbulence of blood flow.

Conclusion
Although various cardiovascular disorders have been reported in snakes, there are many disorders that have not been observed. Thorough diagnostics and necropsies of diseased and dead snakes will certainly assist in the discovery and better understanding of these diseases.

Overall, for thorough cardiovascular evaluation and resuscitative efforts the following should be recognized.

1. Reptile respiratory stimulus is the decrease in oxygen levels,7 and manual ventilation efforts should include periods of pure oxygen with weaning off to room air. Care should be taken that animals are not overventilated, as this will not allow proper stimulus for the animal to attempt spontaneous respiration. The clinician should remember that some reptiles can survive periods of prolonged apnea when compared to mammals.12

2. Care should be taken with cardiovascular stimulating drugs, as reptiles may respond differently than mammals. One study found that reptiles respond to acetylcholine by vasoconstriction, where in mammals this causes vasodilatation.5 There is much work yet to be done to determine the effectiveness and activities of cardiac glycosides, vasodilators and sympatholytics in reptiles.

3. Proper use of ultrasound may aid in diagnosis of certain disorders.11

4. Doppler is usually effective for evaluation of cardiac rate and rhythm in reptiles.7

5. If the reptile is in shock, i.v. access is critical and can be obtained through catheterization of the palatine vein.

6. Fluid therapy is likely to be indicated, especially in shock, but care must be taken not to overload the cardiovascular system.

7. Electrolyte panels may or may not be assistive individually, but could be helpful if taken serially.

Improvement of husbandry practices for reptiles and longevity can be expected to contribute to increased incidence and recognition of cardiovascular disorders. Cardiovascular pathology should be considered as a differential diagnosis if appropriate, and the predisposing factors should be included in history and diagnosis of these patients. If pertinent, these issues (husbandry, congenital, parasitism, and infection) should be addressed and the patient monitored for response to therapy given.

LITERATURE CITED


SOFT TISSUE SARCOMAS ASSOCIATED WITH IDENTIFICATION MICROCHIP IMPLANTS IN TWO SMALL ZOO MAMMALS

Allan P. Pessier, DVM, 1* Ilse H. Stalis, DVM, 1 Meg Sutherland-Smith, DVM, 2 Lucy H. Spelman, DVM, 3 and Richard J. Montali, DVM 4

1Department of Pathology and 2Department of Veterinary Services, Zoological Society of San Diego, PO Box 120551, San Diego, CA 92112-0551 USA; 3Department of Animal Health and  4Department of Pathology, Smithsonian National Zoological Park, 3001 Connecticut Ave NW, Washington, DC 20008 USA

Abstract

Subcutaneously-implanted microchips (Trovan, Electronic Identification Devices, Santa Barbara, CA 93108 USA; AVID, Norco, CA 91760 USA) are used as a convenient method to permanently and individually identify a variety of domestic, laboratory and exotic animal species. 4,6 Microchip implants are considered to be safe with implants eliciting only a mild tissue reaction in prospective studies. 1,4,5 Recently, soft tissue sarcomas associated with microchip implants were described in 36 of 4,279 (0.8%) laboratory mice used in a lifetime carcinogenesis study. 8 This report describes the occurrence of soft tissue sarcomas associated with microchip implants in a degu (Octogon degus) and a feathertail gilder (Acrobates pygmaeus) housed at separate zoological institutions.

Case Reports

A 4-yr-old female degu from the National Zoological Park had an identification microchip implanted in January of 1997. In September of 1997, the animal was presented for evaluation of a “hump-backed” appearance and of an alopecic focus on the dorsal midline. On physical examination, a firm, irregularly-shaped, 2.5 cm in diameter mass was noted in the subcutis between the scapulae, just proximal to the alopecic focus and underlying the palpable microchip. A fine-needle aspirate from the mass demonstrated cells suggestive of a mesenchymal neoplasm and the mass with the peripherally-attached microchip was surgically excised. Histologic examination of the mass showed an invasive neoplasm composed of interlacing streams and bundles of pleomorphic spindle cells within a moderate collagenous stroma. The histologic diagnosis was fibrosarcoma. Two months postoperatively, the degu died due to sepsis caused by Pseudomonas aeruginosa. At necropsy, there was no gross or histologic evidence of the previously-excised neoplasm.

The second case occurred in a 6-yr-old female feathertail glider from the San Diego Zoo. An identification microchip was implanted in September of 1993. In July of 1998, the glider was presented for evaluation of a scabbed wound over the right dorsal midline. Physical examination revealed an underlying subcutaneous mass which was surgically excised. The mass measured 1.7 × 0.6 × 0.6 cm and surrounded the previously implanted microchip. Histologic examination showed osseous metaplasia and necrosis surrounding the microchip, which, in turn, was surrounded by a spindle cell neoplasm in which neoplastic cells appeared to produce a fibrillar to homogenous...
eosinophilic material interpreted as osteoid. The histologic diagnosis was of extraskeletal osteosarcoma. The glider progressively declined in condition after surgery and was euthanatized 5 days postoperatively. There was no evidence of distant metastasis at necropsy.

Discussion

Soft tissue sarcomas have been associated with foreign bodies composed of a variety of materials including metals, glass and plastics. Foreign body sarcomas are likely best known to clinicians as rare complications of surgical implants. Recently, a liposarcoma occurring on the forelimb of a dog was associated with a traumatically-acquired glass foreign body. Experimental studies in rats have shown that important factors associated with development of foreign body sarcomas include size and texture of the implanted material. Larger implants and those with a smooth surface (perhaps like implanted microchips) are considered to be more tumorigenic. In general, foreign body sarcomas are thought to arise by malignant transformation of mesenchymal cells responding to the presence of the foreign body rather than by a direct effect of the foreign body on surrounding tissues.

Subcutaneously implanted identification microchips are frequently used in small mammals at both zoological institutions involved in this report and with the exception of the described neoplasms, complications have only rarely been observed. The direct association between the described soft tissue sarcomas and the implanted microchips is compelling and the possibility that identification microchips may rarely contribute to the development of soft tissue sarcomas in small mammals should be considered.

LITERATURE CITED

ETHICAL CONSIDERATIONS CONCERNING CAPTIVE POPULATIONS

Daniel M. Farrell, PhD

Department of Philosophy, The Ohio State University, Columbus, OH 43210 USA

Abstract

The two principal considerations to be surveyed are the question of the moral justification of maintaining captive populations and the question of the moral requirements for their care in captivity. Arguments for and against the right to maintain such populations will be briefly explored, after which various views about our obligations to captive populations will be discussed. Issues raised by the recent appointment of Peter Singer, well-known animal rights advocate, to the Princeton University faculty will also be explored, along with the recent birth of a new legal speciality called "animal law."
SURPLUS ANIMALS: STEWARDSHIP ON THE ARK

Albert H. Lewandowski, DVM

Cleveland Metroparks Zoo, 3900 Wildlife Way, Cleveland, OH 44109 USA

Abstract

This paper provides a frank, philosophic discussion of what constitutes a surplus animal in a zoological garden and the options that zoological gardens face in dealing with this surplus.

Introduction

For decades, zoological parks and gardens around the world have aspired to attain consistent, reproducible results in breeding and raising the diverse species in their collections. One hallmark of a "good" zoo has always been a good breeding record. Success brings public acclaim, professional accolades, and an unspoken authority and status on the myriad committees of AZA that manage threatened and endangered species.

With the emphasis on providing optimum conditions for reproduction and scientific advances in medicine, nutrition, and animal psychology, an increased number of institutions are becoming successful in reproducing the species in their care. The regularity with which some species are now reproducing leads us to the brink of another problem: too many animals. With limited space in our institutions, limited funds in times of economic crisis, and spiraling costs, maintaining animals not essential to the immediate purpose of maintaining an adequate gene pool borders on irresponsible stewardship of already limited resources.

Discussion

Surplus animals are surplus for myriad reasons. An unpaired male or female may be temporarily surplus and need only be placed in an appropriate breeding situation. The most responsible animal managers are relentless in obtaining suitable matches for their charges, keeping unpaired time down to a bare minimum. Animals whose lifespan may only be 12-15 yr cannot be permitted to languish unmated for 2-3 yr in a holding cage while we procrastinate about their fate.

Historically, some unpaired animals have been important symbols in the zoological community and served an indispensable function for public awareness. The gorilla Massa of the Philadelphia Zoo and Smokey the Bear of the National Zoo have been symbols of their institutions, recognized worldwide, superstars of the animal kingdom. Their role as ambassadors of the animal kingdom superseded other functions they might have had within the gene pool in their time. They provided a focus for media attention, attracting the general public and providing needed revenues.
Hopefully, more enlightened marketing will direct education about programs and populations, with less emphasis on the individual animal, which have been a double edged sword. One recent success story was the transfer of the gorilla, Timmy, from Cleveland to the Bronx. Animal rights activists tried to block the nonreproductive, but popular animal’s move. A federal judge allowed the transfer and Timmy has been a sire many times over. Because of the focus on an individual, the more responsible management might never have occurred.

Excess male offspring pose a surplus problem. With a 50/50 chance of producing either sex, when only one or two males are adequate to cover needs, a most uncomfortable situation is created. Bachelor groups are not particularly popular with zoological park managers or the public. Every zoo director wants baby gorillas.

Recently, the Taronga Zoo broached the subject of aborting a male gorilla fetus. In a bold and revealing statement, the zoo’s general manager, Will Meikle, stated that the zoo industry would have to decide what to do with surplus males in the global population. He noted that we are able to absorb the surplus today, but that it “will be a management problem for someone else in 10 yr.” Unfortunately, some managers are more concerned with preserving the status quo than with the long term effects on animal populations. Ten years will pass quickly.

Non-reproductive animals, whether due to advanced age or infirmary, are frequently in the ranks of surplus. Notable exception to the class of "non-reproductive" animals are those that serve as an integral part of a herd or group, such as the gorilla aunts.

The Species Survival Plans (SSP), in attempting to manage the gene pool for threatened and endangered species frequently single out animals that are over represented and recommend that they not be bred or rebred. They become surplus to the needs of the species.

Hybrid animals pose another class of surplus. In an attempt to maintain pure genetic lines, animals of mixed subspecific heritage have been relegated to nonbreeding desirability and contribute nothing to the gene pool for their species among reputable institutions. Notable examples include Sumatran × Bornean orangutans, tigers of mixed lineage or undocumentable pedigree, and dik-dik rendered sterile by cross subspeciation.

Genetic freaks, bred for their unusual color or pelage pattern, represent an interesting, but evolutionary dead end that contributes nothing to the mainstream gene pool of the species. White tigers, albino wallabies, and black panthers have all generated much interest and filled our coffers with gold, but have contributed little to scientific conservation, unless the arousal of public awareness is touted. If we educated the public properly, these mutants would have no place in an institution managing for the conservation of a species.

Imperfect animals, aged animals, animals with medical conditions that we cannot cure, but because of our advances in medicine are capable of maintaining indefinitely, and animals that medicine can
improve (maybe) their condition at high cost and untold man-hours represent still another class of surplus. Do we maintain these animals for their good, or for our own impotent reasons?²

As responsible animal managers and as institutions dedicated to the advancement of science and the conservation of nature, what options are open for placing these animals?

Within our own institutions these surplus animals are frequently maintained within the primary exhibit or warehoused in an off exhibit area. Housed in the main group, multiple problems are often the result. The surplus animal is frequently low in the social structure and is subject to injury, or worse, injures a valuable breeding animal. Animals that would in the wild be driven from the group are confined in a limited area, interfering with the optimal social structure, interfering with optimal reproductive success, and decreasing the survivability of neonates that may be genetically more valuable to the species. Each exhibit has a limited carrying capacity due to the territorial requirements of each species.

If the surplus animal is housed in an off exhibit area, the quarters are usually less desirable and less suitable than the main exhibit area. Temporarily keeping an animal in less than ideal areas until permanent placement can be made can be justifiable. A moral/ethical question arises when the “temporary” placement drags on for years. The quality of life for that animal now comes into question. Is this moral? Is this truly ethical?

In more callous terms, can my institution or any responsible institution reasonably afford the personnel, the time, the space, or the cost to maintain long-term a surplus animal, an animal that will be warehoused in suboptimal areas for the remainder of its life?

Where can a responsible institution place these animals? To not take up precious funds, time, space, tie up personnel, divert curators, keepers, and veterinarians from animals/species that require intensive management to maintain, to make optimum use of the resources at hand and contribute the maximum to serious conservation effort, these animals must be placed in a suitable situation.

Conservatively, a large carnivore like a tiger will in 1999 U.S. dollars cost a minimum of $25,000 to maintain for its lifetime. Primates generally are more expensive to maintain. Hoofed stock are less expensive to maintain. Extraneous animals, even one extraneous animal, rapidly become a burden on the system. Compounded over years and multiple animals, what institution would not rather focus its attention on progressive, fruitful programs over maintaining the status quo?

What other options are available?

Optimally, another party desires the surplus animal. The first institution of choice would be another zoological park. Good, professional care by an institution involved in a cooperative breeding program is most desirable. Knowing that an animal that we have invested time and effort into is going someplace that friends and colleagues will be caring for it always gives peace of mind.
When a direct zoo to zoo transaction is not possible, another option is the registered animal supplier. With their ear to the ground, licensed broker/dealers are often able to put together transactions with overseas contacts, zoos out of our usual sphere, or other institutions that may not have ready access to us. Often zoos go to considerable effort to limit second party transactions in an attempt to keep unqualified persons from obtaining rare or dangerous animals.

A third alternative encompasses transactions with private breeders. Many fine institutions with high professional standards and impeccable, impressive records breed and raise unusual species, often more out of dedication and interest rather than profit. The use of profile sheets by zoos to document the experience and care afforded by these private breeders also helps to insure proper care and the ability to track genetic lines if required.

Private individuals constitute another pool of possible placement for some species. While I would not advocate the placement of lions or leopards with a private individual to maintain as a pet, judicious placement of neonates, injured animals, and long term care cases might be suitable. Small, private menageries stocked with creatures of questionable parentage, cast-off pets, and zoological oddities contribute nothing to the efforts of conservation. These situations only hamper serious efforts by reputable institutions.

Public animal auctions? No responsible zoological park can justify disposing of surplus stock to the highest bidder. The AZA Code of Ethics condemns such practices. AZA members pledge to “make every effort to assure that exotic animals do not find their way into the hands of those not qualified to care for them.”

An often-maligned outlet for surplus animals is legitimate medical research. An option more suited to primates than deer and politically incorrect in many circles, animals provided to medical research provide an invaluable service to mankind. Our colleagues in Lab Animal Medicine provide no less concern and care for their charges than those of us working at zoological parks. Allowing surplus animals to go to medical research is a public relations nightmare, but perhaps the zoo community needs to voice an opinion in support of sound medical research that has made the lives of man and animal alike more tolerable.

The last option for a surplus animal not suited to medical research, too dangerous to situate with a private individual, too old for a dealer to place, too common for placement with any zoo, tying up valuable exhibit and off exhibit space, costing thousands of dollars to maintain, not a high profile animal, not having a life threatening medical condition, but not “perfect”... is euthanasia.

Here is an animal that is to be warehoused until it dies of old age, an animal that for whatever reason has outlived a useful, productive life, an animal that deserves better than to be relegated to a less than ideal holding area for the remainder of its days. Morally, ethically, the quality of life for this animal becomes nonexistent.
As animal managers, we have the responsibility and obligation to make the effort to advertise that this animal is available. We must make a sincere effort to place this animal. Sometimes we may have this animal for several years before it becomes obvious even to the most optimistic of us that there will be no takers. We must then get on with our lives, assume the role of a responsible steward, make a difficult choice, and manage for the future.

We do not advocate rushing out and eliminating any surplus animal in our collections today. Much soul searching needs to be done and as the need for space becomes more important, perhaps the surplus animals in our care need to make way for more endangered animals, for important breeding stock, and for SSP animals... not on a whim, but as part of a carefully thought out, long-term part of responsibly managing our animal collections, of being a responsible steward.

Conclusion

“...I have moral responsibilities… to the animals under my care...(and need to) make every effort to assure that all animals do not find their way into the hands of those not qualified to care for them properly...(to) display the highest integrity, the best judgement or ethics possible, and use my professional skills to the best interests of all.”

I need to be a responsible steward. The animals in my care, the animals that I manage, belong not to the past, nor to the present, but to the future.

LITERATURE CITED

EUTHANASIA AND HUMAN EMOTIONS IN THE ZOO

Sally O. Walshaw, MA, VMD

University Laboratory Animal Resources, College of Veterinary Medicine, Michigan State, University, East Lansing, MI 48824-1513 USA

Abstract

People form attachments to animals in a variety of situations. The zoo is no exception. Employees, docents, and members of the public may have a special interest in certain animals, within the zoo. The death of a zoo animal may initiate a wide range of emotions in people. Zoo employees must balance feelings of sadness surrounding the death of animals with work, responsibilities that may include euthanasia.

The goal of euthanasia is to provide a peaceful death for an animal. Ideally, veterinarians and other zoo employees work as a team to plan a scheduled euthanasia. This provides the opportunity to explain the procedure, to delegate specific responsibilities, and to acknowledge the kindness given the animal by the animal care staff members.

Many people today have little experience with human death and with the process of grief and mourning. Even those who have experienced significant losses may not have had time to mourn. Unfortunately, grief and loss can be cumulative and complicated. Consequently, issues surrounding grief and human interactions are a potential source of workplace stress. This, presentation will focus on strategies for handling grief-related situations in the zoo.

All of us who work with animals must maintain a reverence for life, both human and animal, and an acceptance of death as a part of life. Acknowledging loss and grief in the zoo is one aspect of this compassionate relationship.

LITERATURE CITED

THE CARE AND FEEDING OF YOUR DIRECTOR

William R. Foster, DVM

Louisville Zoological Garden, P. O. Box 37250, Louisville, KY 40233 USA

Abstract

A twelve-step program has been developed to define the veterinarian’s current positional relationship to the director of his/her institution. The purpose of the program is to provide a guided tour and insights into the magical mystery of the “Ivory Tower” and its environmental impact on the veterinary medical care of the facility. The program focuses on the practical application of the fine points of the husbandry and nutritional requirements of a director and, thus, the implied growth of a successful zoo veterinary program.

Virtual Authority
Effective Communication
Third Party Perspective
Education and Skill Development
Responsibility, Focus, Perspective and Style
Institutional Mission Alignment and Goal-Setting
No Surprises
Assessment of Self and Marketing Your Strengths
Resolution of Conflict
Implementation of Change
Asset or Liability and Measure of Success
Nirvana (any place or condition of great peace or bliss)

Exit Strategy:

Where Do Old Zoo Vets Go?
CHEMICAL IMMOBILIZATION OF EXOTIC SWINE AT THE SAN DIEGO ZOO

Patrick J. Morris, DVM, Dipl ACZM,* Beth Bicknese, DVM, Donald L Janssen, DVM, Dipl ACZM, Meg Sutherland-Smith, DVM, and Lee Young, DVM

Department of Veterinary Services, San Diego Zoo, PO Box 120 551, San Diego, CA 92112-0551 USA

Abstract

In recent years the Zoological Society of San Diego has made a significant effort to establish captive breeding populations of six species of exotic swine including the babirusa (Babyrousa babyrussa celebensis), Bornean bearded pig (Sus barbatus barbatus), Chacoan peccary or tagua (Catagonus wagneri), European wild boar (Sus scrofa scrofa), red river hog (Potamochoerus porcus) and southern warthog (Phacochoerus africanus sundevallii). In addition, the petting zoo maintains two adult castrated Vietnamese pot-bellied pigs (Sus scrofa F. domestica). Routine husbandry and medical management of these species requires consistently reliable chemical immobilization protocols.

In 1992, after encountering problems associated with less than desirable recovery from anesthesia using cyclohexylamines in red river hogs immobilized with tiletamine and zolazepam (Telazol), one of the authors (Morris) began surveying the literature for sedative/anesthetic alternatives to cyclohexylamines in swine species. The main goal of this search was to find a set of drugs that, when used as a combination, would produce complete and reversible immobility with manageable side effects. A literature search for information on anesthetic combinations in swine was conducted. Among the many citations retrieved, combinations of ketamine, xylazine and climazolam;2 midazolam alone;6 and butorphanol, xylazine and ketamine4 were attractive prospects. Another paper reporting on the effects of butorphanol combined with medetomidine in swine5 was of particular interest.

Despite its attractiveness as a possible immobilization agent in exotic swine, medetomidine was not widely available, so combinations of butorphanol with xylazine and detomidine were evaluated in the two pot-bellied pigs at the petting zoo. Doses of 0.3 mg/kg butorphanol combined with 2 mg/kg xylazine, and butorphanol at the same dose combined with 100 µg/kg produced profound sedation but were easily aroused. However, when midazolam 0.3 mg/kg was added to these two combinations complete immobility with relaxation and analgesia was observed in the pot bellied pigs (P.J. Morris, unpublished data). Using this combination one of the authors (Morris) castrated the two petting zoo pigs using nothing more than local lidocaine infiltration into the spermatic cord of the patients during surgical preparation. At the end of surgery a combination of yohimbine (0.3 mg/kg) and naltrexone (50-mg total dose) was given, which resulted in rapid and smooth reversal of immobility. Subsequently, several combinations of xylazine, midazolam and butorphanol or detomidine, midazolam and butorphanol were used in babirusa, European wild boar, red river hogs warthogs, and to evaluate the efficacy of these protocols in other exotic swine species. Though
significant effects were observed in warthogs and European wild boar using detomidine at 100-125 mg/kg with butorphanol at 300 μg/kg and midazolam at 300 mg/kg, these two species were consistently easy to arouse during sedation, forcing the use of adjuncts such as low doses of Telazol and ketamine. However, in red river hogs, the use of this combination consistently resulted in complete immobility with smooth, rapid and complete recovery upon injection of yohimbine at 300 μg/kg and naltrexone at 25-50 mg total dose as a mixture given intravenously.3

With the increased availability of medetomidine in recent years, the authors began testing the combination of medetomidine, butorphanol and midazolam in these seven swine species with significantly improved results compared to similar combinations including xylazine or detomidine. At this point the combination of midazolam, butorphanol and medetomidine (MBM) has provided consistent and optimal results in all the suid species within our collection, and is now the preferred anesthetic option for general chemical immobilization and/or for induction of inhalant anesthesia of all swine at the San Diego Zoo.

Though the results of MBM sedation in swine species studied are highly desirable and consistently reproducible, the volume of injection with this combination is excessive. Frequently two darts must be employed to deliver the entire dose to larger animals. In addition, this combination is more expensive to use than other alternatives such as combinations of Telazol and xylazine.1 Still, one of the most expensive drugs employed routinely in the antagonism of the zolazepam fraction of Telazol (flumazenil) is one of the most expensive drugs in these protocols. As a result, the apparent cost savings associated with the use of Telazol are significantly hampered when flumazenil is used routinely as an antagonist in any protocol.

Sedation in swine immobilized with MBM results in fair to good analgesic effects and excellent relaxation including significant jaw tone relaxation, a quality not observed in our situation using other protocols. Hypoxemia, hypotension, bradypnea and bradycardia are the predictable side effects resulting from the use of MBM in swine (as well as in other species at the San Diego Zoo not reported here). Changes in cardiopulmonary physiology have been reported in swine associated with the use of medetomidine and butorphanol,4,5 as well as with the use of midazolam.6 Minimum patient monitoring recommendations to be used in association with MBM in swine include pulse oximetry with intermittent temperature, pulse and respiration observations. In addition, supplemental oxygen via nasopharyngeal insufflation with a soft rubber cannula at 4-6 L/min effectively combats hypoxemia as measured by observing trends in pulse oximetry. At the time of preparation of this abstract, only one mortality has been associated with the use of this protocol. In this case the patient, an aged babirusa, we observed to have cardiac disease on post mortem examination. This combination has been used repeatedly and successfully in animals less than 1 yr to over 10 yr of age in the swine species listed.

Antagonism of MBM sedation has been achieved mainly through i.m. injection of a mixture of naltrexone at approximately 350-700 mg/kg and atipamezole at 100-350 μg/kg as a single i.m. injection. Time from injection to standing posture vary, but in most cases animals are standing within 10 min post antagonist injection (PAI). In all cases signs of antagonist effects start with
increased respiratory rate and depth. In most cases animals will rapidly regain sternal and/or standing posture within 3-7 min after this initial increase in respiratory rate. However, in some cases, animals remain recumbent despite noticeable increases in respiratory rate and depth. In these cases, flumazenil at 1:10 - 1:20 flumazenil:midazolam have resulted in complete recovery within 5 min of flumazenil injection i.m. in all cases studied to date. The use of flumazenil in every case is warranted for optimal antagonism of effects, but as mentioned previously the cost of this drug may be prohibitive. As a result, we use flumazenil only in refractory cases where naltrexone and atipamezole fail to yield the desired antagonist effects. Finally, through sequential reductions in the dose of atipamezole we now recommend dose ranges of 80-100 mg/kg routinely for reversal of medetomidine effects in swine species. In most cases swine will remain quiet with variable appetite for 24 hr after the use of this combination. In one group of four warthogs immobilized with MBM all four individuals were unusually quiet and inappetent for 48 hr after antagonism. Flumazenil had to be employed to completely antagonize MBM in these individuals. This suggests a lingering effect of midazolam may have played a role in the effects observed in these cases. However, by 72 hr PAI all four animals were normal, and no further problems were observed in this group.

In conclusion, through the clinical evaluation of several combinations (Table 1), MBM has emerged as the preferred combination for chemical immobilization and general anesthetic induction in suid species at the San Diego Zoo.

ACKNOWLEDGMENTS

The authors would like to thank the staff of the Veterinary Services Department of the San Diego Zoo as well as the Mammal Department for years of hard work that have contributed to the development of these protocols. In addition, the authors would like to thank the many veterinarians who have used these combinations over the years for sharing their observations of the use of these combinations in suid species.

LITERATURE CITED

Table 1. Recommendations for various drug combinations for chemical immobilization of swine. All six protocols have been used with favorable results in clinical cases at the San Diego Zoo. Doses for narcotic antagonists are empirical and based on clinical response only. It is recommended that all antagonists be given intramuscularly only to prevent hyper excitability during recovery. Darkened spaces represent untested combinations for which no recommendations are offered. In all cases if flumazenil is required we recommend a dose range of 1:10-1:20 flumazenil:midazolam and only to reverse effects of midazolam when incorporated into the protocol. In cases where a protocol may have more than one recommendation for an antagonist pair, only use ONE PAIR to antagonize the sedative effects for that protocol. Det=Detomidine, Med=Medetomidine, Xyl=Xylazine, Mid=Midazolam, Tel=Telazol, But=Butorphanol, Ati=Atipamezole, Yoh=Yohimbine, Nal=Naltrexone, Nar=Narcan. All doses are in micrograms per kilogram (μg/kg). *The preferred protocol is in bold (protocol # 3).

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Agonists</th>
<th>Other</th>
<th>Antagonist combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α-2 agonists</td>
<td>Other</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Det</td>
<td>Med</td>
<td>Xyl</td>
</tr>
<tr>
<td>1</td>
<td>100-150</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>2</td>
<td>100-150</td>
<td>500-750</td>
<td>300</td>
</tr>
<tr>
<td>3*</td>
<td>70-100</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>4</td>
<td>70-100</td>
<td>500-750</td>
<td>300</td>
</tr>
<tr>
<td>5</td>
<td>2000-3000</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>6</td>
<td>2000-3000</td>
<td>500-750</td>
<td>300</td>
</tr>
</tbody>
</table>
EVALUATION OF AN ADVANCED HANDLING SYSTEM FOR PHYSIOLOGIC DATA COLLECTION, TESTING AND MEDICAL TREATMENT OF LARGE, NONDOMESTIC HOOFSTOCK

Mark W. Atkinson BVSc,1* Thomas H. Welsh, Jr. PhD,2 and Evan S. Blumer, VMD1

1The Wilds, 14000 International Road, Cumberland, OH 43732 USA; 2Departments of Animal Science and Veterinary Anatomy, Texas A&M University System, College Station, TX 77843 USA

Abstract

Introduction

Regular manipulation of large, nondomestic hoofstock species is a necessary component of effective preventative health programs at zoological facilities and is often restricted by the zoo’s limited handling capabilities. In the majority of cases, large ruminants must be chemically immobilized for basic physical examination, research procedures, disease testing and certain types of medical therapy. As an alternative to chemical immobilization, appropriate handling facilities permit the physical restraint of hoofstock species in a manner that is safe for both animal and handler.1,2 The Wilds (Cumberland, Ohio, USA) has developed an effective handling system which has been designed to: reduce adverse visual stimuli, ensure the smooth advancement of animals through the system without the need for human contact and takes advantage of the animals ‘natural’ behavior to accomplish our specific goals. The system incorporates a series of corridors of varying width, strategically placed transfer gates and doors which may be remotely operated and a modified version of a commercially available hydraulic restraint device (The Tamer™, Fauna Products Inc., Red Hook, NY). This system has been regularly used for physiologic sample collection, testing and medical treatment of such species as scimitar horned oryx (Oryx dammah), sable (Hippotragus niger), eland (Taurotragus oryx), Jackson’s hartebeest (Alcelaphus buselaphus jacksoni), Pere David’s deer (Cervus elepharus), Bactrian deer (Cervus elephus bactrianus) and Urial sheep (Ovis vignei). In addition to being used in preventative health programs, this handling system has also proven valuable in several reproductive studies carried out at this facility.

Physical and psychologic stress associated with handling can result in physical injury, capture myopathy and on occasion, may even result in the animal’s death. The handling system developed at the Wilds has been designed to move animals in a step-wise but smooth fashion, from a holding pen, into a transfer corridor and finally into the Tamer™. A series of sliding gates allow animals to be moved through the system without direct human manipulation and also allows the easy separation of individuals within the transfer corridors. The walls of the chute are designed to minimize disturbing visual stimuli to the animals and are designed to prevent animals attempting to jump or climb walls and gates and are spaced to prevent turning while in the transfer corridor. When animals enter the final corridor section, movement into the restraint device is effected by means of a sliding wall pushed up behind the animal. After the subject has been restrained, the
system allows for the animal to either return to the animal holding pens or to be moved into a padded induction/recovery room connected to a medical treatment suite. When in the restraint device, adjustable hydraulic pressure allows the animal to be squeezed laterally between padded walls and then lifted so that its feet cannot gain purchase on the ground, similar to the action of a drop floor restraint system. As this stage, movement of the animal is limited to dorso-ventral flexion of the back and neck. Movement is further reduced by restraining the head. The Tamer™ itself allows access to the head and neck, the muscles of the thoracic and pelvic limbs and provides access to the perineal area for rectal or vaginal examination. In an attempt to reduce the adverse effects of any restraint episode, animals are fed preferred feed (alfalfa) following return to their holding stalls whenever possible.

**Methods**

In order to evaluate the stress associated with the use of this handling system, blood samples were collected to determine the plasma concentration of cortisol during a series of reproduction research studies in eland antelope. Initially, 10 adult, female eland underwent estrus synchronization and superovulation in preparation for non-surgical embryo collection. Exogenous hormone administration and sample collection occurred nine times during the 17-day trial. In addition to per rectum fecal collection carried out at these times, jugular venipuncture was performed on each animal and serum and plasma were collected and subsequently evaluated for progesterone and cortisol, respectively. Plasma and serum was stored at –20°C until the content of cortisol and progesterone could be determined by radioimmunoassay.3,4,7 For specific techniques, see Note 1.

Despite the fact that corticoid levels on their own may not accurately measure stress,5 corticoids are still considered reliable indicators of stress. In this study, the difficulty of interpreting single cortisol values was obviated by the collection of multiple samples and the evaluation of trends. For the collection of further research data and to rule out the possibility that any significant cortisol reduction was a result of adrenal exhaustion, we initiated a follow-up project which involved the regular collection of blood and fecal samples from a subset of the group of eland (n = 5), handled in the same way, for approximately 8 mo, to evaluate endocrine profiles and estrous cyclicity.

Stress associated with multiple physical restraint sessions was evaluated in the following ways:
1. Subjective evaluation: During the initial 17-day trial period, all animals appeared to tolerate regular manipulation, medical treatment and blood and fecal collection with no untoward effects. It became apparent that the more experienced and skilled the operators, the easier and quicker the operation proceeded. As animals became familiar with the procedures of the trial, speed and efficiency of restraint, sample collection and drug administration improved significantly.
Following a restraint episode, animals were observed for a short period to ensure normal behavior and appetite. It was apparent that animals rapidly became accustomed to the physical requirements of the trial.

2. Stress evaluation: During the initial trial period, each animal was investigated by assessing physiologic stress response through the evaluation of plasma cortisol trends. Of the 10 animals evaluated, 7 (70%) showed a decreasing trend in plasma cortisol values over the 17-day study period, 2 animals (20%) showed no significant change with 1 animal (10%) showing a slight increase. First sample cortisol values varied from 1.3831 ng/ml to 8.4810 ng/ml (x = 3.6337 ng/ml) whereas cortisol values from final samples varied from 1.4039 ng/ml to 6.8447 ng/ml (x = 2.6877 ng/ml). Morton, et al. investigated capture associated plasma cortisol levels in Zimbabwe and found a mean of 3.52 ng/ml for 20 wild-caught, physically restrained eland. In this investigation, the trend of decreasing plasma concentration of cortisol in 70% of the animals is an indication that animals became familiar with the requirements of the program and were not unduly stressed by regular physical manipulation.

3. Endocrine profile evaluation: Following the 17-day trial period, a subset (n = 5) of the group of eland being investigated was selected to undergo further evaluation over a longer time period. Five animals were manipulated through the handling system three times per week for a period of 30 wk. At each restraint episode, blood and fecal samples were collected and stored for later evaluation. After 20 wk, a mature male eland was placed with the group, effect on female endocrine profiles was recorded and gestation periods were determined following birth of offspring. Despite being intensively manipulated through the Wilds’ handling system for a period of more than 7 mo, all five study eland showed evidence of regular estrous cyclicity with a mean cycle length of 23.46 days and all five animals conceived and became pregnant with a mean gestation length of 272 days. It is well recognized that ‘management’ stressors can affect both cycle length and time of ovulation in domestic hoofstock. These results indicate that the management stressors associated with handling animals in the manner described above, appeared not to be significant. Plasma cortisol values associated with restraint during this follow-up trial are currently being determined and were not available at the time of writing.

Conclusion

We recommend the use of an appropriate and safe handling system that incorporates a means of physical restraint as described above, as a suitable method for the regular manipulation of large nondomestic hoofstock species such as eland. When carried out in an efficient and professional manner, regular manipulations are not unduly stressful nor detrimental to the health of the animals, as evidenced by decreasing trends in plasma cortisol values, the presence of regular estrous cycles and the ability to conceive and produce healthy offspring.

LITERATURE CITED

Note 1:
Titrated hydrocortisone and progesterone were purchased from New England Nuclear, Inc. (Boston, MA, USA). The cross-reactivity of the cortisol antisera (Pantex, Inc., Santa Monica, CA, USA) with corticosterone, deoxycorticosterone, progesterone, androstenedione and estradiol was 60%, 48%, 0.01%, 0.01% and 0.01%, respectively. The sensitivity of the cortisol RIA was 31 pg/tube. The intra- and inter-assay coefficients of variation were 4.7% and 8%, respectively. The cross-reactivity of the progesterone antisera (GDN 337; obtained from Dr. G.D. Niswender, Colorado State University, Ft. Collins, CO, USA) was 1.1%, 0.1%, 0.3%, 0.3%, 0.2%, 0.2% with pregnenolone, cortisol, estrone, estradiol, testosterone, and androstenedione. The intra- and inter-assay coefficients of variation were 7.3% and 8.9%, respectively. The sensitivity of the progesterone RIA was 31 pg/tube. Radioimmunoassay data were processed by the AssayZap program (Biosoft, Ferguson, MO).
CLINICAL PRESENTATION AND ANTIVIRAL THERAPY FOR POXVIRUS INFECTION IN PUDU (Pudu puda)

Randall E. Junge, MS, DVM, Dipl ACZM,1 Mary C. Duncan, BVMS, PhD,1 R. Eric Miller, DVM, Dipl ACZM,1 Douglas Gregg, DVM, PhD,2 and Mark Kombert, DVM1

1 St. Louis Zoological Park, 1 Government Drive, St. Louis, MO 63110 USA; 2 Foreign Animal Disease Diagnostic Laboratory, United States Department of Agriculture, Greenport, NY 11944 USA

Abstract

A severe poxvirus infection occurred in three pudu (Pudu puda), resulting in two fatalities. Cutaneous ulcers with mucopurulent exudate were present on periorbital skin, nose, lip margins, coronary bands, and teats, and mucosal ulcers were present in the oral cavity, esophagus, and forestomach. In the two fatalities, a secondary systemic fungal infection also occurred. Affected animals were leukopenic, hypocalcemic, hyperphosphatemic, and had elevated serum alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase levels. One case was treated with cidofovir, 5 mg/kg i.v. every 7 days for four treatments. Complete recovery occurred in the treated animal in 4 wk. This is the second report of poxvirus infection in pudu, and the first report describing clinical presentation, presence of secondary fungal infection, and successful treatment.

Introduction

Poxvirus infections in ungulates have been described in a variety of domestic and nondomestic ungulates. A novel poxvirus infection has been described in pudu (Pudu puda) in captivity.9,10 This report details the second outbreak of this virus in a zoological collection, complicated with a significant systemic fungal infection. The report describes clinical presentation and antiviral therapy with cidofovir.

Case Reports

The affected pudu group consisted of an adult pair and a male calf. At 1 mo of age, the calf was examined for a suspected abrasion with purulent exudate on the left side of the muzzle near the preorbital scent gland. Treatment consisted of topical antibiotic cream and systemic antibiotics. Results of complete blood count (CBC) and serum biochemical profile were within normal limits for the species.6 Examination 1 wk later revealed mild hyperthermia (39.6°C), and persistence and extension of the cutaneous lesions, now appearing as ulcers with adherent purulent debris. A small ulcer was also present on the tongue tip, and leukopenia (WBC = 1600 cells/μl), hypocalcemia (7 mg/dl), hypoproteinemia (4.4 g/dl), and elevated aspartate aminotransferase (AST) (387 IU/L) were present. A presumptive diagnosis of poxvirus infection was reached based on lesions and progression consistent with the previous report.9,10 Supportive care (fluid and antibiotic therapy, and nutritional support) was continued for 1 wk. At that time, ulcers were present at the
mucocutaneous margins of the lips and nose, anterior to the preorbital scent glands, and bilaterally on the lateral aspects of the forelimbs near the coronary band. Ulcers had adherent purulent exudate forming crusts. Extensive ulceration was also present on the tongue and at the dorsal oral cavity. The calf was depressed, inappetent, and febrile. Increased respiratory sounds were auscultable in all lung fields, and dyspnea was evident. Results of CBC confirmed the persistence of leukopenia (WBC = 3400 cells/µl) and sickle cells, and serum biochemical profile confirmed hypocalcemia (6.8 mg/dl), hypoproteinemia (5.9 g/dl), and elevated AST (3171 IU/L), and indicated hypoglycemia (37 mg/dl), hyperphosphatemia (12.0 mg/dl), and elevated alanine aminotransferase (353 IU/L). Due to continued deterioration and grave prognosis, the animal was euthanatized (14 days after initial presentation).

The second case involve the adult female, the dam of case 1. First clinical signs were noted 2 days after the appearance of lesions in the calf. Initial cutaneous lesions were present bilaterally on the rear limbs from the coronary bands to the tarsometatarsal joint, and bilaterally on the forelimbs at the lateral aspect of the carpus. Results of CBC and serum biochemical profile were within normal limits, and therapy consisted of topical and systemic antibiotics as with the first case. One week after initial presentation, areas of dermatitis on the extremities were significantly worse, and swelling had developed ventral to the right orbit. Marked respiratory stridor was present. Repeated CBC remained within normal limits and serum biochemical profile revealed hypocalcemia (7.9 mg/dl), hyperphosphatemia (11.6 mg/dl), and elevated AST (1376 IU/L). Two days later, extensive lingual and oral cavity ulceration was noted. In spite of supportive care, the animal’s condition continued to decline. A deep corneal ulcer developed in the left eye, and ulcers were present at the mucocutaneous junctions of the eyelids and oral cavity, planum nasale, distal limbs, and ventral abdomen over the mammary glands. Results of CBC and serum profile taken 2 days later revealed leukopenia (WBC = 3,400 cells/µl), continued hypocalcemia, hyperphosphatemia, and elevated AST, and the development of hypoglycemia, hypoproteinemia, and elevated ALT, and high creatine phosphatase (CK) (4768 IU/L). The pudu died overnight, 12 days after initial presentation.

Post-mortem examinations were performed on the pudu. In both cases, multiple ulcers were present at the tongue tip and hard palate, and on incision of the salivary gland and mandibular lymph nodes a purulent exudate was expressed. Lungs had depressed dark red areas with scattered firmer 3-5 mm foci which were dark red or tan on section. Prescapular and mandibular lymph nodes were enlarged three to five times normal size. Scattered high numbers of ring-like ulcers up to 3 mm diameter were present in the forestomach of the infant. Histologically, tissue from the lip had multifocal epithelial necrosis and areas of hydropic swelling. In the esophagus there was focally extensive thickening of the mucosa with eosinophilic intracytoplasmic inclusion bodies and small areas of full thickness necrosis. Similar lesions were present in the reticulum of the infant. The lungs had discrete areas of lytic necrosis centered on arteries. Multiple dense mats of fungal hyphae were associated with these in the infant. Lymph nodes from both necropsy cases had fungal hyphae and rare bacterial colonies on a background of necrotic lymphoid tissue. Fungal hyphae were noted in pyogranulomatous skin lesions on the infant, with vascular invasion. A diagnosis of disseminated poxvirus infection with secondary fungal infection was made. Poxvirus infection was confirmed via electron microscopy and serologic testing.
Case 3 involved the adult male, which did not show clinical signs at the time the adult female and calf became ill. When the first two pudu began to show clinical signs, the male was moved into isolation. Mild facial swelling was noted 10 days after the female pudu began showing signs; however, no cutaneous pox lesions were noted until the day after the adult female died. At this point, the pudu was examined due to right ocular discharge. Sloughing skin with underlying seropurulent discharge was present caudal to the right eye, over the dorsal nose, and at the coronary bands of both tarsi and the right front foot. The lesions were debrided and cleaned with topical disinfectant. The following day the animal was depressed, febrile (40.6°C) and dyspneic. Results of CBC indicated leukopenia, and serum biochemical profile indicated hypocalcemia, hyperphosphatemia, elevated AST and high CK.

Based on the previous cases, the prognosis for survival were considered poor. Antiviral therapy with cidofovir (Vistide, Gilead Sciences, Foster City, CA 94404 USA) 5 mg/kg i.v. every 7 days for four treatments was initiated. Intravenous fluid therapy and oral probenecid (Benemid, Merck and Co, Inc, West Point, PA 19486 USA) was given concurrently with each treatment in the following regimen. An indwelling catheter was placed in a cephalic vein and 100 ml normal saline was administered i.v. and 250 mg probenecid was given orally. One hour later, cidofovir 5 mg/kg diluted in 30 ml normal saline was given i.v. One hour later, 100 ml normal saline was given i.v., 250 mg probenecid was given p.o., and the catheter was removed. Within 2 days of the initial treatment, the animal appeared less depressed. Superficial lesions were notably less exudative, and facial swelling seemed diminished. Antibiotic delivery was changed to enrofloxacin (Baytril, Bayer Corp., Shawnee Mission, KS 66201 USA) 2.5 mg/kg p.o., s.i.d. At this point, necropsy results from the first two cases were available, and indicated secondary fungal disease was an important factor. Itraconazole (Sporanox, Janssen Pharmaceutica, Inc., Titusville, NJ 08560 USA), 100 mg p.o., s.i.d. for 14 days, was initiated.

At 1 wk after initiation of antiviral therapy, cutaneous lesions were significantly improved. No new lesions had developed, and existing lesions became nonexudative and healed. Repeated examination at weekly intervals confirmed that no new cutaneous or mucocutaneous lesions had developed.

Discussion

Poxvirus infection in pudu has been reported, the virus described, and serologic cross-reactivity evaluated. Based on ultrastructural features, size, and lack of neutralization by antisera to multiple known poxviruses, the investigators suggested that the isolate was a novel poxvirus. That case description indicated illness in eight of nine pudu, with one death. Gross lesions were limited to periorbital area and/or oral cavity in the surviving animals, and also on the palate, esophagus, rumen, and reticulum of the single fatal case. As with the current case, the animals were housed in a single species exhibit and no recent additions had occurred.

The clinical presentation of poxvirus infection in pudu includes cutaneous and mucocutaneous ulcers and vesicles, as with other poxvirus infections. In this outbreak, ulcers at the coronary bands, cornea, and mammae occurred, which were not reported in the previous outbreak. In addition,
significant pneumonia was present in the two cases that died. These animals were consistently febrile and dyspneic, with coarse lung sounds. The presence of disseminated fungal disease was suspected to be due to immunosuppression secondary to viremia. In the two fatal cases, the progression from first evidence of cutaneous lesions to death was approximately 2 wk.

Clinical pathology findings in these cases were consistent, with early development leukopenia typical for viral infection. Electrolyte abnormalities (hypocalcemia, hyperphosphatemia), and high enzyme levels (AST, ALT) may be due to extensive tissue damage that results from the necrosis associated with poxvirus lesions. As cases progress, hypoglycemia and hypoproteinemia develop, probably due to decreased nutritional intake. It is presumed that the high CK values were secondary to repeated i.m. injections during treatment attempts. In the case that recovered, all CBC and serum biochemical profile abnormalities resolved coincident with clinical evidence of improvement.

The source of the virus in this outbreak could not be determined. As with the previously reported case, no recent exposure to new conspecifics or other species occurred. It is possible that a prolonged carrier state exists, and stress, such as the parturition in the recent case, initiated a recurrence. It is also possible that the poxvirus is latent in another species and manifests itself as a more serious disease in the aberrant host. Pudu are South American Cervidae, and at our institution are housed in a barn that is predominantly African antelope (Bovidae). However, no poxvirus disease has been documented or suspected in the remaining collection.

Antiviral drug therapy is not routinely used for animal poxviruses. Cidofovir is a nucleoside analog of deoxycytidine monophosphate that has shown activity against herpesviruses, as well as other virus infections, including poxvirus.\(^1\,7\) This drug is licensed for treatment of cytomegalovirus (CMV) retinitis in human patients with human immunodeficiency virus infection.\(^3\) Cidofovir suppresses viral replication by selective inhibition of viral DNA synthesis.\(^3\) While not approved for other viral infections, experimental research has suggested that cidofovir is effective against poxvirus infection.\(^1\,2\,4\,6\,7\) An anecdotal report of three human patients with HIV being treated with cidofovir for CMV retinitis indicates dramatic improvement of concurrent molluscum contagiosum virus dermatitis.\(^7\) No reports of the use of cidofovir against animal poxvirus infections were found. Dose-dependent nephrotoxicity is the major adverse reaction of cidofovir.\(^3\) To control nephrotoxicity, patient pretreatment evaluation is recommended, along with i.v. normal saline hydration. In addition, the concurrent administration of probenecid is recommended. Probenecid is a renal tubular transport blocking agent.\(^8\) Administration prolongs the half-life of cidofovir, resulting in prolonged period between doses.

The treatment regimen provided concomitant hydration and probenecid therapy, an appropriate dose, and a manageable treatment frequency. Patient monitoring did not reveal any evidence of renal impairment or leukopenia, the most common side effects in humans. Although only a single case is represented, the resolution of lesions, clinical signs, and clinical pathology abnormalities suggest that the treatment was both successful and safe. Attention to possible secondary infection is necessary. The presence of systemic fungal disease in these cases may suggest an immunocompromised state secondary to the viral infection.
LITERATURE CITED


ROLE OF CHRONIC IRON OVERLOAD IN MULTIPLE DISORDERS OF CAPTIVE BLACK RHINOCEROSES (Diceros bicornis)

Donald E. Paglia, MD1* and Pam Dennis, DVM2

1Hematology Research Laboratory, Department of Pathology & Laboratory Medicine, UCLA School of Medicine, 10833 LeConte Avenue, Los Angeles CA 90095-1732 USA; 2Department of Wildlife and Zoological Medicine, University of Florida College of Veterinary Medicine, PO Box 100126, Gainesville FL 32610 USA

Abstract

Necropsy reports of black rhinoceroses (Diceros bicornis) dying in captivity have frequently cited hemosiderosis as residual evidence of hemolytic anemia, a disorder of high morbidity and mortality in this species. Recent necropsy experience, however, and reevaluation of archival materials, revealed histopathologic patterns and quantities of iron deposition that were incompatible with that interpretation. Extensive, sometimes massive, involvement of both reticuloendothelial and parenchymal cells in multiple organs, indicated that this condition represents a true iron overload syndrome and could not result from hemolytic disease alone. This was confirmed by measurements of iron analytes in fresh and stored sera from four species of rhinoceroses, and by quantitative analyses of tissue iron from a limited number of archival specimens. Comparisons with free-ranging rhinoceroses indicated that iron accumulates as a consequence of captive conditions and in direct relation to time in captivity, producing an acquired hemochromatosis. Histopathologic characteristics of iron deposition in affected rhinoceroses were virtually identical to those observed in humans and lemurs with dietary iron overload syndromes. No evidence of iron excess was found in natural grazers (Ceratotherium simum and Rhinoceros unicornis), but sera from three Sumatran rhinoceroses (Dicerorhinus sumatrensis) revealed comparable elevations, indicating that species that normally forage predominantly on browse are most at risk for development of hemochromatosis in captivity. Since enteric absorption was the only possible source of iron excess, these observations suggest that components that bind iron into nonabsorbable complexes (such as tannins, phytates, polyphenolics, etc.), present in dietary browse and/or absent in captive herbivore diets, might be responsible for increased bioavailability of iron and its unregulated uptake. Since free iron induces cell injury by catalyzing production of highly reactive hydroxyl free radicals, to which rhinoceros red cells are highly sensitive, iron overload might contribute directly to cellular impairment in any of the several syndromes affecting black rhinoceroses in captivity. In particular, the suppressive effect of iron excess on “nutritional immunity” (host vs. pathogen competition for essential elements) is likely to contribute to this species’ apparently high susceptibility to a broad range of infectious diseases. Strategies to prevent and/or treat iron overload in captive rhinoceroses may be restricted to the juvenile population, because of the inordinate body burdens present in many older animals and practical limitations on the amounts of iron that can be mobilized by phlebotomy and/or chelation therapy.

Introduction
Necropsy records of captive black rhinoceroses (*Diceros bicornis*) dying over the past three decades have commonly mentioned the presence of hemosiderosis, inordinate tissue deposition of the iron storage pigment hemosiderin. This has generally been viewed as an incidental finding and interpreted as evidence of previous hemolytic episodes in which premature destruction of red cells allows hemoglobin iron to accumulate in reticuloendothelial cells throughout the body. The magnitude and patterns of iron deposition that we have observed in more recent necropsies, however, have been incompatible with that interpretation, conforming instead to an acquired form of hemochromatosis affecting parenchymal as well as reticuloendothelial cells, thereby producing a pathologic iron-overload syndrome with potentially injurious effects on multiple organ systems.

Assessment of this condition by assays of serum iron compounds has now confirmed that captive black rhinoceroses exhibit significant increases in total body iron stores, with serum ferritin concentrations often tenfold to many hundreds of times higher than those in their natural habitats. Assays on a limited number of Sumatran rhinoceroses (*Dicerorhinus sumatrensis*) revealed that they too, are overloaded with iron in captivity.

Hemosiderosis is but one of a number of disorders or syndromes of unknown etiology that occur frequently in captive black rhinoceroses, often with severe to lethal consequences. These include episodic hemolytic anemia; high susceptibility to *Leptospira, Salmonella*, mycobacteria, and fungal pneumonias; diffuse ulcerations of skin and mucous membranes; congenital leukoencephalomalacia; hepatic failure; chronic progressive anemia with severe weight loss and occult infection; and a more recently recognized syndrome involving the microvasculature, idiopathic hemorrhagic vasculopathy. That so many disparate disorders should affect only one species of rhinoceros while sparing others seems intuitively improbable, unless they share some common elements in terms of underlying causes or pathogenesis.

Data presented here suggest that iron overload might serve as a common mediator, potentially contributing to the initiation, clinical course, and severity of a number of these disease syndromes.

**Methods**

Over the past several years, the zoological community has accorded the senior author opportunities to attend and assist in necropsies of black rhinoceroses dying from (or euthanatized for) diverse problems, including traumatic, infectious, hematologic, and hemorrhagic disorders. Histopathologic materials from past necropsies were also made available by a number of institutions for review and reevaluation, including recuts of paraffin blocks and special stains.

Blood specimens from captive and free-ranging black rhinoceroses (and other species) that were referred to the UCLA Hematology Research Laboratory for evaluation of blood cell metabolism were analyzed additionally for serum iron compounds. Archival specimens stored at individual institutions, including the collective repository for black rhinoceroses at the St. Louis Zoo, were also sought, obtained, and similarly studied. Serum iron concentrations, iron binding capacities, and transferrin saturations were determined using the quantitative colorimetric technique employed in Sigma diagnostics (St. Louis, MO) Kit No. 565. Serum concentrations of ferritin and haptoglobin and elemental iron assays of frozen necropsy tissues were contracted to the Cellular and Molecular
Pathology Laboratory at Kansas State University College of Veterinary Medicine where species-specific iron-analyte assays for rhinoceroses were originally developed by the late Dr. Joseph Smith and his associates.12

Results

Necropsy Evidence of Iron Overload

Iron stores in necropsy tissues were significantly (sometimes massively) increased in virtually all captive adult black rhinoceroses studied, regardless of (and out of proportion to) any previous or current hemolytic process. All but three had negative histories for past hemolytic episodes, and normal haptoglobin and bilirubin values ruled out active hemolysis. Hemosiderin deposition appeared far too large in amount, and its distribution in multiple organs too atypical and widespread, to be caused either by reprocessing of normally aged red cells or by their premature destruction. Erythroid hyperplasia and/or extramedullary erythropoiesis, and renal tubular epithelial siderosis characteristic of intravascular hemolysis, were uniformly absent. Bone marrows tended to be hypocellular and even fibrotic with broad sheets of hemosiderin-laden macrophages filling medullary spaces, producing in some instances a myelophthisic siderosis.

Histopathologic characteristics of pigment deposition were virtually identical to dietary iron-overload syndromes occurring in other captive wildlife such as lemurs13 and in sub-Saharan African Bantu tribes whose methods of food and beverage preparation significantly enhance bioavailability of ingested iron.2,3 (In the latter population, as in human idiopathic hemochromatosis, genetic determinants also contribute to increased uptake of dietary iron.4) Organs most consistently involved were spleen, liver, bone marrow, and lungs, with less frequent but prominent siderosis of intestines, lymph nodes, heart, adrenals, thyroid, and other endocrine organs. Distinctive deposits were present in the lamina propria and submucosa of the entire bowel with the highest concentrations occurring at the tips of small bowel villi, a histologic pattern indicative of enteric origin of excess iron.6 Deposition within macrophages in these organs generally dominated, but parenchymal cells were also frequently involved to an extent that would be expected to affect cellular function.

Dense patterns of hepatic iron deposition observed in these cases differed distinctly from those described in uncomplicated hemolytic disease. Portal macrophages and sinusoidal Kupffer cells were heavily engorged with hemosiderin. Hepatocytes contained course and fine iron-positive granules that were most prominent in perportal regions, but extended throughout the lobules. Despite extensive liver deposits in many cases, there was minimal evidence of hepatic fibrosis that characteristically occurs in severe hemochromatosis in humans and other species, but cirrhotic changes and low-grade hepatocellular carcinoma have been observed in archival material from one black rhinoceros.

Serum Iron Analytes in Captive and Free-Ranging Rhinoceroses
These subjective impressions of inexplicably severe hemosiderosis at necropsy were supplemented by quantitative assays of serum iron and ferritin concentrations and iron-binding capacity in seven groups of rhinoceroses (Table 1). Since the six animals in Group D had lived lifelong in the wild until capture approximately 2 wk before sera were obtained, they provided the best control standard for comparative purposes. This was the only group of black rhinoceroses studied in which iron analyte values for all members fell uniformly within established normal ranges for humans and equines.

Average serum iron among long-term U.S. captives (Group A) and recent imports (Group B) was 2 ½ times higher than the mean for control Group D, and individual values ranged as high as fivefold or greater. Mean transferrin saturation in these long-term captives was also more than twice as high as Group D control mean. Group C, with much shorter periods in captivity, displayed correspondingly intermediate values for serum iron concentrations with mean transferrin saturations of 44%. Smith, et al.12 using other assay techniques, reported even higher serum iron (x = 614 ± 200 mg/dl) with similar transferrin saturations (x = 67.5 ± 3.7 %) for long-term captive Diceros. In human hemochromatosis, transferrin saturations of 65-70% are viewed as the threshold region for onset of overt organ damage by chronic iron overload and its sequelae.2

Within certain ranges, serum ferritin concentrations provide the best estimates of total body iron stores short of quantitative assays of hepatic and other tissues. Results shown in Fig. 1 demonstrate concentrations in captive black rhinoceroses that ranged fully over three orders of magnitude, dramatically greater than other species of rhinoceroses and various control groups. Two neonatal samples from calves born in captivity had < 200 ng/ml ferritin, but ferritin concentrations tended to increase logarithmically thereafter as a function of time in captivity (data not shown), confirming similar observations made previously by Kock et al.5 and Smith et al.12 The presence of significant increases in total body burdens of iron, demonstrated by necropsy pathology and serum iron analyte assays, received additional confirmation from quantitative assays of stored frozen tissues from selected animals (data not shown). It is particularly notable that iron analytes in predominantly grazing rhinoceroses (white and Indian) showed no elevations in captivity, but the other browsing species (Sumatran) distinctly did, reaching levels comparable to some long-term captive Diceros.

Discussion

The significance of hemosiderosis in captive black rhinoceroses was brought into sharper focus by two pivotal studies. In 1993, Kock and her associates reported that hemosiderosis was not seen in necropsies of free-ranging Diceros in Zimbabwe that died within 2 wk of capture, where-as it began to appear and progressively increase in those that died during boma confinement > 2 wk-2 yr after capture.5 Similarly, Smith et al. found that serum ferritin levels in U.S. captive black rhinoceroses tended to increase progressively with age or time in captivity, and further, that ferritin and hepatic iron stores were both significantly higher in captive black vs. white rhinoceroses.12 Since haptoglobin concentrations were comparable between the two species, hemolysis could not account for these discrepancies, and the authors presciently suggested that dietary changes resulting in increased iron absorption might provide a more logical explanation.
Taken together, these two important studies present perhaps the most significant differences yet reported between captive black and white rhinoceroses and between *Diceros* in captivity and those residing lifelong in the wild. A pertinent aspect of both studies was an apparent correlation between time in captivity and degree of iron overload. Supplemented by our own more recent necropsy observations and quantitative laboratory assessments of body iron stores reported here, we believe that these data present compelling evidence for existence of a clinically significant iron overload syndrome in black rhinoceroses that is directly related to captive conditions, most likely on a dietary basis. The possibility that enhanced uptake of iron in these animals might also be influenced by genetic determinants, such as those operative in human hemochromatoses, remains to be investigated.

Hemosiderosis has also been a common finding in other species of animals when brought into captivity, including simians, prosimians, and avians, whose natural diets (like the browsing rhinoceroses) normally contain high concentrations of tannins, phytates, fiber, polyphenolics, phosphates, and other compounds that chelate iron into insoluble complexes, most of which then passes through the gastrointestinal tract unabsorbed. Both Sumatran and black rhinoceroses normally browse on trees and shrubs. By contrast, white (*Ceratotherium simum*) and Asian greater one-horned (*Rhinoceros unicornis*) rhinoceroses predominantly graze on grasses, and neither shows biochemical or necropsy evidence of iron overload in captivity. In the case of browsing rhinoceroses, even though the content of iron may be the same in natural-browse vs. captive diets, we believe that the absence of natural chelators in grass-based preparations likely increases the bioavailability of iron, allowing it to accumulate progressively in animals sustained largely on captive rations.

Although speculative, a number of potential connections exist between iron overload and certain disorders affecting these species in captivity. It is well established that susceptibility to infections, for example, is significantly increased in iron-loaded humans and other animals. Since microorganisms compete with their hosts for certain metabolites, a major defense mechanism (so-called “nutritional immunity”) involves rapid sequestration of iron to deprive invading microorganisms of ready access to this essential trace element. Most microorganisms thrive in high-iron environments, both in vitro and in vivo, and many (such as mycobacteria) are notoriously virulent in humans and animals with iron overloads. This presents a major clinical problem for children with thalassemia or other hemolytic disorders who develop iron overloads from frequent transfusions, and it directly underlies the high incidence and virulence of tuberculosis currently rampant among hyperferremic African Bantu.

Another possible connection exists between iron overload and the myelin degenerative changes occurring in congenital leukoencephalomalacia. Among U.S. captive black rhinoceroses, (see Fig. 1), 11 of the 12 highest serum ferritin concentrations were found in immediate relatives of the four female calves known to have died with this disorder. In each instance, their mothers had extraordinary elevations (hundredfold to thousandfold greater than normal), and transplacental transfer (pronounced, but transient, neonatal hyperferritinemia) was clearly demonstrable in the female calves, but, for unknown reasons, not in their male siblings.
Free iron produces cell injury primarily by production of hydroxyl free radicals, to which rhinoceroses are known to be highly vulnerable,\textsuperscript{10} a problem that may be compounded by inherently low levels of the antioxidant, vitamin E. Free radicals actively attack lipid membrane components of cells and organelles, alter critical structural and enzymatic proteins, and cleave DNA, disrupting cellular replication. The consequences of such events during in utero development would likely be disastrous, and might even be related to the male preponderance and survival rates among live births in North America.\textsuperscript{1}

Whether or not iron overload is causally related to any of the diverse disorders affecting captive black rhinoceroses, these findings indicate that iron burdens have reached hazardous proportions in this captive population and (based on a small sample) in Sumatran rhinoceroses as well. Toxic effects of iron overload in humans and other mammals occur at levels far lower than those now prevalent in these two species. Were human clinical criteria to be applied, virtually all captive browsing rhinoceroses that we have been able to evaluate would be immediate candidates for therapeutic intervention by phlebotomy or parenteral chelation.

The potential hazards of progressive body-iron burdens in browsing rhinoceroses merit consideration of both preventative and therapeutic strategies. Addition of high-tannin food components, such as tamarind pods, is already being tried by some zoo veterinary staffs, and mobilization and excretion of tissue iron has been demonstrated in one black rhinoceros by chelation therapy with desferrioxamine preparations. Therapeutic intervention, as well as preventative measures, might require a triage approach, targeting the youngest segment of the captive population with the lowest body iron burdens and the greatest breeding potential. Calves are apparently born with low body iron stores, but ferritin levels may rise tenfold by 3-4 yr of age. The older the animal, or the longer its time in captivity, the less effective intervention is likely be, since their progressively larger storage pools of iron would be relatively less affected by modulation of daily intake or significantly altered by any practical means of iron mobilization within their lifetimes.

For animals trained to tolerate the procedure, phlebotomy has a number of potential advantages: It is relatively non-intrusive and free of side effects, inexpensive, and it allows precise calculation and monitoring of quantities of iron removed, since hemoglobin contains a fixed amount of iron (0.34\% by weight). Phlebotomy also provides an opportunity for secondary benefits: harvested plasma could easily be preserved, and by slightly more complicated procedures, washed red cells could be frozen in glycerol and stored long-term for potential transfusional use.

Because virtually nothing is yet known about the dynamics of iron homeostasis in these species, carefully planned and controlled studies are needed before widespread application of any preventative or therapeutic approach can be recommended. In the interim, it would seem prudent to assess trace- and transition-metal status in all captive black and Sumatran rhinoceroses, particularly those active in breeding programs.

ACKNOWLEDGMENTS
We are particularly grateful to the many individuals and institutions in the zoo and wildlife community who invited the senior author to participate in necropsies over the past 7 yr, and to those who shared valuable archival sera and frozen tissues, and who provided important histopathology materials for reevaluation. Serum ferritin, haptoglobin, and tissue iron assays performed by Sue Chavey in the Cellular and Molecular Pathology Laboratory at the University of Kansas College of Veterinary Medicine, under the direction of Dr. Gordon A. Andrews, were essential for quantitative confirmation of subjective necropsy observations. Angel Tsu provided the technologic expertise for iron, enzyme, and metabolite assays performed in the UCLA Hematology Research Laboratory. Research support was provided by grants from the International Rhino Foundation, the Morris Animal Foundation, the LB Research and Education Foundation, and a Fulbright Senior Research Scholar award from the J. William Fulbright Foreign Scholarship Board.

These studies are dedicated to the memory of Dr. Joseph E. Smith, friend and colleague to so many of us in both veterinary and human medicine, whose tragic untimely passing prevented him from completing his seminal work in this field.

LITERATURE CITED


Table 1. Serum iron assays in various species of captive and free-ranging rhinoceroses.

<table>
<thead>
<tr>
<th>Group</th>
<th>Species and location</th>
<th>Time in captivity prior to sampling</th>
<th>Iron (µg/dl)</th>
<th>Transferrin saturation (%)</th>
<th>Ferritin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A:</td>
<td><em>D. bicornis</em>, longterm US captivity</td>
<td>$x = 144\text{ mo}$ ($1-391\text{ mo}$)</td>
<td>247 ± 94 (34)*</td>
<td>65 ± 22</td>
<td>2,200 ± 2,240</td>
</tr>
<tr>
<td>B:</td>
<td>Wild <em>D. bicornis</em> imported</td>
<td>~10 mo</td>
<td>243 ± 103 (9)</td>
<td>72 ± 20</td>
<td>1,270 ± 566</td>
</tr>
<tr>
<td>C:</td>
<td><em>D. bicornis</em> in Zimbabwe boma confinement</td>
<td>$x = 86\text{ mo}$ ($12-150\text{ mo}$)</td>
<td>144 ± 44 (6)</td>
<td>44 ± 13</td>
<td>372 ± 160</td>
</tr>
<tr>
<td>D:</td>
<td><em>D. bicornis</em> lifelong free-ranging until capture</td>
<td>&lt;2 wk</td>
<td>101 ± 19 (6)</td>
<td>28 ± 6</td>
<td>133 ± 62</td>
</tr>
<tr>
<td>E:</td>
<td><em>C. simum</em>, US captivity</td>
<td>Longterm</td>
<td>134 ± 43 (6)</td>
<td>37 ± 15</td>
<td>47 ± 32</td>
</tr>
<tr>
<td>F:</td>
<td><em>R. unicornis</em>, US captivity</td>
<td>Longterm</td>
<td>123 ± 48 (4)</td>
<td>33 ± 8</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>G:</td>
<td><em>D. sumatrensis</em>, US captivity</td>
<td>Longterm</td>
<td>222 ± 65 (3)</td>
<td>91 ± 3</td>
<td>798 ± 134</td>
</tr>
<tr>
<td></td>
<td>Equine controls:</td>
<td></td>
<td>50-198</td>
<td>22-44</td>
<td>43-261</td>
</tr>
<tr>
<td></td>
<td>Human controls:</td>
<td></td>
<td>65-165</td>
<td>20-50</td>
<td>10-300</td>
</tr>
</tbody>
</table>

*Values shown are $x \pm 1\text{ SD}$. Number of animals assayed is shown in parentheses. Table excludes values from longterm U.S. captive black rhinoceroses with serum ferritin concentrations >10,000 ng/ml (see Fig. 1).
Figure 1. Serum ferritin concentrations in various groups of rhinoceroses (log scale). Eleven of the twelve highest values in group A (>10,000 ng/ml) occurred in kindreds of calves with congenital leukoencephalomalacia. This group also had high incidences of primary hemolytic anemia and mucocutaneous ulcerative disease. Open symbols in Group B are values derived from follow-up samples obtained after residing in U.S. captivity for the periods indicated.
STUDIES ON THE GAMMA HERPESVIRUS CARRIER STATUS OF SCIMITAR-HORNED ORYX (Oryx dammah) AND GEMSBOK (Oryx gazella)

E.J. Flach, A. Klemt, I. Pow, H.W. Reid, and C. Tack

1Veterinary Science Unit, Institute of Zoology, Whipsnade Wild Animal Park, Dunstable, Beds. LU6 2LF, UK; 2Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, EH26 0PZ, Scotland, UK; 3Africa section, Whipsnade Wild Animal Park, Dunstable, Beds. LU6 2LF, UK

Abstract

Malignant catarrhal fever (MCF) is caused by one of two gamma herpesviruses; alcelaphine herpesvirus 1 (AHV-1), first isolated from blue wildebeest (Connochaetes taurinus), and ovine herpesvirus 2 (OHV-2) of sheep which cannot be grown in tissue culture and thus is incompletely characterized. These viruses are non-pathogenic in their true hosts, but infection of other species causes a lymphoproliferative disease with very high mortality. Both the wildebeest-associated and sheep-associated forms of MCF have been responsible for deaths in zoos.

Serologic surveys of artiodactyl collections using AHV-1 antigens have revealed that there are species whose populations have a high prevalence of sero-positives, suggesting that they carry their own endemic gamma herpesvirus. Two of these related viruses have been isolated and named; alcelaphine herpesvirus 2 (AHV-2) from topi (Damaliscus lunatus) and hartebeest (Alcelaphus buselaphus), and hippotragine herpesvirus 1 (HiHV-1) from a roan antelope (Hippotragus equinus). Species of the genus Oryx are also potential carriers of their own gamma herpesvirus and indeed an MCF-like virus has been reported from a neonatal scimitar-horned oryx (SH oryx; Oryx dammah).

In order to investigate the carrier status of the breeding herds of SH oryx and gemsbok (Oryx gazella gazella) at Whipsnade Wild Animal Park, a study was carried out during 1998. The study material consisted of: a) serum samples collected during the previous 10 yr from animals anesthetized for veterinary or management reasons, b) blood samples from 11 SH oryx and 4 gemsbok calves born during 1998 taken within 24 hr of birth, c) tissue samples from the three SH oryx neonates and one gemsbok which died, d) further blood samples from the eight surviving SH oryx calves at 4-5 mo of age, and e) blood samples from two adult SH oryx and four gemsbok anesthetized during the year for veterinary or management reasons. Serum samples were tested for antibodies to AHV-1 in an indirect immunofluorescent antibody test (IFAT) and a virus neutralization test (VNT). The polymerase chain reaction (PCR) was used to amplify DNA derived from peripheral blood leukocytes (PBL) or tissues of calves, using as primers OHV-2 (PCR/1) or two amplifications of a mixture of the non-specific gamma herpesvirus primers POL1 and POL2, on its own (PCR/2), or followed by primers for AHV-1 (PCR/3) or OHV-2 (PCR/4).
Thirty-two out of 34 (94.1%) stored SH oryx sera and all 16 stored gemsbok sera were positive in the IFAT to AHV-1, but only four SH oryx and 10 gemsbok sera had neutralizing antibody to the virus.

Eight of the 11 newborn SH oryx calves had detectable gamma herpesvirus DNA, six had a band at 800 base-pairs (bp) (PCR/2), two responded to AHV-1 POL amplification (PCR/3) and six responded to OHV-2 POL (PCR/4) (Table 1). Two newborn gemsbok calves were positive, one gave a band at 388bp in PCR/2 and one a band at 173 bp after OHV-2 POL amplification in PCR/4 (Table 2). A selection of these PCR products were tested by Southern blot hybridization against a specific AHV-1 probe, but none reacted. All the serum samples from newborn calves which were tested (eight SH oryx and four gemsbok) were positive in the IFAT and four of the SH oryx and two of the gemsbok samples were also positive in the virus neutralization test against AHV-1 (Tables 1 and 2).

When the eight surviving SH oryx calves were re-sampled at 4-5 mo of ages, all eight reacted in PCR/2, none to AHV-1 POL (PCR/3) and six to OHV-2 POL (PCR/4) (Table 1). However, only one animal was still positive in the IFAT to AHV-1. One of the seronegative animals was re-tested 3 mo later and was still PCR/4 positive, but IFAT negative. Pooled PBL from the eight calves were added to a monolayer of kidney cells derived from one of the SH oryx calves that died. Following two sub-passages and 83 days in culture a dense multinucleate focus was observed. Similar foci appeared and increased in number over the next few days. Cells from these cultures were co-cultivated with further SH oryx kidney cells and with cultured bovine turbinate cells. Similar cytopathic effects developed in these cultures. DNA from these cells were positive by the PCR/1, 2 and 4. In addition, when cells from one of the affected cultures were inoculated into a rabbit it developed a rectal temperature of 41°C on day 13 and at necropsy lymphoproliferative lesions, characteristic of malignant catarrhal fever, were observed grossly and histologically.

All of the adult SH oryx and gemsbok were found to have viral DNA in their PBL (Tables 1 and 2).

These results confirm the suspicion that oryx carry their own gamma herpesvirus which is related to, but distinct from, AHV-1 and OHV-2. On the basis of the PCRs it would appear that the viruses of both the SH oryx and the gemsbok are more closely related to OHV-2, which may explain the relative lack of neutralizing antibodies to AHV-1. Interestingly, a higher proportion of gemsbok than SH oryx had virus neutralizing antibodies, suggesting a difference between their respective viruses. Virus has now been isolated from the SH oryx which should allow a more detailed analysis of the viral DNA and a more specific diagnostic test in scimitar-horned, and other, oryx. That a rabbit developed MCF following inoculation with this virus demonstrates its capability of inducing disease, though there is no evidence for transmission to susceptible species in zoos. The probable designation of the virus will be hippotragine herpesvirus-2, after the subfamily to which SH oryx are ascribed. It would appear likely that the virus is transmitted in utero, because so many of the neonates had virus DNA in their PBL before they were 24 hr old. The antibodies detected were probably maternal and in all but one case had disappeared by 4-5 mo of age. It is hoped to retest the animals to find out when they mount their own antibody response.
LITERATURE CITED


### Table 1. Summary of serologic and PCR results from scimitar-horned oryx.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Sex</th>
<th>Age</th>
<th>IFAT⁴</th>
<th>VN⁵</th>
<th>PCRc</th>
<th>Age</th>
<th>IFAT</th>
<th>PCRd +ves</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf 1</td>
<td>F</td>
<td>&lt;1day</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>Died &lt;2 days</td>
</tr>
<tr>
<td>Calf 2</td>
<td>M</td>
<td>&lt;1day</td>
<td>NT</td>
<td>NT</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>Died &lt;1 day</td>
</tr>
<tr>
<td>Calf 3</td>
<td>F</td>
<td>&lt;1day</td>
<td>+</td>
<td>+</td>
<td>2,4</td>
<td></td>
<td></td>
<td></td>
<td>Died 4 days</td>
</tr>
<tr>
<td>Calf 4</td>
<td>M</td>
<td>&lt;1day</td>
<td>+</td>
<td>-</td>
<td>2,4</td>
<td>5 mo</td>
<td>-</td>
<td>2,4</td>
<td>Died 9 mo</td>
</tr>
<tr>
<td>Calf 5</td>
<td>F</td>
<td>&lt;1day</td>
<td>+</td>
<td>+</td>
<td>2,3,4</td>
<td>5 mo</td>
<td>-</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Calf 6</td>
<td>F</td>
<td>&lt;1day</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>5 mo</td>
<td>-</td>
<td>2,4</td>
<td></td>
</tr>
<tr>
<td>Calf 7</td>
<td>M</td>
<td>&lt;1day</td>
<td>+</td>
<td>-</td>
<td>2</td>
<td>5 mo</td>
<td>-</td>
<td>2,4</td>
<td></td>
</tr>
<tr>
<td>Calf 8</td>
<td>F</td>
<td>&lt;1day</td>
<td>NT</td>
<td>NT</td>
<td>-</td>
<td>5 mo</td>
<td>-</td>
<td>2,4</td>
<td></td>
</tr>
<tr>
<td>Calf 9</td>
<td>M</td>
<td>&lt;1day</td>
<td>NT</td>
<td>NT</td>
<td>4</td>
<td>5 mo</td>
<td>-</td>
<td>2,4</td>
<td></td>
</tr>
<tr>
<td>Calf 10</td>
<td>F</td>
<td>&lt;1day</td>
<td>+</td>
<td>-</td>
<td>2,4</td>
<td>5 mo</td>
<td>+</td>
<td>2,4</td>
<td></td>
</tr>
<tr>
<td>Calf 11</td>
<td>F</td>
<td>&lt;1day</td>
<td>+</td>
<td>+</td>
<td>3,4</td>
<td>4 mo</td>
<td>-</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Adult 1</td>
<td>F</td>
<td>5 yr</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>2,4</td>
<td></td>
</tr>
<tr>
<td>Adult 2</td>
<td>F</td>
<td>17 yr</td>
<td>+</td>
<td></td>
<td>2,3,4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

⁴Indirect immunofluorescent antibody test against AHV-1 infected cell culture
⁵Virus neutralization test. Positive if serum protected more than 50% of cell culture wells at a dilution of 1:10 or greater.

<table>
<thead>
<tr>
<th>PCR1</th>
<th>OHV-2 PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR2</td>
<td>POL1/POL2 PCR (2 amplifications)</td>
</tr>
<tr>
<td>PCR3</td>
<td>POL1/POL2 (2 amplifications) then POL2/AHV POL</td>
</tr>
<tr>
<td>PCR4</td>
<td>POL1/POL2 (2 amplifications) then POL2/OHV POL</td>
</tr>
</tbody>
</table>

⁶PCR/1 not tested, PCR/3 and PCR/4 not tested on calves 1,2 & 8
Table 2. Summary of serologic and PCR results from gemsbok.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Sex</th>
<th>Age</th>
<th>IFAT&lt;sup&gt;a&lt;/sup&gt;</th>
<th>VN&lt;sup&gt;b&lt;/sup&gt;</th>
<th>PCR&lt;sup&gt;c&lt;/sup&gt; +ves&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf 1</td>
<td>M</td>
<td>&lt;1day</td>
<td>+</td>
<td>-</td>
<td>4</td>
<td>Died 3 days</td>
</tr>
<tr>
<td>Calf 2</td>
<td>M</td>
<td>&lt;1day</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>Calf 3</td>
<td>M</td>
<td>&lt;1day</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Calf 4</td>
<td>M</td>
<td>&lt;1day</td>
<td>+</td>
<td>NT</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Adult 1</td>
<td>F</td>
<td>12 yr</td>
<td>+</td>
<td>NT</td>
<td>2,4</td>
<td></td>
</tr>
<tr>
<td>Adult 2</td>
<td>F</td>
<td>3 yr</td>
<td>+</td>
<td>NT</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Adult 3</td>
<td>F</td>
<td>4 yr</td>
<td>+</td>
<td>NT</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Adult 4</td>
<td>F</td>
<td>9 yr</td>
<td>+</td>
<td>NT</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Indirect immunofluorescent antibody test against AHV-1 infected cell culture

<sup>b</sup>Virus neutralization test. Positive if serum protected more than 50% of cell culture wells at a dilution of 1:10 or greater.

<sup>c</sup>PCR/1 OHV-2 PCR

<sup>d</sup>PCR/2 POL1/POL2 PCR (2 amplifications)

<sup>e</sup>PCR/3 POL1/POL2 (2 amplifications) then POL2/AHV POL

<sup>f</sup>PCR/4 POL1/POL2 (2 amplifications) then POL2/OHV POL

<sup>g</sup>PCR/1 not tested on calves or adult 2
USE OF PERCUTANEOUS ABOMASAL FEEDING TUBES IN SMALL RUMINANTS

Bonnie L. Raphael, DVM, Dipl ACZM,* Stephanie B. James, DVM, Sharon L. Deem, DVM, Dipl ACZM, and Robert A. Cook, VMD

Wildlife Health Sciences, Wildlife Conservation Society, Bronx, NY 10460-1099 USA

Abstract

Hand rearing neonatal ruminants can be a challenge, especially for those animals with small body mass. Nutrient intake must begin early and be adequate to prevent weight loss and subsequent organ compromise. There is a limited time period in which an animal can accept a bottle and nurse on it effectively before its body stores are depleted.

If neonatal ruminants do not begin active nursing behavior within a reasonable period of time, gastric tube feedings can be used on a limited basis. Copper sulfate placed on the back of the tongue is required to stimulate closure of the esophageal groove so that milk bypasses the rumen and goes directly to the abomasum. Repeated tubing may cause pharyngeal and esophageal trauma, safe copper levels may be exceeded over time, and the risk of milk induced rumenitis increases over time.

Additionally, if an attempt is being made to initiate normal suckling behavior from a bottle, repeated manual restraint in order to pass a tube will be counterproductive.

Placing a feeding tube percutaneously into the abomasum provides an avenue for direct feeding, which eliminates the need for copper sulfate, insures that formula is delivered into the abomasum rather than the rumen, reduces the stress due to manual restraint during conventional tube feeding and allows delivery of adequate nutrients regardless of the nursing behavior of the animal. A technique described by Ensley et al.1 for placement of abomasal feeding tubes via nasal cannulation has the advantage of being a nonsurgical procedure and thus not requiring anesthesia, but requires manufacture of specialized equipment. However, percutaneous placement of a feeding tube allows the animals head to be free of any apparatus and may facilitate more rapid transition to bottle feeding.

Case #1

A maxwell duiker (Cephalophus maxwelli), which had weighed 1.02 kg at birth, was removed from its dam at 6 days of age due to weight loss and maternal neglect. At that time the animal weighed 788 g, was dehydrated and thin, and the abdomen felt empty on palpation. Initial attempts at bottle feeding were unsuccessful. Due to its compromised condition, percutaneous abomasal tube placement was performed. The animal was anesthetized via isoflurane via face mask, intubated with a 3.0 mm cuffed endotracheal tube and maintained on 3-5% isoflurane in 2 L/m O₂ with intermittent positive pressure ventilation for 70 min. An 18-ga, 2.5-inch spinal needle was placed into the left femoral intraosseous space for administration of fluids and antibiotics. An abomasal feeding tube
was placed using the technique described below. Direct abomasal feeding with 5% dextrose in lactated ringers solution began 4 hr later. Subsequently a formula of whole cows milk, colostrum and fluids was used. Volume infused into the abomasum increased to 15 ml per feeding every 3-4 hr over the next 4 days. In addition, attempts were made to get the animal to begin nursing from a bottle. On day 6 after placement of the tube (11 days of age), the animal was consistently taking formula orally. On day 7 the animal was anesthetized and the tube was surgically removed. The i.o. catheter remained in place through 15 days of age. Amikacin sulfate (Amiglyde-V, Fort Dodge Animal Health, Fort Dodge, Iowa 50501 USA) 10 mg/kg i.o., s.i.d. and sodium ampicillin (Ampicillin Sodium USP, Apothecon, Princeton, New Jersey 08540 USA) 50 mg/kg i.o., t.i.d was administered for 10 days followed by ampicillin 50 mg/kg s.c., b.i.d. for an additional 10 days. Solid food was offered beginning at 8 days of age and the animal was eating consistently at 7 wk of age, at which time it weighed 3.3 kg.

Case #2

A 293-g, female, larger Malayan chevrotain (Tragulus napu), was born to a dam with a history of repeated maternal neglect of offspring. Within 6 hr of birth an abomasal feeding tube was placed in the animal using the technique described below. The calf was anesthetized with isoflurane via facemask and an i.o. catheter consisting of a 22-ga, 1.5-inch needle was placed in the left femur. Formula, consisting of 100% cow’s colostrum, was begun 4 hr after tube placement at a rate of 2 ml every 3 hr for four feedings. The formula was then changed to colostrum, Esbilac® (PetAg Inc, Elgin, Illinois 60120 USA) and Multi-Milk® (PetAg) in a ratio of 1 ml colostrum to 1 ml of a mixture of 100 ml Esbilac + 13 g Multi-Milk. Over the ensuing 4 wk the volume delivered through the tube was increased to 9 ml 5 times per day. No attempt was made to initiate bottle feeding. The animal was encouraged to eat solid foods consisting of an herbivore pellet, kale and carrots and to drink water starting on day 5. Amikacin 10 mg/kg s.i.d. and sodium ampicillin 50 mg/kg t.i.d. were administered i.o. for 3 days. Subsequently the amikacin was administered i.m. for 5 days and the ampicillin s.c., t.i.d. for 13 days followed by the ampicillin being given 20 mg/kg s.c., b.i.d. for an additional 8 days. Penicillin G Benzathine and Penicillin G Procaine (Flo-Cillin, Fort Dodge Animal Health, Fort Dodge, Iowa 50501 USA) 50,000 IU/kg was administered s.c., s.i.d. from day 25-34. The animal’s weight when the feeding tube was removed at day 28 was 670 g.

Percutaneous abomasal tube placement: The animals are placed in right lateral recumbency to facilitate clipping and surgical preparation of the left dorso-lateral abdomen. A 1-2-cm paramedian skin incision, dorsal lateral to the 8th-10th rib is made. Blunt dissection through muscle layers is performed until the peritoneum is encountered and penetrated. The abomasum is blindly grasped by passing a tissue forceps or hemostat ventrally along the body wall cranio-medial. When the abomasum is encountered it is grasped and gently retracted toward the incision from underneath the rumen. A stay suture is placed in the wall of the abomasum and an incision is made into the lumen so that an appropriate size catheter or feeding tube can be placed. The feeding tube is directed caudally towards the pylorus, a purse string suture is placed around the tube and through the wall of the abomasum. If a Foley catheter is used it is partially inflated at that time. One suture is placed through the serosa of the abomasum to attach the abomasum to the body wall, and then peritoneum
and muscle layers are closed in routine fashion around the tube. The tube is sutured to the animal’s back in two sites. A sterile dressing is placed over the incision and around the base of the tube and a light body wrap is applied over that. The end of the tube is closed with an adapter and an injection cap.

Discussion

Mother reared maxwell duikers typically gain 30-70 g/day in the first 2 wk of life. The fact that the animal in this report had lost almost 25% of its body wt in the first week meant that it was severely calorically challenged and in danger of an imminent crisis. Usually animals that are this stressed are presented comatose, hypoglycemic and close to death. In these cases immediate rehydration and early nutrient delivery is essential for survival. It is our experience that waiting for an animal to learn to nurse at this stage often results in death. Teaching or developing an appropriate nursing behavior takes time and can not be reliably used with animals that are “on the edge.” The abomasal feeding tube allowed for appropriate feeding while bottle training ensued.

Chevrotain birth wt range from 260-320 g for healthy neonates. Mother reared calves gain 30-50 g/day and double their birth wt within10-14 days. Previous attempts at hand rearing underweight babies or those that had experienced maternal neglect and weight loss, using conventional methods, were usually unsuccessful. In this case, the calf was removed for abomasal feeding tube placement and hand rearing before its health became compromised. Nutrient delivery began within 12 hr of birth before weight loss or dehydration could occur. Subsequent weight gains were less than a mother reared animal but adequate for growth.

LITERATURE CITED

PRELIMINARY INVESTIGATIONS OF A NON-PATHOGENIC Theileria IN MHORR GAZELLES (Gazella dama mhorr)

Lee A. Young, DVM,¹* Tom Robinson,² and Patricia Conrad, DVM, PhD²

¹Veterinary Services, San Diego Zoo, PO Box 120551, San Diego, CA 92112 USA; ²School of Veterinary Medicine, University of California, Davis, CA 95616 USA

Abstract

Members of the genus Theileria are small protozoal parasites that infect erythrocytes and lymphocytes of wild and domestic ruminants. The organism has been most frequently described in Africa and India but is also observed in North American wildlife and cattle. The susceptibility of the host species and pathogenicity of the particular Theileria species are the determining factors of the severity of the infection. Many Theileria species cause only benign infections but severe, fatal infections are well described. The most significant of these is East Coast Fever (Theileria parva and Theileria annulata), a frequently fatal disease of domestic cattle in East Africa.² A fatal Theileria infection of sable antelope (Hippotragus niger) calves has also been described in South Africa.³

Recent identification of a non-pathogenic Theileria in Mhorr gazelles (Gazella dama mhorr) at the San Diego Zoo has presented interesting questions concerning common concepts of transmission of the organism and concerns about interstate and international transfer of this species.

The Mhorr gazelle is an endangered species, considered to be extinct in its native Morocco and Western Sahara. The North American Mhorr gazelle population descends from animals captured in Spanish Sahara in the late 1970's and transferred to Spain. Most European stock are considered to be from this same collection. This initial population was brought to the San Diego Zoo and its descendants have been shipped to zoos throughout North America.

Historically, a small erythrocyte parasite has been frequently observed in peripheral blood smears obtained as part of a neonatal examination of 1-day-old Mhorr gazelles. No clinical disease has been seen in any Mhorr gazelles at the San Diego Zoo that could be attributed to this organism. Specific identification of the parasite was recently made possible due to advances in diagnostic technology. Due to concerns about this parasite and eventual release of Mhorr gazelles in their native range, blood samples from a 1-day-old and a 1-yr-old Mhorr gazelle were sent to the University of California-Davis for identification and molecular characterization.

Small, rod or ring-shaped piroplasms were identified in Giemsa-stained blood smears. Parasite DNA was extracted from each sample and the 18s ssrRNA gene was sequenced for identification of the organism. A similarity search of the entire GenBank database revealed that the parasite was a member of the genus Theileria. The two isolates were shown to be extremely similar if not identical to each other. The isolate from the older gazelle was most similar (96% similarity) to the Theileria sp.-Thung song isolate, a benign bovine species, and Theileria parva isolate (96%
similarity). The isolate was not homologous with other piroplasm species originating in California wildlife. This suggests that this isolate did not originate in North America and may have been naturally occurring at the time of capture.

*Theileria* infection in all mammals has been traditionally considered to require a tick vector. Identification of this organism considered to require tick transmission in this group of animals was of interest due to the management of this species at the San Diego Zoo and the age at which the *Theileria* are observed. Observation of this parasite in 1-day-old calves suggests that tick transmission is unlikely due to the short period of time available to become parasitemic. There is also no recent history of tick infestation in any animals in the San Diego Zoo collection. This is attributed to the low-humidity, semi-desert climate and hoofstock exhibits that are typically free of vegetation. The extremely young age of some of the infected animals and lack of a tick vector are very suggestive of transplacental transmission of the organism.

Review of 224 CBCs obtained from 69 Mhorr gazelles over the past 5 yr revealed 27 individuals with the organism observed on at least one peripheral blood smear (37% of sampled population). Of these 27 infected individuals, 14 (51%) did not demonstrate the parasite at the time of the neonatal exam but were observed to be infected at subsequent examinations. No gazelles over 2 yr of age were observed to have the parasite.

Obvious concerns exist about interstate and international transportation of this species due to the presence of this organism. It is presumed that other Mhorr gazelles in North America and Europe may be infected as most descend from a single founder group. There are no known reports of *Theileria*-like disease in these captive Mhorr gazelles. Cross-species infection, especially to domestic cattle must also be considered as a possibility. The variety of management situations, including mixed-species exhibits, in which this species is kept throughout the world would appear to give the organism the opportunity to have infected other species. The absence of the specific (and as yet unknown) tick vector for this *Theileria* isolate in North America may prevent transmission of the organism to other species. Most *Theileria* have a very limited number of species of ticks which can successfully act as a vector.¹

The U. S. Department of Agriculture has been informed of the presence of this *Theileria* isolate and has not expressed concern at this time. Individual state veterinarians may be more interested in this organism and could be more resistant to importation of Mhorr gazelles into their state. As of this time, animals have been shipped to a pre-existing herd in Oregon with the approval of the state veterinarian after extensive discussions of the preliminary findings. Further investigations should be pursued before release of these animals into their native habitat. These investigations will include a survey of other captive Mhorr gazelle populations for the presence of the organism. Ideally, transmission studies in domestic cattle and surveillance of domestic livestock in the country of origin should also be performed.

ACKNOWLEDGMENTS
The authors wish to thank the Veterinary Services and Clinical Pathology Laboratory staff for their assistance in sample collection and diagnostic support.

**LITERATURE CITED**


SUSCEPTIBILITY TO COLD IN CAPTIVE GIRAFFE (Giraffa camelopardalis)

Marcus Clauss, MSc, 1* Wm. Kirk Suedmeyer, DVM, 2 and Edmund J. Flach, MA, MSc, VetMB, CertZooMed, MRCVS 3

1 Institute of Zoo Biology and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany; 2 Kansas City Zoological Gardens, 6700 Zoo Drive, Kansas City, MO 64132 USA; 3 Veterinary Science Unit, Institute of Zoology, Whipsnade Wild Animal Park, Dunstable, Beds., LU6 2LF, United Kingdom

Abstract

There are numerous reports of dead captive giraffe with no discernible cause of death except serous atrophy of adipose tissues. The term “peracute mortality syndrome” has been utilized to describe this finding. 11 Although the common linolenic acid deficient status of captive animals might contribute to the problem; 5 there must be another triggering mechanism. As cold stress is a well-known inducer of lipolysis, the probability of captive giraffe being cold-stressed was examined by a literature search.

In the wild, giraffe are renowned for their ability to withstand extreme heat and radiation. 21 In captivity, they rarely seek shade even on hot days. 7 Two hypotheses explaining this phenomenon exist: The unusual body shape of the giraffe is believed to maximize heat dissipating surface relative to volume, 3 and an obligatory heterothermia allows giraffe to save energy for body temperature (BT) regulation by simply allowing body temperature to rise along with ambient temperature. 17,18 The correlation found between ambient and body temperature is demonstrated in Fig. 1. In contrast, the zoo medicine literature gives a constant normal BT of captive giraffe of about 37.5-38.8°C. 20

While these adaptations allow giraffe to withstand their share of heat, in theory, the same adaptations make them especially susceptible to cold. If they have a comparatively greater heat-dissipating surface, any circumstance that increases heat convection (e.g., wind or humidity) should have a more pronounced effect on them. If they can depend upon ambient temperature to maintain their BT, and allow it to decrease with nighttime temperatures, any condition that forces them to keep their BT elevated against colder temperatures should impose an additional energetic cost on them-possibly to the extent that cannot be compensated. For free-ranging giraffe, high mortalities have been reported after a period of exceptionally cold and wet weather. 29 Gastrointestinal capacity and insufficient diets limit the ability to compensate for the energy demands during these periods.

With prolonged cold exposure, blood glucose (Glc), phosphorus (P) and thyroid hormones (TH) should be increased, while calcium (Ca) and magnesium (Mg) should be decreased. 24,26 In a state of prolonged hyperthyroidism, as might be induced by constant cold stress, leukocytosis, lymphopenia, hyperglycemia and hyperphosphatemia can develop. 22 Blood values related to the energy status of the animal such as Glc and TH should be related to basal metabolic rate; such a relation has been demonstrated for Glc. 28
Selected blood values from free-ranging and captive giraffe are presented in Table 1. In general, captive giraffe sampled appear to have a leukocytosis, lymphopenia, hypocalcemia, hyperglycemia, and hyperphosphatemia. While Glc levels may increase due to handling stress, it is debatable whether these consistent high values can be explained by excitement alone. Mg values are generally unchanged, but the sample size for this mineral was extremely small \((n = 3)\). In theory, giraffe showing signs of peracute mortality syndrome might suffer from electrolyte imbalances.

A deficiency in linolenic acid, as has been reported for captive giraffe\(^6\) could, in theory, contribute to an increase in metabolic rate and diminished adipose tissue stores,\(^13\) lymphoid depletion,\(^10\) a decrease in both insulin responsiveness and insulin secretion, and a disturbance of myocardial Ca homeostasis.\(^1\) A zinc deficiency could, in theory, contribute to a leukocytosis and a lymphopenia,\(^8\) and a decrease in adipose tissue stores.\(^14\) Both deficiencies should manifest themselves in skin lesions, which have rarely been reported in captive giraffe.

Conclusions

While precise studies are lacking, reported data confirms the hypothesis that captive giraffe should be prone to cold stress. In eland (*Tragelaphus oryx*), another browsing ruminant with body temperature regulation similar to that of giraffe,\(^27\) two cases of death related to stressful events revealed serous atrophy of fat as the only necropsy finding.\(^25\) These appear to be the only cases of “peracute mortality” in another ungulate species to date.

Recommendations for indoor housing temperatures for giraffe range from 13-16°C\(^2\) to 21-22°C.\(^20\) These temperatures are below or close to the ambient temperature that led to minimal BT in free-ranging giraffe.\(^18\) A consistent recommendation is that giraffe should not be housed outdoors if temperatures are below 10°C.\(^20\) In light of the high incidence of peracute mortality syndrome,\(^16\) and its possible link to cold stress, these husbandry guidelines might have to be re-evaluated.

A survey of peracute mortality syndrome related to temperature management and housing latitude is currently being conducted. Fatty acid evaluations are continuing in captive giraffe.

ACKNOWLEDGMENTS

The authors would like to thank the staff at the Whipsnade Wild Animal Park, England, and the Kansas City Zoological Gardens giraffe management staff, for conditioning efforts with their giraffe. In addition, a special thanks to Dr. Murray Fowler, whose insight into the initial syndrome, and his contributions to the field of zoological medicine are greatly appreciated.

LITERATURE CITED

Figure 1. Correlation of ambient temperature and rectal temperature in three giraffe.

Table 1. Selected blood values in cattle,\textsuperscript{1,2} wild\textsuperscript{9,19} and captive giraffe;\textsuperscript{4,15,23} ranges for captive giraffe are the range of means from the different sources.

<table>
<thead>
<tr>
<th></th>
<th>Cattle</th>
<th>Wild Giraffe</th>
<th>Captive Giraffe</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC $\times 10^9$</td>
<td>4-12</td>
<td>-</td>
<td>9.8-15.9</td>
</tr>
<tr>
<td>Lymph %</td>
<td>45-75</td>
<td>-</td>
<td>14-34</td>
</tr>
<tr>
<td>Ca (mmol/L)</td>
<td>2.1-2.8</td>
<td>3.0</td>
<td>2.0-2.4</td>
</tr>
<tr>
<td>P (mmol/L)</td>
<td>1.4-2.5</td>
<td>1.6</td>
<td>2.0-3.6</td>
</tr>
<tr>
<td>Mg (mmol/L)</td>
<td>0.7-1.2</td>
<td>1.1</td>
<td>0.9-3.1</td>
</tr>
<tr>
<td>Glu (mmol/L)</td>
<td>2.3-4.1</td>
<td>3.0</td>
<td>4.6-11.3</td>
</tr>
<tr>
<td>Glu (mg/dl)</td>
<td>42.1-74.5</td>
<td>54.7</td>
<td>83.3-203.9</td>
</tr>
</tbody>
</table>

FIELD STUDIES OF WILD BACTRIAN CAMELS (\textit{Camelus bactrianus ferus}) IN MONGOLIA
FIELD STUDIES OF WILD BACTRIAN CAMELS (Camelus bactrianus ferus) IN MONGOLIA

Evan S. Blumer,1* Z. Namshir,2 T. Tuya,2 B. Mijiddorj,3 Richard P. Reading,4 and Henry Mix5

1The Wilds, 14000 International Road, Cumberland, OH 43732 USA; 2Mongolian Institute of Biology; 3Mongolian Ministry for Nature and the Environment; 4Denver Zoological Foundation; 5Nature Conservation International

Abstract

Wild Bactrian camels (Camelus bactrianus ferus), the sole extant form of the Old World Camelidae, have a range which is now limited to the Gobi ecosystem of southern Mongolia and north central China. Population estimates for wild Bactrian camels have variously been estimated at 500-900 individuals, with substantial evidence of further decline. In recent years, several investigators have suggested that this decline is due primarily to poor recruitment of young into the population. To identify the causes for this continued population decline, a consortium representing three U.S. zoological institutions (Denver Zoo, the Wilds, Zoo Montana) an NGO (Nature Conservation International, Germany) and two Mongolian governmental agencies (Mongolian Academy of Sciences and Ministry for Nature and the Environment) has developed a multi-faceted initiative to conserve wild Bactrian camels.

In March of 1997, a large-scale aerial census was conducted to determine as accurately as possible current population parameters. This survey covered all of Region A of Great Gobi Strictly Protected Area (39,865 km²) in southern Mongolia, which is believed the home to all wild Bactrian camels in Mongolia. North-south, parallel transects, approximately 15 km apart, were flown, covering a distance of more than 1,700km. All sightings of wild Bactrian camels, as well as all other mega-vertebrates, were recorded by a team of 10 observers. Results of these observations were analyzed using the computer program DISTANCE. The team observed 277 camels in 27 groups (mean group size = 10.26 ± 12.38 S.D.). Population estimates for wild Bactrian camels were 1,985 ± 803 S.E., with a density of 5.0 ± 2.0 camels/100 km².

In a second phase of the project, veterinarians and biologists traveled to the Great Gobi Strictly Protected Area in November of 1998 to develop field anesthesia protocols and to collect initial biologic samples for detailed analysis. Working with a small “semi-tame” population that has resulted from an earlier attempt to establish a captive-breeding program, several anesthetic combinations were evaluated. Over the course of 7 days, 15 anesthetic procedures were conducted on 14 individuals (2 domestic Bactrian camels and 12 wild Bactrian camels). Anesthetic regimens used consisted of variations of:

- Etorphine + Ketamine + Xylazine (EKX) with antagonism by Naltrexone + Yohimbine
- Telazol (Tiletamine + Zolazepam) + Ketamine + Detomidine (TDK) with partial antagonism by Yohimbine
- Ketamine + Xylazine (KX) with partial antagonism by Yohimbine
Drugs were delivered to the animals either by CO₂ powered Pneu-Darts or by hand injection (Original plans to simulate field capture procedures by darting with a Pneu-Dart Rifle were complicated by the confiscation of the rifle by Chinese authorities in Beijing on 10/30/1998). As a result of the approachability of these "hand raised" animals, darting distance was generally >15 m, however manual restraint of non-sedated animals was not possible.

- Eight procedures (three males and five females) used Etorphine (2.5-4.0 mg Total Dose; $\bar{x} = 3.2$ mg) + Ketamine (0.3-0.6 mg/kg; $\bar{x} = 0.5$ mg/kg) + Xylazine (0.1-0.4 mg/kg; $\bar{x} = 0.2$ mg/kg)
- Five procedures (five females) used Telazol (1.1-1.6 mg/kg; $\bar{x} = 1.3$ mg/kg) + Ketamine (1.0-1.3 mg/kg; $\bar{x} = 1.1$ mg/kg) + Detomidine (0.03-0.04mg/kg; $\bar{x} = 0.04$ mg/kg)
- Two procedures (one male and one female) used Ketamine (1.0-2.4 mg/kg; $\bar{x} = 1.7$ mg/kg) + Xylazine (0.4-0.5 mg/kg; $\bar{x} = 0.45$ mg/kg)

All animals were extensively monitored during anesthesia. In addition to basic monitoring of heart rate, respiratory rate and body temperature, attempts were made to monitor multiple physiologic processes during anesthesia through the use of portable, electronic monitoring devices provided by the Heska Corporation. Attempts to monitor SpO₂, heart rate (electronically), end-tidal CO₂, respiratory rate (electronically), ECG, serum chemistry and blood gases met with variable success as a result of the unique physiology of the camels, and the harsh field conditions present in the Gobi Desert. There were no mortalities resulting from the anesthetic procedures and a preliminary assessment suggests that wild Bactrian camels do respond differently to various anesthetic agents than do domestic camels.

Significant differences were noted in the effectiveness of the anesthetic protocols (Table 1). Time to effects varied significantly. Time to first effect (TFE) and time to recumbency were shortest for EKX followed by TKD and KX respectively. However, despite complete antagonism of the anesthetic drugs in the EKX protocol, recovery was slower. Subjectively, this appears due to the fact that EKX produced a deeper plane of anesthesia than either TKD or KX.

Physiologically, the protocols produced differing effects. While there were fluctuations in the level of oxygen saturation of the peripheral blood, as measured by pulse-oximeter, with all of the protocols, TKD ($\bar{x} = 86.1\%$) and KX ($\bar{x} = 84.6\%$) resulted in better oxygenation than EKX ($\bar{x} = 80.4\%$). This trend may however reflect the lighter plane of anesthesia resulting from these protocols. Cardiovascular parameters showed more remarkable differences. Mean heart rates were highest for EKX (52.8bpm) with TKD (40.4bpm) and KX (33.1 bpm) following respectively. Additionally, subjective evaluation of mucous membrane perfusion appeared better in EKX.

Evaluations of venous blood gases (chosen over arterial samples due to practicalities of collection from camels in the field and with heavy hair coats) and peripheral blood ph, suggest that animals anesthetized with the EKX protocol experienced substantial acidosis when compared to either a “standard” mammalian model or the camels anesthetized with the other protocols. Based on samples collected at the end of each anesthetic episode, the EKX group showed a mean peripheral blood pH of 7.15, compared to 7.34 for both the TKD and KX groups. Analysis of mean PCO₂ for the three
groups showed a notable elevation only for the EKX group (EKX = 58 mm Hg; TKD = 39 mm Hg; KX = 47 mm Hg). These results can be best interpreted as resulting from a deeper plane of anesthesia in the EKX group resulting in both central nervous system depression and less effective ventilation. The reported acidosis is most likely respiratory in nature, which is further supported by a lack of disturbance in the anion gap for any of the groups (EKX = 15 mEq/L; TKD = 12 mEq/L; KX = 11 mEq/L).

In addition to samples collected for physiologic monitoring, a large number of biologic samples were collected for hematologic, parasitologic, genetic, and endocrine evaluations. Samples collected included:

1. Blood for complete blood count, serum chemistry, exposure to infectious diseases, genetic studies, reproductive and stress hormones, hemoparasites and serum banking
2. Feces for gastrointestinal parasites, fecal hormone metabolites, fecal DNA and diet evaluations
3. Hair for genetic studies
4. Ectoparasites and skin scrapings
5. Body measurements for weight estimations, development of body condition scoring system and identification of transmitter placement for future telemetry and satellite tracking studies
6. Biopsies and cultures of atypical lesions

Analyses of these samples are currently underway.

Based on the findings of these studies, several individuals from wild herds will be captured in 1999 and 2000 for the application of either radio-telemetry or satellite collars. These animals will serve both as focal individuals and sentinels for studies of the ecology and behavior of the wild population. Efforts are currently underway to identify several Mongolian biologists who will be trained in modern ecologic techniques to serve as the primary field researchers for this phase of the project.

Another component of the program is the development of non-invasive endocrine monitoring techniques to assess reproductive patterns in the population. Working initially with captive, domestic Bactrian camels in U.S. zoos, efforts are underway to develop techniques for the assessment of estrogen, progesterone, and testosterone metabolites in Bactrian camel fecal samples. Once adequately validated in the laboratory using the domestic animal model, wild Bactrian camels will be monitored to determine if any reproductive abnormalities exist.

The ultimate goals of this project are to determine the causal factors for population decline in wild Bactrian camels, and to develop strategies to prevent a continuation of this pattern. While this species clearly has great cultural, biologic and economic value, it may be most important as a “flagship species” for the Gobi ecosystem. In addition to the inherent benefits of conserving one of its most important wildlife taxa, Mongolian conservation efforts should benefit from the extensive training of numerous Mongolian biologists and veterinarians as well as the transfer of technology to in-situ conservation initiatives.

ACKNOWLEDGMENTS
LITERATURE CITED


Table 1. Effectiveness of the anesthetic protocols (x time).

<table>
<thead>
<tr>
<th>Drug Protocol</th>
<th>T-First Effect</th>
<th>T-Recumbency</th>
<th>T-Sternal</th>
<th>T-Stand</th>
</tr>
</thead>
<tbody>
<tr>
<td>M99+Ket+Xyl</td>
<td>0:04:20</td>
<td>0:06:30</td>
<td>0:05:40</td>
<td>0:09:05</td>
</tr>
<tr>
<td>Tel+Ket+Det</td>
<td>0:05:30</td>
<td>0:12:00</td>
<td>0:01:00</td>
<td>0:02:00</td>
</tr>
<tr>
<td>Ket+Xyl</td>
<td>0:07:00</td>
<td>0:16:00</td>
<td>0:02:00</td>
<td>0:05:00</td>
</tr>
</tbody>
</table>
DIAGNOSTIC THERMOGRAPHY: APPLICATIONS IN ZOO ANIMAL MEDICINE

Tracy L. Clippinger, DVM* and Robert A. Cook, VMD

Department of Wildlife Clinical Sciences, Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, NY 10460 USA

Abstract

Skin serves as a thermoregulatory interface between the metabolic processes of the body and the state of the environment. Skin temperature depends on many internal and external factors, including: body metabolic state, thermal conduction from internal heat sources, vascular activity adjacent to skin surface, evaporative heat losses, air current convection, and reflection from environmental energy sources. Heat radiation is distinctive at anatomic sites and a high degree of symmetry is present between right and left sides of the normal whole body. Inflammatory processes (e.g., trauma or infection) and neurologic processes (e.g., nerve damage or irritation) may alter circulation and metabolism, and thus, the surface temperature of the skin.

Thermal imaging identifies and quantifies comparative skin surface temperature. Invisible infrared energy emitted from the skin surface is captured by an infrared scanning device and converted into electric impulses that are displayed in color on a monitor as a map of body temperature, or thermograph. Increased or decreased emissions of heat radiation are indicated by a spectrum of colors. Thermography is a real-time, non-invasive diagnostic aid to identify abnormalities in comparative body temperature. Early stages of injury may be detected as a “hot spot” since heat is one of the cardinal signs of inflammation.

The Diagnostic Thermal Imaging System (DTIS)-500 (eMERGE Vision Systems [eVS], XL Vision and Safeguard Scientiﬁcs, Inc, Sebastian, FL, 32958) features an uncooled, handheld infrared camera, proprietary image processing and database software, and access to a webbed clinical database to provide state of the art technology in thermography for practicing veterinarians. The camera produces a color video image, which is recorded as a digital image that may be stored on a FLASH Memory card. This card may be used to download images into the eVS image processing software, where images may be viewed, catalogued, annotated, enhanced, and archived. The software allows the generation of written reports and image prints. In addition, images that are produced in any video or digital format can be archived in the software system. The webbed clinical database is designed to be a reference tool for the customer base which will obtain and manage information, share knowledge, and document images and the imaging environment.

The DTIS-500 detects and maps temperature differences as small as 0.2°C as it measures the surface temperature of an animal or object from -15°C to +100°C. Control buttons on the camera allow specification of settings for optimal data collection related to patient and environment. Several color palettes with multiple discriminatory shades in each are available for user selection: black/white,
rainbow, blue, red-hot black/white, and lava. For example, in the commonly used rainbow color scheme, areas of increased heat (i.e., injury and inflammation) appear as bright reds, oranges, and yellows; areas of decreased heat (i.e., nerve damage and scar tissue) appear as greens, blues, and purples. The red-hot black/white palette facilitates the rapid location of “hot spots” during a quick scanning procedure. The distance range of the DTIS-500 falls between 1-20 m, thus allowing remote examination of unrestrained animals.

For collection of optimal thermograms, several factors must be addressed (some of which are difficult to control in the zoological setting). Animal motion should be controlled where possible or images should be collected when the animal is relatively still. Low-level lighting with elimination of extraneous radiant energy sources (e.g., the sun) should be sought. An ambient temperature of (68°F) is ideal, while temperatures below (86°F) are recommended. Debris on the haircoat, scar tissue, irregular hair lengths, ointments, and wraps may produce artifacts. Plexiglass and glass interfere insurmountably with image reception, while many screens and cage bars may be focused away for minimal interference. Elimination of artifacts and optimization of the viewing situation is important as asymmetry of even 0.5°C merits consideration for possible pathologic change.

In general symmetry is essential to image interpretation. Identical anatomic images should be compared so that thermal changes can be accurately evaluated. Normal thermal patterns can often be predicted by vascular patterns and surface contour. For example, the “normal” warmer areas of an ungulate include the dorsal midline, ventral midline, between rear legs, and the routes of major vessels through the appendicular musculature (i.e., forelimb cephalic vessel, and rearlimb saphenous vessel). The operator must strive for objectivity in symmetry evaluation of animals, in particular for the great diversity of zoological specimens.

Thermography is limited to the measurement of skin surface temperature as it reflects local conditions. Deeper structures may have increased radiant heat but may be shielded by massive musculature. Coat color pattern and hair coat length cause differential external energy absorbency and reflectivity. Motion and position are difficult to control in zoological patients and so the optimal views to evaluate symmetry (left to right, side to side, front to back) may be very challenging to capture. In contrast to radiography, but similar to ultrasonography, thermograms are not collected at distinct camera settings. The subjective adjustment of camera settings may cloud the comparison of structures or abnormalities over time and dependent upon operator.

Diagnostic thermal imaging serves as a complementary tool for evaluation of soft tissue structures to be used in conjunction with visual examination and other imaging modalities. Critical evaluation of areas of asymmetry in the thermogram may allow zoo veterinarians to more accurately direct attention towards a traumatized or diseased region and highlight regions worthy of further diagnostic study. Thermography has applications in zoo animal medicine in the areas of general health assessment, lameness evaluation, wound management, dental evaluation, localized inflammation, and reproduction.
LITERATURE CITED

TRANSRECTAL ULTRASOUND EVALUATION OF CHEETAHS

Frank Goeritz, DVM,1* Julia Maltzan, DVM,2 Robert Hermes, DVM,1 Henning Wiesner, DVM,2 Lucy H. Spelman, DVM,3 Steffen Blottner, PhD,1 Guido Fritsch, DVM,1 and Thomas B. Hildebrandt, DVM1

1Institute for Zoo Biology and Wildlife Research (IZW), D-10315 Berlin, Germany; 2Zoological Park Munich-Hellabrunn, D-81543 Munich, Germany; 3National Zoological Park, Smithsonian Institution, Washington DC 20008 USA

Abstract

Zoological institutions and private facilities have become increasingly proactive in the management of cheetahs (Acinonyx jubatus) and helping establish conditions necessary for successful reproduction in captivity. Although the number of facilities breeding cheetahs has recently increased, only 10% of them reported successful reproduction.7 The mortality rates of cheetahs has increased gradually over the last 40 yr. The predominant causes of death in animals over 6 mo of age (n = 1266) include kidney and/or liver diseases (21%).8,9 High mortality rates coupled with low reproductive success have left a captive population which is not self-sustaining. The development of reliable methods for health monitoring and reproductive assessment is needed to optimize breeding management of captive cheetahs. This type of program could also assist in managing and conserving the other 36 extant wild felid species maintained in zoos.6

There is relatively little information on ultrasonography in cheetahs.10 Although ultrasonography has been described as a diagnostic and research tool in zoological medicine,5 laparoscopy is still the most common technique for visualizing the internal genital organs. This is particularly true for evaluation of the ovaries and nonpregnant uterus in felids.1 However, ultrasonography could be very useful for non-surgical monitoring of the reproductive status in cheetahs. Furthermore, sonographic examination of the major abdominal organs (such as liver, spleen, adrenals, and kidneys) may provide additional information to determine the relative fitness and breeding potential of individuals.

To develop an ultrasonographic examination protocol, seven captive cheetahs (Acinonyx jubatus; three males and four females) were anesthetized and imaged using transrectal and transcutaneous ultrasonography. Two females and one male were examined four times, before and after hormonal treatment for artificial insemination and electroejaculation, respectively. A portable real-time, B-mode computer sonograph (EUB 405, Hitachi) equipped with a small curved, 7.5 MHz and a 10.0 MHz linear array as well as a 3.5 MHz convex transducer was used for the study. For transrectal application the small 7.5 MHz probe was fitted into an extension specially designed for medium sized mammals which has been applied in several exotic species.2,5

In general transcutaneous ultrasonography allowed successful imaging of the major abdominal organs, such as liver, spleen, kidney, and urinary bladder. In contrast to transcutaneous ultrasound, transrectal ultrasonography allowed excellent visualization of the entire urogenital tract including vagina, cervix, uterus and ovaries in all females independent of their reproductive status. Follicles and corpora lutea
were clearly distinguishable in males, the accessory sexual glands and testes were visualized by transrectal and transcutaneous ultrasonography, respectively.

Due to the knowledge of the sonomorphology of the abdominal organs it was possible to differentiate normal structures from pathologic alterations (i.e., nephropathy, nephromegaly, splenic myelolipoma, hydrosalpinx, endometrial cysts, cholecystopathy with gallbladder sediment). With the exception of one animal with clinical renal disease, all alterations described were incidental findings.

In conclusion ultrasonography provides a diagnostic tool for visualization of the abdominal organs in cheetahs. In contrast with transcutaneous ultrasound, the advantages of the rectal application are detailed imaging of the ovaries including follicles and corpora lutea, and the ability to detect the nongravid uterus. Transrectal ultrasonography offers an accurate, non-invasive practical method for health monitoring and reproductive assessment in cheetahs and possibly in other endangered felid species.

LITERATURE CITED

MAGNETIC RESONANCE IMAGING FOR LESION LOCALIZATION IN PSITTACINES WITH CHRONIC SINUSITIS

Geoffrey W. Pye, BVSc, MSc, R. Avery Bennett, DVM, MS, Susan M. Newell, DVM, MS, and Rick Johns, RT(R)(MR)

1Department of Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32609 USA; 2Department of Radiology, Shands Hospital, University of Florida, Gainesville, FL 32609 USA

Abstract

Magnetic resonance imaging (MRI) of the skull and beak was used as a diagnostic tool in eight cases of chronic sinusitis in psittacine birds (three African gray parrots, Psittacus erithacus, two blue and gold macaws, Ara ararauna, a scarlet macaw, Ara macao, a harlequin macaw, Ara ararauna × macao, and a yellow-naped Amazon parrot, Amazona oratix auropalliata). Six of the birds had abnormal magnetic resonance images, including lesions involving the infraorbital and beak sinuses. The MR images allowed localization of the lesion within the sinuses and this enabled the most appropriate surgical approach to be used for the removal of the lesion. Contrast MRI was used in one bird to determine the nature of the lesion. Two of the birds had normal MR images. MRI is superior to radiography and computed tomography (CT) in lesion localization and differentiation. MRI is an excellent diagnostic tool for the localization of lesions in psittacines with chronic sinusitis.
COMPARISON OF BARIUM AND IOHEXOL AS GASTROINTESTINAL CONTRAST MEDIA IN AVIAN RADIOGRAPHY

Edward J. Gentz, MS, DVM, Dipl ACZM,†* Nathan L. Dykes, DVM, Dipl ACVR, George V. Kollias, Jr. DVM, PhD, Dipl ACZM, and Victor T. Rendano, VMD, MS, Dipl ACVR

Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, 14853 USA; †Present address: Wildlife Center of Virginia, PO Box 1557, Waynesboro, VA 22980 USA

Abstract

Barium sulfate, iohexol, and half-strength iohexol solutions were compared as gastrointestinal contrast media in three different psittacine species. Gastrointestinal contrast studies utilizing iohexol were of comparable diagnostic quality to those using the more routinely recommended barium sulfate. Iohexol should be considered as an equivalent alternative when planning avian gastrointestinal contrast studies. Half-strength iohexol did not provide adequate opacification as a gastrointestinal contrast agent. Although not isotonic, no adverse effects were associated with the use of iohexol as a gastrointestinal contrast agent. In all three species, films made within the first hour of administration of contrast media will show all portions of the gastrointestinal tract opacified, with the exception of the cloaca.

Introduction

The indications for gastrointestinal contrast radiography in avian medicine are generally the same as in mammals. Studies to assess different contrast media in birds are limited.2,5 The primary indications for a gastrointestinal contrast study in birds include regurgitation, vomiting or diarrhea, palpation of an abnormal crop or abdominal mass, or abnormal radiographic findings such as an obstructive bowel pattern, organomegaly or organ displacement.4 Similar to mammals, barium sulfate suspension is contraindicated if gastrointestinal perforation is suspect.7 However, the routine use of iodinated gastrointestinal contrast agents have not been recommended for GI studies in birds,6 probably because of the hypertonicity of the solutions.

The use of a non-ionic, iodinated contrast media, iohexol (Omnipaque, Winthrop Pharmaceuticals, New York, NY, USA), as a gastrointestinal contrast agent was reported in a cockatiel (Nymphicus hollandicus) with crop stasis.9 Iohexol (240 mgI/ml) has low osmolality (520 mOsm). A recent study in pigeons (Columba livia) described the safety and efficacy of iohexol.3

The purpose of this study was to evaluate gastrointestinal studies made with either barium sulfate suspension or iohexol solution in three psittacine species of differing body size.
Materials and Methods

Nine birds were used in a cross-over design study, each bird serving as its own control. We used three African grey parrots (*Psittacus erithacus*), body wt range 371-440 g; three monk parakeets (*Myiopsitta monachis*), body wt range 104-148 g; and three budgerigars (*Melopsittacus undulatus*), body wt range 43-47 g. On three separate occasions, each bird underwent a complete gastrointestinal radiographic study. Contrast media used was barium sulfate 25% w/vol suspension, iohexol 240 mgI/ml solution, and iohexol 120 mgI/ml solution (normal saline diluent). The volume of contrast media administered was the same for each study but the smaller species were given relatively more contrast media. The African grey parrots received 0.025 ml/g body wt (approximately 10 ml), and the monk parakeets and budgerigars received 0.05 ml/g body wt (approximately 6 ml and 2 ml, respectively). Birds were fasted overnight preceding the study. All birds were dosed using a flexible feeding tube to deposit the contrast media into the crop. Radiographs were made with the bird physically restrained on a plexiglass restraint board; neither sedation nor anesthesia was used to avoid chemical effects on gastrointestinal motility. Radiographs were made from the right lateral recumbent and ventrodorsal positions at 5, 15, 30, 60, 120, 180 and 240 min after contrast media administration. All radiographs were made on Lanex Fine screens (Kodak X-Omatic Cassette, Kodak, Rochester, NY, USA) with TML-RA film (Kodak, Rochester, NY, USA) exposed with a 40” SFD at 2.5 mAs and either 52 kvp (budgerigars) or 54 kvp (parrots). The birds were observed closely during and for 48 hr following the procedures. This protocol was approved by the Cornell University Institutional Animal Care and Use Committee.

Each study was evaluated individually and separately by two radiologists (NLD, VTR). While the species was obvious, the contrast medium was not known during the reading. Each examiner was asked to comment on intestinal transit, clarity of viscera and ability to identify specific regions in the gastrointestinal tract. Evaluations were considered qualitative; no statistical analyses were made. There was good agreement among the two radiologist examiners and results are presented as consensus.

Results

In African grey parrots the undiluted iohexol opacified the proximal portions of the gastrointestinal tract longer than either dilute iohexol or barium, although this was not true in every case. The diluted iohexol study was less opaque overall and had faster transit times through the bowel. Lumen margins were harder to see and opacity tended to decrease with time. Some luminal contents appeared frothy with indistinct margins.

In monk parakeets all contrast agents tended to leave the upper gastrointestinal tract more rapidly. The intestines remained opacified for several hours with any contrast agent. Overall transit times were rapid with either iohexol concentration. For the diluted iohexol, readers felt there was more rapid GI transit and poor opacification of structures.

In the budgerigars the iodinated contrast agents tended to stay in the crop and esophagus though emptied faster from the proventriculus and ventriculus. As in other species studied, films made within the first hour will show all portions of the gastrointestinal tract except the cloaca.
None of the birds regurgitated contrast media after administration. However, some birds did reflux some contrast (any type) later in the study. This was documented by appearance of contrast media in the nasal sinus cavity. This usually occurred after 2 hr. No clinical abnormalities were associated with this phenomenon or in general during the 48-hr observation period following each study.

In general, diluted iohexol, not surprisingly, produced the least opacification of bowel. Some studies showed decreasing opacity with time most likely as a result of further dilution. Barium produced consistently well opacified bowel lumens in all the study species. Iohexol-240 mgI/ml also produced well opacified bowel lumen and was not distinguished from barium by the observers of this study.

**Discussion and Conclusions**

Gastrointestinal contrast studies are performed to enhance visualization of the morphology of the intestinal tract. Alteration in the size, shape, opacity, location and distribution of the components of the bowel are interpreted to various disease conditions. Contrast materials make it easier to see the bowel and discern these changes. Functional aberrations of transit time, partial or complete obstruction, or malabsorption and malabsorption are interpreted from changes in morphology of the bowel. Morphologic abnormalities are easier to describe and functional conclusions are subject to interpretation error. This study confirms that different contrast materials are suitable to visualize the gastrointestinal tract in avian species and there is variation between species, among individuals, and between contrast media in the ability to demonstrate bowel morphology.

Results suggest that either 25% w/v barium suspension or iohexol-240 mgI/ml are useful for avian GI radiography. Diluted iohexol produced less opaque bowel and increased rate of transit through the proximal portions of the intestine making evaluation of these regions more difficult. Also, dilute contrast makes lumen margins less distinct and defeats the major purpose of contrast radiography. These results contrast with those of a recent study that concluded that half and full strength iohexol were equally efficacious in such studies.¹

Both barium and full strength iohexol provide excellent morphologic distinction and choice of agent is mediated by consideration of possible bowel perforation. When suspected, the non-ionic, iodinated contrast medium, iohexol is preferred over the particulate barium suspension. While not confirmed, we strongly suspect that barium in the coelomic cavity would induce an inflammatory response as it does in mammals. Though not isosmotic at the undiluted concentration, there were no ill effects observed with iohexol in any of the birds.

All contrast media moved rapidly into the intestines in all birds. In most studies, the intestine was opaque very early and stayed so for nearly 4 hr. From these results, a contrast study of the avian gastrointestinal tract can be performed in 1 hr in most birds. Films should be made immediately after contrast is given and subsequent examinations timed by the rate of transit through the bowel. If the “upper GI” regions are of prime interest, more films should be made early in the hour. We could recommend every 15 min, though this may be very stressful for ill birds. We do recommend that sufficient films be made to visualize all portions of the gastrointestinal tract and that any morphologic
abnormalities are persistent in more than one film. This prevents transitory phenomenon such as incomplete lumen filling, peristalsis, or ingesta being confused with pathologic processes. A true morphologic abnormality should be persistent on multiple films.

This study suggests that either iohexol 240 mgI/ml or 25% w/v barium suspension can be used for safe, effective, and efficient contrast studies of the avian gastrointestinal system.

ACKNOWLEDGMENTS

This study was supported by a grant from the Joint Alumni/Faculty Committee on Unrestricted Funds of the Cornell University College of Veterinary Medicine.

LITERATURE CITED

TECHNIQUES IN ADVANCED IMAGING OF FISH

Reid Tyson, BS,* Nancy E. Love, DVM, Dipl ACVR,1 Gregory Lewbart, MS, VMD,2 and Robert Bakal, MS, DVM2

1Department of Anatomy, Physiological Sciences, and Radiology and 2Department of Companion Animal and Special Species Medicine, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606 USA

Abstract

The usefulness of imaging in fish has been demonstrated.4 However, there is limited information regarding imaging protocols or the normal anatomy of fish using conventional radiography or alternate imaging modalities. The strict environmental factors required by fish limit the availability of diagnostic tests available to the practitioner. With proper preparation and planning, imaging can be a beneficial non-invasive addition to the diagnostic plan. The purpose of this presentation is to (1) present selected modalities that can be used to image fish, (2) describe selected successful imaging protocols for fish, and (3) present an example that highlights the strength of each modality discussed. Imaging modalities that have been utilized in fish include conventional radiography, digital fluoroscopy, ultrasonography, computed tomography (CT), nuclear medicine and magnetic resonance imaging (MR).1,2,4 While protocols have been published for non-contrast conventional radiography in fish, there is limited information on the imaging protocols of fish using CT and MR. Three normal koi (Cyprinus carpio) were imaged to determine normal anatomy and to develop and optimize imaging protocols for koi pairing conventional radiography and oral contrast, CT, and MR.

Conventional radiography is excellent for looking at bone and swim bladder abnormalities.4 Indications for use in clinical cases include suspected swim bladder abnormalities, bone changes (e.g., fractures, scoliosis), suspected mechanical ileus and identification of coelomic or superficial masses. To better evaluate abdominal coelomic structures, an upper gastrointestinal (UGI) contrast examination may be needed. This is due to the inherent poor soft tissue detail fish have on radiographs. Oral administration of positive contrast media can be used to improve visualization of the GI tract and aid in the evaluation of GI transit times. Fish for this project were imaged in right to left lateral, dorsoventral, and left lateral decubital views. A red rubber catheter was used for p.o. administration of the contrast media, Iohexol (OmnipaqueÆ 240, Nycomed, Princeton, NJ).

CT provides good soft tissue information and excellent bony detail. The advantages of CT over MR include cost, time of scan, patient handling, and identification of osseous changes. Both modalities are excellent for reducing anatomic complexity and providing anatomic detail. Helical whole body CT scans were used because of the short imaging times and increased flexibility for image reformatting and manipulation. Indications for CT use in clinical patients include detailed examination of bone, swim bladder problems and visualization of abdominal masses.
MR provides superior soft tissue detail compared to the other modalities, but is complicated by the time required for each scan, logistics of anesthesia, and cost. MR imaging presents novel difficulties. Ferromagnetic metals are not allowed in the MR chamber and the time needed for MR scans can be lengthy, therefore we adapted a previously described anesthesia machine for this function. Life support and anesthesia were achieved using an extended, out of room, open circuit system. Fish were imaged using a resin coated human wrist radiofrequency (RF) coil. Clinical indications for MR imaging include evaluation of abdominal masses, detailed soft tissue visualization, and swim bladder abnormalities.

Immobilization and anesthesia for imaging procedures was achieved with tricaine methane sulfonate or MS-222 (Finquel®, Argent Chemical Laboratories, Redmond, WA), which has been proven to be efficacious in a variety of fish. Many fish will not need anesthesia for conventional radiography examinations. Immobility is imperative for CT examinations, for CT scans less than 4 min, anesthesia was achieved by inducing the fish in 200 ppm of MS-222 until cessation of opercular movement. Species susceptibility to MS-222 varies widely, therefore dosages are somewhat species specific. Fish were placed on the CT gantry and imaged in sternal recumbency. Recovery was achieved by placing the fish in fresh water. For procedures lasting more than 4 min (MR) fish were managed using the previously described general anesthesia machine.

ACKNOWLEDGMENTS

Project supported by Paul Fisher, Director of the Biomedical Imaging Resource Facility, North Carolina State University. A special thank you to Cecil Charles, Magnetic Resonance Imaging Center, Duke University.

LITERATURE CITED

ULTRASOUND MONITORING OF THE SEXUAL MATURATION IN THE MALE ELEPHANT

Thomas B. Hildebrandt, DVM,1* Guido Fritsch, DVM,1 Robert Hermes, DVM,1 Katharina Jewgenow, PhD,1 Michael Rudolph, PhD,1 Julia Maltzan, DVM,2 Henning Wiesner, DVM,2 Nancy C. Pratt, PhD,3 Don Neiffer, DVM,3 Dennis L. Schmitt, DVM, PhD,4 and Frank Göritz, DVM1

1Institute for Zoo Biology and Wildlife Research, Berlin, D-10315 Germany; 2Tiergarten Hellabrunn, München, D-81543 Germany; 3Disney’s Animal Kingdom, Lake BuenaVista, FL 32830-1000 USA; 4Dickerson Park Zoo, Springfield, MO 65803 USA

Abstract

In general, the reproductive rate of elephants in captivity is low. This is partly because of logistic difficulties associated with transporting these large animals for breeding purposes and there may be physiologic problems which also contribute to this low reproductive rate. In context with a reproductive assessment1 of potential breeding bulls it appears that many adult bulls of both species (Loxodonta africana and Elephas maximus) are not producing viable sperms and/or sufficient ejaculate. Our current understanding of incomplete sexual maturation or temporary infertility in male elephants is at best fragmentary.2,3 The following study was performed for characterizing the physiologic sexual maturation process in young male elephants. Two adolescent individuals of both species have been examined in order to investigate the time of their sexual maturity. The examination utilized transrectal ultrasonography of the urogenital tract, rectal stimulation for the collection of ejaculates as well as blood samples for plasma testosterone determination. The development of the testes, the accessory glands (especially the ampullae), the concentration of the testosterone, the body-height and the success of ejaculation after manual stimulation3 was documented and evaluated over a 3-yr period. The results were compared with data from other adult bull elephants which had ultrasonographic examinations or post mortem investigations.2 The findings of this study led to important conclusions about the characterization of the reproductive status of male elephants by means of ultrasonographic examinations. We established criteria for reproductive soundness in connection with the recruitment of potential semen donors for future artificial insemination projects. The ultrasonographic examination combined with the semen collection were appropriate methods for characterizing the exact state of sexual maturity or for identifying potential reproductive disorders in male elephants.

ACKNOWLEDGMENTS

The authors are grateful for the assistance from the elephant staff of the Pittsburgh Zoo, the Muenich Tiergarten Hellabrunn and the Disney’s Animal Kingdom for training the male elephants to stand for the ultrasound examination and semen collection. The authors thank the zoo veterinarians Willem Schaftenaar from the Rotterdam Zoo, Reinhard Göltchenboth and Andreas Ochs from the Zoological Garden Berlin for their contributions to our study.

LITERATURE CITED
DIAGNOSTIC IMAGING - HANDLING DIGITAL IMAGES

John R. Thompson, BS

Emerge Vision Systems, Inc., Sebastian, FL 32958 USA

Abstract

Over the last decade, digital imaging technology has made major strides within the commercial market. Digital imaging has come a long way with major improvements in computer performance and capacity. There are several digit formats including TIFF (tag image file format), JPEG (joint photographic experts group), and BMP (bitmap). Diagnostic imaging modalities are moving very quickly into the digital domain. This presentation is intended to provide insight into the state-of-the-art of digital imaging. This presentation will also address requirements for digital image storage including image compression and manipulation.

Digital imaging has moved out of the hands of high-end computer workstations and into the world of commercially available off-the-shelf personal computers (PC). Image handling does require noted RAM requirements and large enough hard drive space to move and store images with little concern to the user. Minimum specifications are now based on such issues as how fast do you need to bring the images up for display, display resolution, how large are these images (digital size), and the size of the total database.

Digital cameras, scanning devices, and video capture cards that produce and or convert optical to digital images are now on the commercial market and have well defined format specifications. This discussion will not go into great detail on these systems other than to say that digital image capture and conversion (optical to digital) is becoming a well established commercial technology. One on-going issue regarding digital image capture is resolution. How much resolution is good enough? In the case of radiographs, standards are currently being set to establish which technology should be used to provide the quality needed to resolve the points of interest. This is a major issue in establishing the system specifications and requirements for diagnostic digital imaging modalities.

On the market today, there are many commercially available image processing packages. These software systems allow you to digitally enhance images. These functions include magnification (pan and zoom), filtering (edge enhancement), contrast, brightness, hue, as well as annotation, overlay capabilities, and database development. Some systems will also allow image fusion or being able to overlay various imaging modalities on to one another.

Other systems can create three-dimensional modeling. These systems are intended to enhance the image and to bring out features that might not otherwise be identified. For the most part these systems will be compatible with PC based systems (Windows based).
Digital image storage is important to consider when defining your computer system requirements. There is a huge difference when working with $320 \times 240$ images vs. $4 \times 4$ kbyte images. Space can be used up very quickly. In most cases, images of large size can and should be compressed (JPEG) for storage and segmented for handling ease. When archiving images, an external device could be used to store images offline (Zip Disks). In the case of networked systems, a computer could be designated as a repository for your images. With the interest in global networking (e-mail), a consideration should be given to image management and handling.

Digital imaging is a rapidly expanding area of technology which offers the veterinarian tremendous diagnostic and research opportunities. The application of diagnostic imaging and medical record-keeping databases will merge over the next couple of years. Digital Images will provide the information that once was filed away with hard copy film or stored in disjointed files. Over the next few years, universities and zoological facilities will be using systems which encompass digital imaging to manage and maintain the health and well-being of their animal population. As the old adage goes, “an image can speak a thousand words,” a digital image can speak mega bytes of information.
ANTIMICROBIAL THERAPY: STRATEGIES FOR EFFECTIVE USE OF ANTIMICROBIALS

Mark G. Papich, DVM, MS, Dipl ACVCP

College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 USA

Abstract

Selecting the correct antibacterial drug is one of the most challenging therapies encountered in veterinary medicine. Optimum selection should be based on a logical, systematic approach. This paper will focus on some of the important strategies that can guide antibacterial drug selection in small animal patients.

Penetration to the Site of Infection

For most tissues, antibiotic drug concentrations in the serum or plasma approximate the drug concentration in the extracellular space (interstitial fluid). This is because there is no barrier that impedes drug diffusion from the vascular compartment to extracellular tissue fluid.1 Pores in the endothelium of capillaries are large enough to allow drug molecules to pass through unless the drug is highly protein bound in the blood. Fortunately, there are no antibiotics that have been shown to have high drug protein binding in the plasma to the extent that it is clinically relevant.

Diffusion of most antibiotics in tissues is limited by tissue blood flow, rather than drug lipid solubility. This has been called perfusion-rate limited drug diffusion. If adequate drug concentrations can be achieved in plasma, it is unlikely that a barrier in the tissue will prevent drug diffusion to the site of infection, as long as the tissue has an adequate blood supply. Drug diffusion into an abscess or granulation is sometimes a problem because these conditions lacks adequate blood supply and drug penetration relies on simple diffusion. Low drug concentrations in an abscess also is possible because in a cavitated lesion there is low surface area to volume ratio (low S:V ratio) rather than a physical barrier to diffusion.

In some tissues a lipid membrane (such as tight junctions on capillaries) presents a barrier to drug diffusion. This has been called permeability-rate limited drug diffusion. In these instances, a drug must be sufficiently lipid-soluble, or be actively carried across the membrane in order to reach effective concentrations in tissues. These tissues include: the central nervous system, eye, and prostate. There also is a barrier between plasma and bronchial epithelium. This limits drug concentrations of some drugs in the bronchial secretions and epithelial fluid of the airways, but not to the interstitial fluid of the lung. To achieve effective drug concentrations in bronchial secretions, drugs must be sufficiently lipid soluble to diffuse through the lipid barrier.
Intracellular infections

Most bacterial infections are located extracellularly, and a cure can be achieved with adequate drug concentrations in the extracellular (interstitial) space rather than intracellular space. Intracellular infections present another problem. For drugs to reach intracellular sites, they must be carried into the cell or diffuse passively. For passive diffusion to occur, only lipid-soluble drugs will be able to diffuse through the cell membrane. Intracellular organisms such as *Brucella, Chlamydia, Rickettsia, Bartonella* and *Mycobacteria* are examples of obligate intracellular pathogens. Staphylococci may in some cases become resistant to treatment because of intracellular survival. Fluoroquinolones and tetracyclines such as doxycycline are frequently administered to treat *Rickettsia* and *Ehrlichia* infections. Examples of drugs that accumulate in leukocytes, and other cells are fluoroquinolones, lincosamides (clindamycin, lincomycin), macrolides (erythromycin, clarithromycin), and the azalides (azithromycin). Beta-lactam antibiotics and aminoglycosides do not reach effective concentrations within cells.

Local Factors that Affect Antibiotic Effectiveness

Local tissue factors may decrease antimicrobial effectiveness. For example, pus and necrotic debris may bind and inactivate vancomycin or aminoglycoside antibiotics (for example, gentamicin or amikacin), causing them to be ineffective. Foreign material in a wound (such as material surgically implanted) can protect bacteria from antibiotics and phagocytosis by forming a biofilm (glycocalyx) at the site of infection. Cations such as calcium and magnesium can adversely affect the activity of antimicrobials at the site of infection. Two important groups diminished in activity by cations are fluoroquinolones and aminoglycosides.

An acidic environment of infected tissue may decrease the effectiveness of clindamycin, erythromycin, fluoroquinolones, and aminoglycosides. Penicillins and tetracycline activity is not affected as much by tissue pH, but hemoglobin at the site of infection will decrease the effectiveness of these drugs. An anaerobic environment decreases the effectiveness of aminoglycosides, whereas metronidazole has no activity against aerobic bacteria.

As mentioned previously, an adequate blood flow is necessary to deliver an antibiotic to the site of infection. Effective antibacterial drug concentrations may not be attained in tissues that are poorly vascularized (e.g., extremities during shock, sequestered bone fragments, and endocardial valves).

Bacterial Susceptibility

If the bacteria is accurately identified, antibiotic selection is simplified because the susceptibility pattern of many organisms is predictable. For example, if the bacteria is likely to be *Pasteurella*, or *Streptococcus*, or *Actinomyces*, susceptibility is expected to penicillin or an aminopenicillin such as ampicillin, amoxicillin, or amoxicillin-clavulanic acid (Clavamox). Other susceptibility patterns are discussed below:
**Staphylococcus intermedius**

Staphylococcus isolated from small animals is most likely to be *S. intermedius* rather than *S. aureus*. *S. intermedius* will usually have a predictable susceptibility to β-lactamase resistant β-lactam antibiotics such as oxacillin or dicloxacillin, amoxicillin combined with a β-lactamase inhibitor, or a first-generation cephalosporin such as cephalexin or cefadroxil. Reports of studies on *S. intermedius* have shown that, despite frequent use of the above mentioned drugs in small animals, the incidence of resistance has not increased.3

**Anaerobes**

If the bacteria is an anaerobe (for example, *Clostridium, Fusobacterium, Prevotella, Actinomyces*, or *Porphyromonas*) predictable results can be attained by administering a penicillin, chloramphenicol, metronidazole, clindamycin, amoxicillin-clavulanic acid, or one of the second-generation cephalosporins such as cefoxitin. The activity of first-generation cephalosporins, trimethoprim-sulfonamides/ormetoprim-sulfonamides, or fluoroquinolones for an anaerobic infection is unpredictable.

**Gram-negative bacilli**

If the organism is *Pseudomonas aeruginosa, Enterobacter, Klebsiella, E. coli*, or *Proteus*, resistance to many common antibiotics is possible and a susceptibility test is advised. However, for initial therapy we usually expect the gram-negative enteric bacteria to be susceptible to fluoroquinolones and aminoglycosides. An extended-spectrum cephalosporin (second- or third-generation cephalosporin) usually is active against enteric-gram-negative bacteria, but not *Pseudomonas aeruginosa*. If the organism is a *Pseudomonas aeruginosa*, inherent resistance to many drugs is common, but it may be susceptible to fluoroquinolones, aminoglycosides, or an extended-spectrum penicillin such as ticarcillin or piperacillin. Among the fluoroquinolones, ciprofloxacin is the most active against *P. aeruginosa*. In rare cases, one of the carbapenems such as imipenem is used to treat infections caused by resistant gram-negative bacteria.

**Bacterial Susceptibility Testing**

The *minimum inhibitory concentration* (MIC) of an antibiotic is the lowest concentration of the drug that inhibits visible bacterial growth. This is determined directly, by performing serial dilutions of the antibiotic and determining growth against each dilution. An indirect measurement of the MIC is the agar-disk-diffusion test (ADD). The ADD test results are only qualitative (that is, it determines only resistant vs sensitive), but if performed using standardized procedures, this test is valuable.4

It is becoming more common for laboratories to directly measure the MIC for an organism.4 In this test, resistance and susceptibility are determined by comparing the organism's MIC to the drug's breakpoint. If bacteria have a MIC above the “resistant” breakpoint, the organism is resistant regardless of the dose administered or location of the infection. The MIC can be determined for each bacteria isolated. MIC tests are more quantitative than an ADD test, but must be performed according to strict guidelines.4
**Pharmacokinetic-Pharmacodynamic Optimization of Doses**

To achieve a cure, the drug concentration in plasma, serum, or tissue fluid should be maintained above the MIC, or some multiple of the MIC, for at least a portion of the dose interval. Antibacterial dosage regimens are based on this assumption. However, antibacterial drugs vary with respect to the peak concentration and the time above the MIC that is needed for a clinical cure. Pharmacokinetic/pharmacodynamic (PK-PD) relationships of antibiotics attempt to explain how these factors correlate with clinical outcome.\textsuperscript{5,6} Shown on Fig. 1 are some terms used to describe the shape of the plasma concentration vs time profile. The $C_{\text{MAX}}$ is simply the maximum plasma concentration attained during a dosing interval. The $C_{\text{MAX}}$ is related to the MIC by the $C_{\text{MAX}}$:MIC ratio. The AUC is the total area-under-the-curve. This measurement is taken from time zero to infinity after a single dose. The AUC is related to the MIC value by calculating the AUC:MIC ratio (also called the area-under-the-inhibitory curve - AUIC). Also shown in Fig. 1, is the relationship of time to MIC measured in hours. Examples of how these relationships affect drug regimens are described below.

**Aminoglycosides**

Aminoglycoside (e.g., gentamicin, or amikacin) are bactericidal when the peak plasma concentration are several times above the MIC. If a high enough dose is administered once daily it will produce a peak of 8-10x the MIC. This regimen is at least as effective, and perhaps less nephrotoxic than lower doses administered more frequently.\textsuperscript{7} Our regimens in small animals employ this strategy. Gentamicin has been administered safely and effectively at a dose of 7-10 mg/kg once daily, i.v. The corresponding dose for amikacin is 10-15 mg/kg, i.v.

**Fluoroquinolones**

For the fluoroquinolone antimicrobials, either the $C_{\text{MAX}}$:MIC ratio, or the AUIC may predict antibacterial success. A peak concentration that is 8-10x the MIC, or a AUC:MIC ratio of 125 to 250 has been associated with the optimum antibacterial effect.\textsuperscript{8,9} In some immunocompetent animals, lower AUIC ratios that are 50-60 also may be effective. We administer doses to achieve high $C_{\text{MAX}}$:MIC ratios whenever possible because high ratios have been associated with a lower incidence of development of resistance.\textsuperscript{6,9} To achieve this goal, low doses of fluoroquinolones (for example enrofloxacin 5 mg/kg/day) have been administered to treat susceptible organisms with low MIC, such as *E. coli* or *Pasteurella*, but for bacteria with a higher MIC, (for example gram-positive cocci) a larger dose will be needed. To achieve the necessary peak concentration for a bacteria such as *Pseudomonas aeruginosa*, that usually has the highest MIC among susceptible bacteria, the highest dose in a range is administered (for example, 15-20 mg/kg/day for enrofloxacin). When the MIC values are above the breakpoint, the effectiveness of fluoroquinolones is doubtful because even at high doses, a sufficient $C_{\text{MAX}}$:MIC ratio or AUC:MIC ratio will be difficult to achieve.

**Beta-lactam antibiotics**

$\beta$-lactam antibiotics such as penicillins, potentiated-aminopenicillins, and cephalosporins are slowly bactericidal. Their concentration should be kept above the MIC throughout most of the dosing
Dosage regimens for the β-lactam antibiotics should consider these pharmacodynamic relationships. Therefore, for treating a gram-negative infection, especially a serious one, administer penicillin derivatives and cephalosporins 3-6 times per day. Some of the third-generation cephalosporins have long half-lives and less frequent regimens have been used for some of these drugs (for example cefotaxime and ceftiofur). Gram-positive organisms are more susceptible to the β-lactams than are gram-negative bacteria. Additionally, since the MICs are lower for gram-positive bacteria, and antibacterial effects occur at concentrations below the MIC, longer dose intervals may be possible for infections caused by gram-positive as compared to gram-negative bacteria. For example, cephalexin or amoxicillin-clavulanate have been used successfully to treat staphylococcal infections when administered twice daily.

**Bacteriostatic drugs**

The drugs such as tetracyclines, macrolides (erythromycin and derivatives), sulfonamides, lincosamides (lincomycin and clindamycin), and chloramphenicol derivatives act in a bacteriostatic manner. These drugs inhibit, but may not always kill the bacteria. Subsequently, the drug concentrations should be maintained above the MIC throughout the dosing interval. In this way, they act in a time-dependent manner. Most of the bacteriostatic drugs must be administered frequently to achieve this goal, but some have long half-lives or concentrations persist in tissue that maintain inhibitory drug concentrations throughout the dosing interval with infrequent administration (for example macrolides or trimethoprim-sulfonamides). Most dosage regimens are designed to take these drug’s pharmacodynamic action into account.

**Update on Recent Developments in Drug Therapy**

There have been several new developments in antibacterial therapy in the last ten years. Many of these changes affect drug therapy in zoo and exotic animals because some of these drugs have important uses in these species. Most of these developments have come from a need to treat resistant bacteria with safe drugs. There also is a need to find effective drugs that can be administered more conveniently. In this section is a brief summary of a few of the recent developments for drugs in these animals.

**Cephalosporins**

The 1st generation cephalosporins include common drugs such as cephalexin (Keflex), cefadroxil (Cefatabs), and cepfazolin. Cephalexin and cefadroxil are popular oral drugs; cefazolin is a common injectable antibiotic used for acute treatment and surgical prophylaxis. The spectrum of 1st generation cephalosporins includes most gram-positive cocci, including staphylococcus and some gram-negative bacilli. However, resistance among gram-negative Enterobacteriaceae is common. Extended-spectrum cephalosporins have been classified into the 2nd, 3rd, and 4th generation. The situations in veterinary medicine for which extended-spectrum cephalosporins are most often used are treatment of bacterial infections resistant to other drugs. The bacteria often implicated in resistance problems have been E. coli, Klebsiella pneumoniae, Enterobacter species, and Proteus species. Of the 2nd generation cephalosporins, cefoxitin and cefotetan are the most often administered in veterinary medicine. Their
use has been valuable for treating organisms resistant to the 1st generation cephalosporins or in cases in which there are anaerobic bacteria. When anaerobic bacteria of the \textit{Bacteroides fragilis} group become resistant, they produce a cephalosporinase. Cefoxitin and cefotetan are resistant to this enzyme and may be active against these bacteria. Therefore, these drugs may be valuable for some cases such as septic peritonitis that may have a mixed population of anaerobic bacteria and gram-negative bacilli.

The 3rd-generation cephalosporins are the most active of the cephalosporins against gram-negative bacteria, especially enteric bacteria resistant to other cephalosporins. Compared to other drugs in this group, cefotaxime and ceftizoxime have the best activity against staphylococci. Most of the drugs in this group, must be administered i.v. or i.m. (for convenience, some have been administered to animals s.c.). One should be aware that i.m. or s.c. administration of these drugs can be painful. Cefotaxime, ceftiraxone and ceftazidime are the only cephalosporins that can achieve adequate concentrations in the CSF and for this reason are a rational choice for treating septic meningitis. Compared to other cephalosporins, ceftazidime is the most active against \textit{Pseudomonas}.

Cefixime has been examined in dogs because it is one of the only 3rd generation cephalosporins that can be administered orally. Ceftiofur (Naxcel®) fits most criteria for a 3rd generation cephalosporin, but also has activity against gram-positive bacteria and some have suggested that it should be in a separate class. Ceftiofur is a popular cephalosporin for use in cattle, but also has been used in pigs, sheep, horses, and dogs. In small animals it has been used for urinary tract infections caused by \textit{E. coli}. In large animals it has been used primarily for respiratory infections. When comparing the 3rd generation cephalosporins, one should recall that a susceptibility test that indicates an organism is sensitive to one of the 3rd generation cephalosporins (for example cefotaxime), does not necessarily imply that the organism is sensitive to other 3rd generation cephalosporins.

There is little pharmacokinetic information for cephalosporins in animals other than domestic species. Fortunately, for most mammals, distribution and elimination are similar and dosage regimens do not vary much among species. Absorption differences occur and oral absorption is minimal in ruminants and horses. In non-mammals, some important differences have been identified that are most likely related to prolonged excretion from the kidneys. Elimination in reptiles is slower. For example in a study of ceftazidime in sea turtles, we found that excretion is much longer and half-lives of 20 hr allow for dosing as infrequently as every 72 hr. Regimens for cephalosporins in other reptiles have been published. In birds, poor oral absorption, and necessity for high doses and frequent administration are a disadvantage for cephalosporins.

\textit{Penicillins}

Penicillin G is most often used in the form of procaine penicillin G that is injected i.m. or s.c. to produce long-lasting plasma concentrations for up to 24 hr. Penicillin G has a spectrum that includes gram-positive cocci, such as streptococci, anaerobic bacteria, and a few other susceptible bacteria.
Resistance is common. Amoxicillin and amoxicillin, which are called the *aminopenicillins*, have extended spectrums that include some gram-negative cocci, but resistance also is common to these drugs. Combinations of these drugs with $\beta$-lactamase inhibitors in *Unasyn* (ampicillin + sulbactam) or *Clavamox* (amoxicillin + clavulinate) extends the spectrum to include $\beta$-lactamase-producing strains of staphylococci and gram-negative bacteria.

When penicillins are needed that have an extended-spectrum, penicillin derivatives ticarcillin and carbenicillin (carboxypenicillins) have been used. Other extended-spectrum penicillins include the ureidopenicillins (piperacillin, mezlocillin). These drugs are most often administered for treating *Pseudomonas* infections, although they also have activity against other gram-negative bacteria and anaerobes. Piperacillin has activity against some enterococci. Ticarcillin has been one of the most commonly used of this group for treatment of *Pseudomonas* infections and a few other gram-negative bacteria. (There also is an intrauterine form approved for use in horses.) Dose ranges are higher than one is accustomed for other antibiotics. Ticarcillin (Ticar® ticarcillin disodium) may be administered i.v., i.m., or s.c. at doses of 40-80 mg/kg every 6 hr. For non-i.v. administration, ticarcillin may cause pain and it is acceptable to reconstitute ticarcillin with 1% lidocaine (without epinephrine) instead of water or saline solution prior to injection.

For all the penicillin class, there is little pharmacokinetic or dosing information available for animals except the usual domestic species. Among the domestic mammals, the distribution and elimination are similar for penicillin and penicillin derivatives, therefore doses are equivalent for most animals. Oral absorption of these drugs in ruminants or horses is minimal. In non-mammals there is limited dosing information. In birds, the extended-spectrum penicillins have been used for infections resistant to other drugs, but poor oral absorption and necessity for high doses and frequent administration are a disadvantage. There is limited information available based on studies performed in reptiles. In general, these drugs are eliminated slowly in reptiles and dosing regimens are a reflection of the long half-lives.

*Fluoroquinolones*

The fluoroquinolones include enrofloxacin (*Baytril*), marbofloxacin (*Zeniquin*), difloxacin (*Dicural*), and orbifloxacin (*Orbax*), which are currently approved for small animals (dogs, and for some, only for cats). Enrofloxacin and sarafloxacin are approved for poultry, and enrofloxacin 100 mg/ml injection is approved for cattle. There are other fluoroquinolones approved for use in human medicine (ciprofloxacin, lomefloxacin, enoxacin, ofloxacin), but except for ciprofloxacin, their used has been limited in veterinary medicine.

The fluoroquinolones have the advantage of a broad spectrum of activity that includes most gram-negative bacteria, many gram-positive bacteria (including staphylococci), *Rickettsia*, *Chlamydia*, and some *Mycoplasma*. Important deficiencies in the spectrum of activity include gram-positive cocci, especially enterococci (*Enterococcus faecalis* and *Enterococcus faecium*), and anaerobic bacteria.
**Pharmacokinetic characteristics:** Pharmacokinetic information is available for a great variety of mammals, as well as many zoo and exotic animals. Most of the data has been accumulated for enrofloxacin since it has been available for the longest time. The data on pharmacokinetics allows dosing estimates to be made for many reptiles, birds, and small mammals.\(^{13-17}\) In mammals, the pharmacokinetics of enrofloxacin are similar across species (i.e., half-lives of 2-6 hr, volume of distribution of 3-4 L/kg) and dosing regimens are similar. Oral absorption generally is good in animals with simple stomachs, but decreased in horses and ruminants. Although the extent of absorption may be lower in ruminants after oral administration, the half-life is longer, probably caused from delayed absorption from the rumen. In birds, there is low oral absorption and need for higher concentrations for some bacteria. Subsequently, doses cited for birds are generally higher than for mammals\(^4\) (for example 15 mg/kg/day for routine infections). In reptiles there is great variation in the pharmacokinetics, but enrofloxacin has been a valuable antibacterial drug for these animals.\(^{13,16}\) Our laboratory has measured pharmacokinetics and showed that half-lives for alligators, monitors, turtles, pythons, and tortoises were 55, 36, 18, 6, and 5 hr, respectively.\(^18\) Volume of distribution tended to be large as for mammals. The variation in elimination showed that all reptile species should not be treated with the same dosing intervals, and that for some, the long half-life allows for infrequent administration schedules. We also found that in the reptiles for which oral doses were administered, absorption is generally good, but that oral absorption prolonged the terminal half-life. All studies cited were done in reptiles in which temperature was kept relatively constant.

**Dosage regimens:** Dosages for fluoroquinolones in the approved species are flexible: enrofloxacin, 5-20 mg/kg once daily; orbifloxacin, 2.5-7.5 mg/kg once daily; marbofloxacin, 2.75-5.55 mg/kg, once daily; and difloxacin, 5-10 mg/kg once daily. The flexible dose is intended to allow for a differences in susceptibility among bacteria and increased doses for infections that may be more refractory to treatment. Gram-negative bacilli such as *Escherichia coli* that have a low MIC (e.g., <1.0 µg/ml) can be treated with the lowest dose in the range. Organisms that have a high MIC, (0.5-1.0 µg/ml) should be treated with the highest dose in the range. Organisms with intermediate susceptibility can be treated with moderate doses. Doses also have been published for small mammals, reptiles, and birds.\(^{13,14,17}\) These doses reflect the dosing requirements for susceptible bacteria in these animals based on available pharmacokinetic data and susceptibility information.

**Aminoglycosides**

The aminoglycosides include amikacin, gentamicin, kanamycin, and tobramycin. They have good activity against most gram-negative bacilli, including *Pseudomonas aeruginosa*. They have less activity against streptococci and no activity against anaerobic bacteria. Among the aminoglycosides, amikacin is the most active against *Pseudomonas*. Aminoglycosides can cause nephrotoxicosis, ototoxicosis, and vestibulotoxicosis. Because nephrotoxicity has been linked to persistently elevated trough concentrations, dosage regimens that increase the interval between doses has been advocated.\(^7\) The optimal bactericidal effects from these drugs is concentration-dependent. Therefore, a dose regimen that administers a single high dose, compared to two or three lower doses are equally effective. Since a single high dose is perhaps, less nephrotoxic than multiple daily doses, once-daily dosing has been adopted as routine clinical practice.\(^7\)
As an example of dose regimens in mammals, gentamicin is administered at a dose of 7-10 mg/kg and amikacin at a dose of 10-15 mg/kg. Either drug is administered once daily (preferably in the morning to reduce toxicity) i.v., s.c., or i.m. To decrease emergence of resistance, and produce a more bactericidal effect, many clinicians recommend coadministration of an aminoglycoside with a $\beta$-lactam (a penicillin derivative, or cephalosporin). In addition to a broader spectrum, the $\beta$-lactam enhances bacterial penetration of an aminoglycoside.

In non-mammal species, pharmacokinetics of aminoglycosides vary greatly. Pharmacokinetics in birds do not vary significantly from mammals, but there are some differences among birds, and some dosages have been published. Clearance of aminoglycosides is slow in reptiles and subsequently they are at a greater risk for nephrotoxicosis. For most reptiles, dose intervals are 48-96 hr. Route of injection in animals has been i.v., i.m., and s.c. In reptiles, there is a renal portal system that may affect systemic clearance for some drugs. The concern is that a drug injected i.m. in the rear leg may be cleared by the kidney before it reaches the systemic circulation. However, site of injection does not appear to affect aminoglycosides. We observe similar pharmacokinetic profiles when gentamicin was injected i.m. in the rear leg vs front leg in turtles.

**Tetracyclines**

Tetracycline antibiotics remain popular because of their wide spectrum of activity, good distribution to most body fluids, and oral absorption in most animals. Oral formulations of doxycycline have been used to treat bacterial infections as well as intracellular pathogens (Rickettsia, Chlamydia, Ehrlichia) in small animals. Injectable formulations of oxytetracycline (for example LA-200) have been used for i.m. injection because they produce prolonged plasma concentrations. Dosing information for tetracyclines is available for most domestic animals. Additional pharmacokinetic and dosing information exist for camels, wild ungulates, rabbits, fish, reptiles and birds based on pharmacokinetic studies. In reptiles, there is evidence of prolonged elimination of tetracyclines, which is probably caused by low renal clearance. In one study, the average half-life of oxytetracycline administered i.v. in alligators was 74 hr (131 hr after i.m. injection of long-acting oxytetracycline), compared to a range of approximately 3-10 hr in most mammals. Tetracyclines have been used to treat mycoplasma infections in reptiles and dose intervals of 72-96 hr have been used.

Doxycycline has become a treatment of choice for chlamydial infections in birds because of its good oral absorption and efficacy. It can be dosed to pet birds by simply adding doxycycline hyclate to drinking water. When doxycycline hyclate was added to drinking water at concentrations in drinking water of 0.28 mg/ml and 0.83 mg/ml plasma concentrations were maintained high enough for susceptible organisms during a 45-day treatment. Other oral dose regimens are available. The oral route is preferred for doxycycline because i.m. injections cause pain and tissue irritation. Formulations of oxytetracycline have been added to drinking water of poultry to control bacterial infections.

**Macrolides and Derivatives, and Lincosamides**
Erythromycin is an effective drug that has been available for many years and used in a variety of domestic species. Its advantages include intracellular and tissue distribution and good safety profile. Its disadvantages include a narrow spectrum, adverse gastrointestinal effects (nausea and vomiting), poor oral absorption, short half-life, and need for frequent dosing intervals. There are new derivatives of this macrolide drug that are designed to improve therapy and produce fewer adverse reactions. Among these newer derivatives, azithromycin (Zithromax®) is the first drug in the class of azalides. Azalides are derived from erythromycin and the mechanism of action is similar. (Erythromycin is a 14-member ring, and azithromycin has a 15-member ring structure.) The important difference between azithromycin and erythromycin is the good oral absorption, it is better tolerated, has a much longer half-life (especially in tissues), and has a broader spectrum of activity.

Azithromycin is active against gram-positive aerobic bacteria (staphylococci and streptococci) and anaerobes. The activity against staphylococci is not any better than erythromycin, but it has good activity against many intracellular organisms including Chlamydia, and Toxoplasma. It is also active against mycobacteria and Mycoplasma.

Azithromycin is concentrated in tissues, particularly leukocytes, macrophages and fibroblasts. The tissue concentration can be as much as 100x serum concentrations and the concentrations in leukocytes may be 200x the concentrations in serum. There has been limited use of this drug for treating infections in dogs, cats, and birds, but its popularity is increasing. Results of treatment of intracellular infections such as Toxoplasma and Mycobacterium has been encouraging in people, but not yet reported for animals.

LITERATURE CITED


**Figure 1.** Terms used to describe the shape of the plasma concentration vs time profile.
ACID-BASE BALANCE AND IMBALANCE: A REVISED APPROACH

William W. Muir, III, DVM, PhD, Dipl ACVECC, Dipl ACVA

The Ohio State University, Department of Veterinary Clinical Sciences, 601 Tharp St., Columbus, OH 43210 USA

Abstract

(Of innovations) ... when a thing was new people said “It is not true.” Later, when its truth became obvious, people said, “Anyway, it is not important,” and when its importance could not be denied, people said “Anyway, it is not new.”

William James (1842-1910)

Traditionally, the clinical evaluation of acid-base balance of blood focused on the relationship between pH or hydrogen ion concentration ([H+]), carbon dioxide tension (PCO2), and bicarbonate concentration ([HCO3]), as described by the Henderson-Hasselbach equation. The relationship of these variables to one another being described by the CO2 hydration or carbonic acid equation (H2O + CO2 = H2CO3 = H+ + HCO3). In 1981 Peter Stewart applied physiochemical principles to provide a quantitative analysis of acid-base balance in biologic fluids. Since its introduction into clinical medicine, Stewart’s approach has been adapted by almost everyone that evaluates acid-base abnormalities in patients. The basis for Stewart’s approach to acid-base balance centers on three fundamental laws: 1) the dissociation constants of all weak electrolytes in a solution must be satisfied simultaneously, 2) electroneutrality must be maintained, and 3) mass must be conserved. With these laws firmly in mind the second and most important concept of Stewart’s approach to acid-base balance can be stated: H+ and HCO3 are dependent variables in biologic fluids. There are three primary independent variables in acid-base physiology that determine H+, HCO3 and PCO2. All other variables (e.g., [H+], pH, [HCO3], concentration of non-volatile weak acids in dissociated form [A-N]) are dependent variables. They cannot change primarily or individually. They all will change simultaneously if one or more of the independent variables changes. At any given time, knowledge of the independent variables allows one to calculate the dependent variables. The independent variables are: the PCO2 that can be changed by changes in alveolar ventilation, the strong ion difference ([SID]), the difference between all fully dissociated (strong) cations and anions, and the total concentration of non-volatile weak acids ([ATOT]). This last independent variable represents the sum of the dissociated and non-dissociated forms of the weak acids ([ATOT] = [A-N] + [HA]). In plasma, the main components of [ATOT] are plasma proteins (> 90% under normal circumstances) and inorganic phosphate.

By definition, strong ions are completely dissociated in water. The most important strong ions in determining acid-base balance are sodium, potassium, magnesium, chloride, sulfate, lactate, β-hydroxybutyrate and acetoacetate. The net effect of the presence of strong ions can always be expressed in terms of the difference between the total concentration of strong cations and strong anions [SID], and
consequently $[\text{HCO}_3^-]$ can change as a result of changes in free water (as indicated by $\text{Na}^+$), chloride, or unmeasured strong anions ($\text{UA}'$).

Using the Stewart approach, several insights can be gained concerning acid-base regulation in body fluids. First, $[\text{H}^+]$ and $[\text{HCO}_3^-]$ change only if $\text{PCO}_2$, $[\text{SID}]$ or $[\text{A}_{\text{TOT}}]$ change. Since protein and inorganic phosphate concentrations in plasma ($[\text{A}_{\text{TOT}}]$) normally are constant, even in the face of an acid-base disturbance, long-term acid-base homeostasis acutely controlled by changes in $\text{PCO}_2$ (mediated by the lungs) and changes in $[\text{SID}]$ (mediated by the kidneys). Furthermore, the exchange of $\text{H}^+$ and $\text{HCO}_3^-$ between fluids separated by membranes (e.g., extracellular and intracellular fluid) is dependent upon the exchange of strong ions. $[\text{A}_{\text{TOT}}]$ is comprised mainly of proteins that do not cross cellular membranes, and therefore, do not participate in transmembrane exchange. Carbon dioxide ($\text{CO}_2$) is highly permeable in all membranes of the body. The local $\text{PCO}_2$ in a fluid compartment therefore is determined mainly by arterial $\text{PCO}_2$ and local $\text{CO}_2$ production, which in turn is a function of local tissue perfusion. Hydrogen ion concentration and $[\text{HCO}_3^-]$ are dependent variables, and transport of $\text{HCO}_3^-$ or $\text{H}^+$ across cell membranes cannot result in changes in $[\text{HCO}_3^-]$ or $[\text{H}^+]$ unless these ions are accompanied by strong ions. The effects of transported strong ions on $[\text{SID}]$ in the individual fluid compartments are what determine the new $[\text{HCO}_3^-]$ and $[\text{H}^+]$ in body fluid compartments.

**Clinical Application**

The clinical application of Stewart’s concepts has been articulated at many continuing education seminars and never more clearly than by Dr. David Leath. In summary these concepts are:

1) Acid-base balance is determined by three independent variables: $\text{SID}$, $\text{A}_{\text{TOT}}$, and $\text{PCO}_2$.
2) Evaluation of acid-base balance requires the evaluation of $\text{SID}$, $\text{A}_{\text{TOT}}$, and $\text{PCO}_2$.
3) All acid-base abnormalities occur because of derangements in $\text{SID}$, $\text{A}_{\text{TOT}}$, and $\text{PCO}_2$, and
4) Clinically $\text{A}_{\text{TOT}}$ (total protein) is infrequently manipulated in order to correct acid-base imbalance, therefore, most if not all acid-base disturbances are corrected by manipulating the SID and $\text{PCO}_2$.

It is important to realize that the correction of all nonrespiratory acid-base disturbances requires the correction of SID only!

**Respiratory acid-base balance: Disorders of $\text{PCO}_2$**

Alterations in $\text{PCO}_2$ (breathing) can change $[\text{H}^+]$ and $[\text{HCO}_3^-]$. An increase in $\text{PCO}_2$ (respiratory acidosis) or decrease in $\text{PCO}_2$ (respiratory alkalosis) are considered as a change in an independent variable in the traditional and “new” approach to acid-base balance. One important relationship to remember is that for every increase in $\text{PCO}_2$ of 10 mm Hg the pH decreases by 0.05 units, and for every decrease of $\text{PCO}_2$ by 10 mm Hg the pH increases 0.1 units. A $\text{PCO}_2$ of 70 mm Hg therefore would be expected to decrease the pH from normal (7.40) to approximately 7.25. If the measured pH were 7.25, then the patient can be assumed to have a primary respiratory acidosis.
Non-respiratory acid-base balance

**Disorders of $A_{TOT}$**

Changes in $[A_{TOT}]$ will change $[HCO_3^-]$. Disorders of $[A_{TOT}]$ include hypoproteinemic alkalosis, hyperproteinemia acidosis and hyperphosphatemic acidosis.

Disorders of plasma protein concentration: Plasma proteins are non-volatile weak acids. Consequently, changes in total protein and albumin concentrations will change $[H^+]$. A decrease in albumin and total protein concentration will lead to hypoproteinemic alkalosis. Hypoproteinemia decreases $[A_{TOT}]$ and causes alkalosis without changing $[SID]$. Although uncommon, an increase in protein concentration is expected to cause acidosis due to an increase in $[A_{TOT}]$.

Disorders of phosphate concentration: Phosphate is a potentially important component of $[A_{TOT}]$. At normal serum phosphorus concentration, it accounts for only 5% of $[A_{TOT}]$, therefore, hypophosphatemia is not expected to cause a non-respiratory alkalosis. Severe hyperphosphatemia, however, can cause $[A_{TOT}]$ to change resulting in non-respiratory acidosis. Changes in serum phosphorus concentration have been observed in renal failure, after the administration of a hypertonic sodium phosphate enema to cats or in cats receiving phosphate-containing urinary acidifiers.

Treatment of hyperphosphatemic acidosis, hyperproteinemia acidosis and hypoproteinemic alkalosis should be directed at the underlying disease process. When mixed acid-base disorders occur such as hyperchloremic acidosis with hypoproteinemic alkalosis, hypoproteinemia (or hypo-albuminemia) may limit (compensate for) changes in plasma $[H^+]$. Correction of one variable, therefore, without considering the changes caused by the other, could allow acidosis or alkalosis to emerge unopposed.

**Disorders of $[SID]$**

Changes in $[SID]$ usually are recognized by changes in $[HCO_3^-]$ or base excess (BE). There are three general mechanisms by which $[SID]$ changes: (1) changing the water content of plasma (contraction alkalosis and dilutional acidosis), (2) changing the $[Cl^-]$ (hyperchloremic acidosis and hypochloremic alkalosis), and (3) increasing the concentration of UA (organic acidosis).

Disorders of plasma free water content: Changing the water content of plasma without any change in the content of strong ions will dilute or concentrate both strong anions and strong cations. Consequently, $[SID]$ will change by the same proportion (e.g., if $[X^+] - [Y^-] = [SID]$, then $k[X^+] - k[Y^-] = k[SID]$). If all strong ions increase, for example by 15%, $[SID]$ also will increase by 15%. The new $[SID]$ will change all dependent variables ($[H^+]$, $[HCO_3^-]$) resulting in an increase in $[HCO_3^-]$. This alteration classically has been called “contraction alkalosis.” The same rationale is used for addition of water to plasma so-called “dilutional acidosis.” The resultant non-respiratory acid-base disturbance is detected by the presence of hyponatremia or hypernatremia.
Disorders of [Cl−]: When water balance is normal and does not change, the [SID] only changes as a result of changes in strong anions. Chloride is the only strong anion present in sufficient quantities (concentration) to cause a significant change in [SID]. If [Na+] remains constant, changes in [Cl−] can substantially increase or decrease [SID]. A decrease in [Cl−] (relative to Na+) in extracellular fluid is referred to as hypochloremic alkalosis, whereas an increase in [Cl−] (relative to Na+) is called hyperchloremic acidosis. In clinical patients the [SID] increases causing hypochloremic alkalosis when [Cl−] decreases. On the other hand, an increase in the strong anion concentration and resultant decrease in [SID] can be caused by increases in [Cl−] (hyperchloremic acidosis) and by increases in organic anions and sulfates.

Treatment of hypochloremic alkalosis should be directed at correction of [SID]. In cases where expansion of extracellular volume is desired, i.v. infusion of 0.9% NaCl (addition of extra chloride) is the treatment of choice. This solution has an [SID] of 0 and will decrease plasma [SID]. If hypokalemia is present, KCl should be added to the fluid. If volume expansion is not necessary, [Cl−] can be administered using salts without [Na+] (e.g., KCl, CaCl2, MgCl2). Potassium chloride is the salt of choice for most clinical situations. Ammonium chloride and acetazolamide can be given orally to decrease [SID] and correct hypochloremic alkalosis. Treatment of hyperchloremic acidosis is based on administration of solutions with an elevated Na+ concentration (elevate [SID], sodium bicarbonate).

Disorders of [UA−]: Accumulation of metabolically produced organic anions (e.g., lactate, acetoacetate, and β-hydroxybutyrate) will produce non-respiratory acidosis because they behave as strong anions at body pH, thus decreasing [SID] and causing an increase in [H+] in order to maintain electroneutrality. Organic alkalosis does not occur because there is no organic base present in plasma at sufficiently great enough concentrations to influence (increase) [SID]. The addition of exogenous anions such as salicylate or glycolate (e.g., ethylene glycol poisoning) or an increase in serum sulfate concentration will mimic organic acidosis because these substances behave as strong anions.

Organic acidosis in small animal medicine is usually due to hypoxemia-ischemia (lactic acid accumulation) or diabetic ketoacidosis (e.g., β-hydroxybutyrate). An increase in inorganic strong anions can contribute to acidosis in uremia (e.g., sulfates). Treatment should be directed at the underlying cause of the organic acidosis. When the underlying cause cannot be corrected and the pH is < 7.2 alkali (NaHCO3) therapy should be considered. It should be remembered that organic anions can be metabolized, reestablishing normal [SID]. Therefore, the administration of NaHCO3 (a solution with a high [SID]) may result in an increase in [SID] after the organic acids have been metabolized causing a non-respiratory alkalosis.

Quantitation of nonrespiratory acid-base disorders:

1) Determine the serum Na+, Cl− and total protein (TP).
2) Calculate the free water, chloride and TP abnormalities.
3) Sum the values of the free water, chloride and TP abnormalities and compare that sum with the observed base excess (BE). A BE less than the summed values indicates the presence of unmeasured anions in equal magnitude to the difference.
Free water abnormalities: $0.3([\text{Na}^+] - 140) = \phantom{0000000000000}$
Chloride abnormalities: $102 - [\text{Cl}^-] \text{ corr} = \phantom{0000000000000}$
Hypoproteinemia: $3(6.5 - \text{TP}) = \phantom{0000000000000}$
Unidentified anions make the balance: $= \phantom{0000000000000}$

Total is the observed (reported) BE: $= \phantom{0000000000000}$

Note: $[\text{Cl}^-] \text{ corr}$ is $[\text{Cl}^-] \text{ obs} - 140/[\text{Na}^+]$.
Note: For $[\text{Albumin}]$ instead of $[\text{TP}]$, use $3.7(4.5 - [\text{Albumin}])$.

The concepts expressed by Stewart have changed our view of acid-base physiology in health and disease, have provided a more mechanistic and quantitative approach to the pathophysiology of acid-base disorders, and permit a more complete understanding of electrolyte and plasma protein imbalances in relation to changes in blood $[\text{H}^+]$. Changes in $[\text{HCO}_3^-]$ can be explained by changes in $[\text{SID}]$, $\text{PCO}_2$, and $\text{A}_{\text{TOT}}$, and quantification of the individual roles of these variables should be routine in veterinary patients.
FISH MEDICINE

Ruth Francis-Floyd, DVM, MS, Dipl ACZM

University of Florida, Department of Fisheries & Aquatic Sciences, 7922 N.W. 71st St., Gainesville, FL 32653 USA

Abstract

The essentials of fish medicine can be divided into a few basic areas; water quality, preventive medicine, parasite control and control of bacterial infections. Each of these areas needs to be addressed to achieve a successful fish health medicine program. In most situations, water quality management and preventive medicine are more important, and rewarding, than treatment of infectious disease.

Water Quality (it's the environment....)

Water quality management is the component of fish medicine that is often most foreign to veterinarians. The importance of mastering a basic understanding of this area, as it relates to animal health, cannot be overemphasized. The veterinarian must understand water quality well enough to interact effectively with environmental engineers and fisheries biologists or aquarists. Veterinarians can and should be a vital member of the water quality team in a zoo setting.

For day-to-day fish health management there are only a few basic parameters that need to be mastered. Some of these have a direct impact on animal health (e.g., dissolved oxygen), others may indirectly affect animal health because of interaction with another component of the water (e.g., ammonia and pH), or because of the effect on a drug or chemical (e.g., total alkalinity and copper toxicity).

The basic water quality parameters for freshwater systems are: dissolved oxygen, temperature, pH, ammonia, nitrite, chloride, total alkalinity, and total hardness. In marine systems, nitrate and salinity should also be monitored on a regular basis. In addition, some parameters may be of special concern in certain types of systems. For example, a system using city water as it’s source water should be tested for chlorine and/or chloramine. A system using a deep well for source water should be evaluated for nitrogen gas and carbon dioxide to avoid potential problems with “Gas Bubble Disease.” Heavy metals are occasionally of concern, older facilities with copper piping can be disastrous for fish. Zinc will leach from galvanized metals that are located somewhere on the system, and hydrogen sulfide or iron can be problematic. Every effort should be made to thoroughly understand the water being used to set up a system, the treatment of the water on the system, and the essentials of the engineering such as flow rates, turnover rates, filter construction and capacity. Initially this can be tiresome or intimidating but it is essential to develop this expertise to successfully manage aquatic species.
Dissolved Oxygen

Dissolved oxygen is simply oxygen gas in solution in water. Most fish prefer ≥ 5 mg/L for optimal health, and coldwater species often have a higher oxygen requirement than warmwater species. Clinical distress often becomes apparent at dissolved oxygen concentrations of 2-4 mg/L, depending on species, and mortalities usually occur when levels drop to ≤ 1-2 mg/L. Some species are more sensitive and may die at significantly higher concentrations, for example, hybrid striped bass are severely stressed and may start to die at dissolved oxygen concentrations of 4 mg/L. When an oxygen depletion occurs large fish are usually more severely affected than small ones. There are also important species-specific differences in tolerance for low dissolved oxygen.

In outdoor systems in which algae is allowed to grow there is a diurnal oxygen cycle driven by photosynthesis. Dissolved oxygen is lowest at dawn, following respiration all night long by fish and other biota in pond. Dissolved oxygen is highest late in the afternoon, following maximum exposure of the water surface to sunlight. Anything that decreases the intensity of sunlight hitting the surface of the pond, such as cloudy weather, can significantly decrease the amount of oxygen produced. The “greener” the water, the more extreme the diurnal fluctuation, therefore management of “blooms” becomes very important. Algal blooms can be monitored by measuring the visibility of the water with a “secchi disc”. Any time visibility is less than 18 inches the danger of a catastrophic oxygen depletion caused by an excessive bloom is significant. Algal blooms die, turning the water brownish, gray or even black. The visible color change often precedes an oxygen depletion, therefore managers or keepers should be educated to pay attention to the appearance of water.

Certain physical properties of water and oxygen are important for the clinician to keep in mind. First, oxygen concentration (at saturation) increases as temperature, salinity and altitude decrease. Therefore, hot water is able to hold less oxygen in solution - hence the increase in oxygen depletions in summer. Salt water is also able to hold less oxygen than freshwater at the same temperature, however the influence of salinity (or altitude) is far less significant than the influence of temperature.

Temperature

Fish are poikilothermic, therefore environmental temperature has a huge influence on metabolism. Each species has a preferred temperature range, some are very tolerant of temperature fluctuation, others are not. Temperature has a huge influence on the immune system of fish, changes of 5°C have been shown to shut down T-cell function of channel catfish for 3 wk, and to transiently slow the specific immune response to the point where it is essentially non-functional. Many infectious diseases have a "preferred" temperature window, therefore manipulation of environmental temperature is a legitimate therapeutic strategy for some problems.

pH
pH chemistry in water is complex and controlled, in part, by the carbonate cycle. Hydrogen ions complex with carbonates to form bicarbonates. The total alkalinity is a measure of how much carbonate is present, and therefore is closely tied to pH. Any time pH of water is inappropriate the carbonate concentration, or alkalinity, should be checked. This is also discussed below in the section on alkalinity.

For freshwater fish, pH in the range of 6.5-9.0 is a good “normal” range. Some fish, such as Amazon species, prefer more acidic water, while others, such as the Rift Lake cichlids from Africa, prefer more alkaline conditions. In general, pH of 4.0 and 11.0 are lethal to most freshwater species. pH outside the “normal” range may not be lethal but lead to “stress”, reflected in poor growth and reproduction. For marine species, pH of 8.2-8.3 is considered “normal.” The marine environment is very stable, and these animals are much less tolerant of inappropriate pH. A pH less than 7.5-7.7 or greater than 8.3-8.5 can be stressful for many marine species.

In “green water,” such as outdoor ponds with an algal bloom, carbon dioxide fluctuates daily, as does oxygen. Carbon dioxide provides the carbon source for plants during photosynthesis, therefore the concentration decreases dramatically during the day, resulting in a rise in pH. At night, carbon dioxide accumulates because respiration is occurring in the absence of photosynthesis, and as carbon dioxide increases the pH falls. These changes are not only important because of their impact on fish health, but also because of the impact on other chemicals in the water, particularly ammonia (see below).

Ammonia

The most important source of ammonia in aquatic systems is usually fish food because it is very high in protein. In an established system there is an ammonia cycle that removes nitrogen and recycles it. The first two steps in the process are aerobic and only occur in the presence of oxygen. *Nitrosomonas* bacteria oxidize ammonia to nitrite, and *Nitrobacter* oxidize nitrite to nitrate. Under anaerobic conditions, nitrate can be reduced to nitrogen gas. That is then released to the atmosphere. Nitrate can also be used directly by plants.

In aquatic systems, ammonia exists in two forms, ammonia and ammonium. Ammonia (NH₃) is highly toxic and concentrations as low as 0.05 mg/L can cause gill damage, while concentrations of 2.0 mg/L are often lethal. Ammonium (NH₄⁺) is sometimes considered “non-toxic”, but “less toxic” is probably a better way to consider it. The form of ammonia present in water is controlled by pH, the more acidic the water, the greater the concentration of ammonia.

Acute ammonia toxicity is often associated with neurologic signs including spinning and convulsing. Chronic toxicity may result in renal compromise and opportunistic infections. Aminoglycosides are renal toxic and this may be exacerbated by high ammonia conditions. The best short-term treatment for an ammonia problem is a water change. It is also important to determine why the ammonia has spiked and to correct any underlying problems. The most common causes of ammonia accumulation are overfeeding, biofilter failure, or phytoplankton die-off

Nitrite
The second breakdown product in the nitrogen cycle is nitrite. Nitrite causes methemoglobinemia and results in hypoxia. This condition is often referred to as “brown blood disease” because the gills and blood become chocolate brown in color as the percentage of hemoglobin present as methemoglobin increases to about 80%. There are species-specific sensitivities to nitrite toxicity, with channel catfish and freshwater angelfish being extremely sensitive, while centrarchids (bass and bluegill) are refractory.

The treatment for nitrite toxicity of freshwater fish is chloride, usually delivered as sodium chloride. The chloride molecule has approximately the same size and ionic charge as nitrite. Increasing the chloride concentration to at least six times the nitrite concentration sets up a competitive inhibition at the epithelial surface of the gills. The result is a decrease in the amount of nitrite crossing into the blood and a consequent reduction in the amount of methemoglobin that forms. Fish can recover from active disease within 24 hr when treated with chloride.

Chronic nitrite exposure has been associated with development of anemia in channel catfish. It is assumed that the lifespan of the red blood cell is shortened due to the constant need to reduce methemoglobin, using up cell's energy reserves. Chronic nitrite toxicity can be difficult to diagnose unless excellent water quality records are available for several weeks preceding the suspect event.

**Nitrate**

Nitrate is the final product of the aerobic part of the nitrogen cycle. Nitrate is rarely a problem in freshwater systems because water changes flush the ion from the water, reducing the concentration. In marine systems, however, where water changes either are not done at all, or are rare, nitrate can accumulate to several hundred mg/L. The exact impact of this on fish health is poorly understood, but there are some species that do not seem to do well in high nitrate environments.

Nitrate can be removed from closed marine systems by an anaerobic process. There seem to be limits to this technique but it is being used more commonly in large marine systems for which water changes are not possible.

**Total Alkalinity**

As mentioned above, total alkalinity is a measure of the carbonate concentration (buffering capacity) of water. For freshwater systems, total alkalinity should be increased if it is below 50-100 mg/L for most species. This can be done using agricultural limestone (calcium carbonate) in large bodies of water (>0.25 surface acres) but dolomite (calcium and magnesium carbonate) is often easier to use in smaller systems.

Marine systems should have very high alkalinity (> 250 mg/L). Crushed coral or limestone can be used to raise and maintain high alkalinity in these conditions.

When the alkalinity is very low a pH crash can occur as organic acids accumulate in the system. Anytime excessively low pH is detected, low alkalinity should be suspect.
Copper sulfate toxicity is directly related to the total alkalinity of the water. If total alkalinity is below 50 mg/L copper sulfate cannot be used safely in freshwater systems. Copper sulfate dose can be titrated with total alkalinity for safe use of this compound in freshwater (total alkalinity % 100; dose not to exceed 2.5 mg/L).

**Total Hardness**

Total hardness measures the divalent cations in water. These include calcium, magnesium, manganese, iron and zinc. Frequently, the total hardness and total alkalinity are about the same value as carbonates usually form ionic bonds with calcium and magnesium.

The total hardness of water is very important for rearing fry as fish absorb minerals from water for bone development and other metabolic processes. Calcium concentrations < 20 mg/L have been demonstrated to be very detrimental to channel catfish fry. The calcium concentration of water can be increased by adding calcium chloride.

**Chloride**

For freshwater fish a chloride concentration of 100 mg/L is recommended to prevent nitrite toxicity in susceptible species. Chloride and other ions should be avoided in tanks housing fish in the family Morimidae (e.g., elephant nose, black ghost etc.). These animals are from very soft Amazon water and navigate by creating a small electrical current. Excessive ions in the water totally disrupt this process.

**Salinity**

Seawater is a 3% salt solution, which is the same as approximately 30 ppt or 30,000 ppm. Salinity of 0.02% (200 ppm) may be enough to prevent or decrease some protozoal infections of freshwater fish. Salinity of 5 ppt (0.5% or 5000 ppm) is good for short-term transport (hours to days) of most freshwater fish. This concentration is also an excellent anti-protozoal treatment.

**Chlorine/Chloramine**

Chlorine and chloramine are commonly used in municipal water supplies to eliminate bacteria. Chloramine is a combination of chlorine and ammonia. When the chlorine is removed, the ammonia remains. Chlorine concentrations of 0.02 mg/L are toxic to fish and concentrations of 0.2 mg/L are lethal to many species.

Chlorine can be removed from water using sodium thiosulfate. For every 1 mg/L of chlorine present, 7.4 mg/L sodium thiosulfate can be added to remove it. Sodium thiosulfate can remove oxygen from water so excellent aeration is necessary when using dechlorinators.

**Gas Bubble Disease**
“Gas bubble disease” is usually caused by supersaturation of water with nitrogen gas. This is most common when water is brought up from a deep well. Excess gas can be eliminated by spraying water before it comes into contact with fish. A pathognomonic lesion for gas bubble disease is gas emboli within gill capillaries. These can be seen on gill biopsy.

Control of Protozoal Diseases

There are four basic chemicals used to control protozoal diseases of fish: formalin, copper sulfate, potassium permanganate and salt. Selection of one of these agents is often based on constraints imposed by the system, rather than differences in efficacy.

Formalin

Formalin is FDA approved as an ectoparasiticide for fish. FDA-labeled formalin products contain 37% formaldehyde gas in aqueous solution, and methanol may be added as a preservative. Product that appears cloudy or has a white precipitate may contain paraformaldehyde, a highly toxic substance that forms when formalin gets cold (< 45°F).

Formalin concentrations of 12-25 mg/L (1-2 drops per gallon) are effective as an indefinite bath. The concentration can be increased to 170-250 mg/L for 30 min but it is a harsh treatment. Formalin usually has excellent efficacy against protozoans, and moderate efficacy against monogeneans, columnaris bacteria, and saprolegnia. It chemically removes oxygen from water so excellent aeration during treatment is important.

Copper Sulfate

Copper sulfate is EPA approved as an algicide. It is also effective as an anti/protozoal agent, however FDA approval for this use has never been issued. It is extremely toxic to fish and even more toxic to invertebrates and plants. NEVER use copper in a system with plants or invertebrates that you like!

For freshwater fish the concentration of copper sulfate can be titrated with the total alkalinity of the water. If total alkalinity is < 50 mg/L do not use copper. If the total alkalinity is 50-250 mg/L, the concentration of copper sulfate is equal to 1% of the total alkalinity. For example, if the total alkalinity is 100 mg/L, then the concentration of copper sulfate that can be used safely and effectively is 1 mg/L. Never use more than 2.5 mg/L copper sulfate, regardless of how high the alkalinity is.

For marine systems, slowly bring the copper concentration (NOT copper sulfate concentration) up to 0.02 mg/L and try to hold it there for 3 wk. Copper concentration will need to be monitored closely (1-2 times/day) and additional chemical added as needed.

Copper solutions are extremely effective against protozoal infections and moderate efficacy against monogeneans, columnaris and Saprolegnia.
**Potassium Permanganate**

Potassium permanganate does not have FDA approval for aquaculture use but is used routinely in water softening systems. It functions as an oxidizing agent and essentially sanitizes the external surface of fish, removing parasites, fungus and bacteria. It works very well with salt in small tanks.

Potassium permanganate is primarily used on freshwater fish, and there is very little information on safety for marine fish. It can cause significant gill damage, especially when used repeatedly. A safe recommendation is no more than one treatment per week.

Chemical concentration is usually 2 mg/L as an indefinite bath, or 10 mg/L as short-term bath (30 min). The chemical will oxidize whatever organic matter is present and therefore the concentration must be increased when used in organically rich environments.

**Salt**

Salt is mentioned briefly above. It is a wonderful anti-protozoal treatment for many freshwater fish. As mentioned above, most freshwater fish will tolerate a mild to moderate increase in salinity for a fairly long period of time. Freshwater fish probably tolerate an increase in salinity better than marine fish tolerate a decrease in salinity.

**Control of Crustacean Parasites**

**Difluorobenzuron (Dimilin)**

Difluorobenzuron has recently been approved by EPA for use on aquatic sites, however a Restricted Use Pesticide License is required to apply the compound. It inhibits chitin synthesis and is an excellent product for treatment of anchor worms and other crustacean parasites of fish. The chemical should not be used in water with an effluent due to it’s prolonged half-life (no effluent for at least 14 days following treatment). The compound should be used at a concentration of 0.03 mg/L as a prolonged bath.

**Control of Monogenetic Trematodes**

**Praziquantel**

Praziquantel has no approval for use in aquatic sites or on aquatic species. It is effective, however in controlling monogenetic trematodes and intestinal tapeworms, and is most commonly used in closed marine systems. Concentrations as low as 2 mg/L have been effective in controlling monogeneans in
marine fish and the chemical may remain active for 2 wk, killing juvenile forms that hatch after the initial chemical application.

**Control of Internal Parasites**

*Metronidazole*

Metronidazole is not approved for use in any aquatic species or site, however it has excellent efficacy against intestinal flagellates found in ornamental fish. It can be delivered in a medicated feed at a dosage of 50 mg/kg body weight (4.5 g/lb food). Fish should be fed the medicated feed for five days. If fish are not eating it can be delivered as a bath treatment at a concentration of 6 mg/L (250 mg/10 gal water), repeated daily for 5 days. A water change (50%) 4-8 hr after treatment is recommended to remove residue.

*Fenbendazole*

Fenbendazole has not been approved for use on any aquatic species or for any aquatic site. Some efficacy has been demonstrated against intestinal nematodes when given orally, however there is more anecdotal information than actual efficacy data. The compound can be fed at a dose of 3.5 g/lb food for 3 days, and repeated in 3 wk.

**Antibiotics for Fish**

*Terramycin (oxytetracycline)*

Terramycin is FDA approved for use in salmonids, channel catfish, and lobsters. It is a broad spectrum antibiotic, effective against many gram-negative organisms, although resistance to terramycin is common. The dose is 55 mg/kg body weight, and it is usually sold as manufactured medicated feed. The drug should be fed for 10 days, and in food species, followed by a 21-day withdrawal period. Terramycin is sold as a SINKING medicated feed as oxytetracycline is destroyed by heat required to process pellets for floating.

*Romet*

Romet is a potentiated sulfa containing ormetoprim and sulfadimethoxine. It has been approved by FDA for use in salmonids and channel catfish. The dose is 50 mg/kg body weight and it is usually sold as pre-manufactured medicated food. The drug should be fed for 5 days. It has a 3 day withdrawal time in channel catfish and a 6-wk withdrawal period for salmonids. Romet is heat stable and therefore is available in floating pelleted rations.
**Erythromycin**

Erythromycin has not been approved for use in food animals but is generally considered the treatment of choice for infections by gram-positive organisms. It can be fed at a dose of 150 mg/kg body weight for 14 days. It has also been delivered by i.m. injection to salmonids to prevent infection by *Renibacterium salmonarum* (bacterial kidney disease agent).

**Aminoglycosides**

Aminoglycosides have not been approved for use in any aquatic species, however they are used by koi enthusiasts to treat gram-negative infections, particularly *Aeromonas salmonicida*. These compounds are renal toxic, Gentamicin has been injected into goldfish to create a biomedical model for polycystic kidney disease- Not recommended when ammonia levels are elevated.

**Anesthetics**

*MS-222 (methane tricaine sulfonate)*

Methane tricaine sulfonate has been approved by FDA as an anesthetic for use in fish. It is usually delivered at concentrations of 50-200 mg/L. Essentially, the compound should be used to effect, and opercular beats carefully monitored. Aeration is important during any anesthetic procedure, and if the fish is to be anesthetized for a period of time the concentration should be reduced following induction. MS-222 has a 21-day withdrawal period when used on food species.
UPDATE ON WOUND MANAGEMENT TECHNIQUES

Steven Swaim, DVM, MS

Scott-Richey Research Center, College of Veterinary Medicine, Auburn University, AL 36849 USA

Abstract

This class will involve a discussion of new medications and dressings used for wound management, as well as some new and practical wound management techniques. There will also be material presented on skin grafting procedures in birds. Several pieces of literature are useful as background material.1-3

LITERATURE CITED

LONG TERM MEDICAL MANAGEMENT OF POLYCYTHEMIA IN A BLACK LEMUR (Eulemur macaco macaco) WITH HYDROXYUREA

Douglas P. Whiteside, DVM,1,2* Kathy J. Topham, DVM1,3 Michelle R. Bowman, DVM,1 and Roy B. Burns III, DVM1

1Louisville Zoological Gardens, 1100 Trevilian Way, Louisville, KY 40233 USA; 2Toronto Zoo, 361A Old Finch Avenue, Scarborough, Ontario M1B 5K7; 3Audubon Park and Zoological Garden, 6500 Magazine Street, New Orleans, LA 70188 USA

Abstract

Absolute polycythemia is an infrequent clinical finding characterized by an increased mature erythrocyte count, hematocrit, and hemoglobin concentrations. It is classified as primary or secondary. Primary polycythemia, or polycythemia vera, is a rare, idiopathic myeloproliferative disorder manifested by abnormal proliferation of the hematopoietic bone marrow elements, with an absolute increase in red blood cell mass and total blood volume in the absence of increased erythropoietin levels. Secondary polycythemias are further classified as either appropriate or inappropriate depending on their etiology, and arise due to increased circulating levels of erythropoietin or erythropoietin-like substances, which cause an increase in circulating mature erythrocytes.1,3,4

Polycythemia was diagnosed in a 13-yr-old female black lemur (Eulemur macaco macaco) that was examined for progressive lethargy, anorexia and visible weight loss. Further diagnostic tests, utilizing established algorithms for differentiating between polycythemia vera and secondary polycythemias, implicated a membranous glomerulopathy as the probable cause for inappropriate secondary polycythemia.2,3 Intermittent phlebotomies based on the rise in hematocrit were initially used to control the disease. Aspirin was administered orally at 10 mg/kg three times weekly for the prevention of thrombotic events that are often associated with polycythemia. When monthly phlebotomies failed to prevent the hematocrit from rising above 60 L/L, alternative therapeutic options were explored. Hydroxyurea (Hydrea®, Bristol-Myers Squibb Company, New York, New York) is a non-alkylating, reversible myelosuppressive agent that specifically inhibits cells in the synthetic phase of the cell cycle. This drug was initiated at a dosage of 50 mg/kg p.o. once weekly in addition to monthly phlebotomy. The anabolic steroid stanozolol (Winstrol®, Abbott Laboratories, North Chicago, Illinois) was later added to the treatment regime at 2 mg p.o. daily due to progressive weight loss. The hydroxyurea treatment was also increased to twice weekly at this time. Additional medical complications, including hypoalbuminemia and hypoferremia, which can be associated with the long-term medical management of polycythemia were addressed in this lemur.

The lemur has been on therapy with hydroxyurea for over 14 mo, and the hematocrit has been maintained below 60 L/L. No drug-specific adverse reactions have been observed. To the authors’ knowledge this is the first report of hydroxyurea usage in a nonhuman primate species. In this case, we conclude that hydroxyurea in combination with phlebotomy is an effective treatment modality for controlling the clinical and peripheral blood abnormalities associated with polycythemia.
ACKNOWLEDGMENTS

The authors gratefully acknowledge the invaluable assistance of the veterinary technicians Virginia Crossett and Judy Tucker, the veterinary and veterinary technician preceptors, and the animal care staff at the Louisville Zoo. In addition, we thank Dr. Alan Hammer for his expertise, and his staff at Kentucky Veterinary Specialists in Louisville, Kentucky.

LITERATURE CITED

ANTIOXIDANT STATUS IN A SQUIRREL MONKEY (*Saimiri sciureus*) WITH CHRONIC PANCREATITIS AND DEGENERATIVE MYOPATHY

Carles Juan-Sallés, DVM,1* Neus Prats, DVM, PhD,2 Josep Maria Ruiz, BS,3 Xavier Valls, DVM,1 Jordi Giné, DVM,4 Michael M. Garner, DVM, Dipl ACVP,5 Javier Vergés, DVM,1 and Alberto Marco, DVM, PhD, Dipl ECVP2

1Clínica Exòtics, c/ Balmes 454, E-08022 Barcelona, Spain; 2U.D. Histologia i Anatomia Patològica, Facultat de Veterinària (UAB), E-08193 Bellaterra, Barcelona, Spain; 3Laboratorio de Diagnóstico General, c/ Verdi 78 baixos, E-08012 Barcelona, Spain; 4Clínica Veterinària Dr. Florit, c/ París 163, E-08036 Barcelona, Spain; 5Northwest ZooPath, 18210 Waverly Drive, Snohomish, WA 98296-4815 USA

Abstract

A young-adult male squirrel monkey (*Saimiri sciureus*) presented with a 1-wk history of anorexia, weakness, wasting, diarrhea and vomiting. Upon admission, it was dehydrated, postrated and had diarrhea with blood, generalized muscle atrophy and pale mucous membranes. The haircoat was dry and dull and there was focal hypotrichosis in the tail and hindlimbs. The animal was stabilized with lactated Ringer’s (i.v. and s.c.), enrofloxacin (5 mg/kg i.m., s.i.d., 7 days; Baytril® 2.5%, Bayer, Leverkusen, Germany) and dexamethasone (1.75 mg/kg i.m.; Resdex, Schering-Plough, Segré, France). A direct fecal revealed eggs similar to those of *Strongyloides*; the animal was treated with ivermectin (200 µg/kg s.c.; Ivomec®, Merck Sharp & Dohme BV, Haarlem, The Netherlands) within 18 hr. Fat-soluble vitamins (5,000 IU A, 1,250 IU D3 and 25 IU E, i.m.; Veterín-Vit A+D3+E®, Hoechst-Roussel, Barcelona, Spain) and B-complex vitamins (10 µg B12, 5 mg B6 and 2.5 mg B1, i.m.; Hidroxil® B12 - B6 - B1, Laboratorios Almirall, Barcelona, Spain) were administered on days 0 and 5 respectively. From day 0 fluid therapy was furthered with an oral energetic product containing water-soluble vitamins and minerals (Pet Energy Punch, PER®, Boaz, AL) that the animal began to drink voluntarily from day 4. It recovered from shock and started urinating within 16 hr of presentation. Blood disappeared grossly from feces (day 0) and diarrhea stopped on day 3; culture of a rectal swab was negative for enteropathogenic bacteria (day 1). Hematology revealed prominent elevations of CPK, ALT and LDH, consistent with muscular damage, elevated amylase and lipase, hypercalcemia with normal phosphorus and AlkP, and mild hypoalbuminemia and azotemia. On day 5, azotemia worsened despite rehydration and there was a 3.8- and 2.8-fold rise in amylase and lipase respectively; hypoalbuminemia was more prominent and calcium dropped slightly below normal. Vitamin E (13 mg/kg i.m.) and selenium (0.14 mg/kg i.m.) (Vitasel, Laboratorios Ovejero, León, Spain) were administered based on a presumptive diagnosis of nutritional myopathy (day 5). On days 2-6 the animal was fed a high protein, high fat mix (Nutribird A19, Prestige Products NV Versele-Laga, Denze, Belgium) despite weakness and impaired deglutition. On day 6, it developed cardiorespiratory arrest and died; blood was collected and serum frozen at -80 °C.

At necropsy, generalized muscular atrophy was in contrast with moderately abundant fat stores. The pancreas had white nodules up to 3 mm in diameter. The tongue had well-demarcated reddish swollen areas on cut surfaces. A complete set of tissues was preserved in 10% buffered formalin and routinely
processed for histology; special stains on selected tissues included periodic acid Schiff (PAS), Ziehl-Neelsen (ZN), Gram, von Kossa, Warthin-Starry, Perls’ iron and Congo red. Liver and hair were frozen at –20 °C.

Lesions of chronic pancreatitis (CP) consisted of acinar and ductal dilatations by plugs, ductal hyperplasia and mucous metaplasia, acinar atrophy and degeneration, fibrosis, lymphocytic infiltrates, and granulomatous and necrotizing pancreatic steatitis. There was diffuse degeneration of gastric glands associated with chronic lymphocytic gastritis and no spiral bacteria. Chronic degenerative myopathy (DM) with dystrophic mineralization was generalized but most pronounced in limb and tongue muscles. The skeletal fibers in the esophagus had vacuoles containing von Kossa- and ZN-negative, PAS-positive basophilic material. The tongue had two other lesions: fungal pseudomembranous superficial glossitis and bacterial necrotizing glossitis. The heart had diffuse lipofuscinosis. A ZN-negative, PAS-positive, finely granular intracytoplasmic material was seen in adipocytes (brown fat), and cardiac and tongue myocytes. In the kidneys, distal and collector tubules contained casts of a finely granular to fibrillar eosinophilic material, most likely myoglobin; the epithelium of affected tubuli usually had prominent intracellular edema or was flattened. The testes had early atrophy and degeneration (presence of multinucleate giant spermatids, almost complete absence of tailed spermatids, and predominance of Sertoli’s cells and primary spermatids).

Vitamins E and A, and selenium determinations were done on serum collected at the time of death; hair was tested for zinc and liver for selenium. Vitamins E and A were determined by high performance liquid chromatography, whereas flame and hydride-generation atomic absorption spectrophotometry were employed for zinc and selenium analyses respectively. Serum vitamin E levels (0.0092 µg/ml) were extremely low; most primates range 5-20 µg/ml7,13 (E. Dierenfeld, personal communication); hepatic selenium (<0.1 ppm) and hair zinc (171 µg/g) levels were low.8,12 Vitamin A was at 6 µg/dl, that may also be deficient.13,23

CP is thought to have played a major role in the wasting syndrome associated with DM and apparent deficiency of multiple antioxidants in this animal. Morphologically, the pancreatic lesions are reminiscent of chronic calcifying pancreatitis (CCP) of man, that is characterized by the formation of pancreatic calculi sometime during the disease.4,17 However, some findings (acinar plugs and ductal hyperplasia) are not expected in CCP and have been described in children with kwashiorkor, a form of protein-calorie malnutrition (PCM) that causes pancreatic acinar atrophy,2 after refeeding with a balanced diet; together with ductal plugs and fibrosis, these lesions have been considered a possible transitional form between the pancreatic lesions of PCM and those of CCP.15 It is not possible to rule out underlying PCM in this squirrel monkey as its hematologic and histologic diagnostic features may have been masked, if present, by CP and the other diseases found. In man, possible causes of CCP include a low fat and protein diet, antioxidant deficiencies, hypercalcemia, and alcoholism coupled with a high fat and protein diet;4,5,17,19 despite some associations with PCM,5,15 PCM alone does not seem to be a cause of CCP.18 Despite normal calcemia on day 5, the cause of initial hypercalcemia associated with mild hypoalbuminemia is unknown and may have been involved in the development of CP. The etiology of CP in this squirrel monkey may be multifactorial and the lack of knowledge on its diet precludes further discussion on possible nutritional causes. Trichospirura leptostoma, the spirurid
nematode commonly found in the pancreatic ducts of New World primates, seems to have little impact on their health but can be associated with CP. T. leptostoma was found in a colony of common marmosets with wasting, apparent malabsorption and CP. T. leptostoma was not found in 75 sections from seven different areas in the pancreas of this squirrel monkey.

In man, fat-soluble vitamin deficiencies are common in CP as a result of steatorrhea and inappropriate nutrition. This animal was apparently deficient in multiple antioxidants, including vitamin E, selenium, zinc and probably vitamin A. In humans, deficiency of multiple antioxidants has been well documented in CP and may be involved in its pathogenesis. Elevations of CPK, LDH and ALT, vitamin E deficiency and apparent selenium deficiency correlated well with DM. Whenever DM is suspected panels of CPK, LDH, ALT and AST should be selected for their higher sensitivity and specificity for the diagnosis of DM. DM in vitamin E-deficient primates has been described, and the disease has been associated with wasting and pancreatic acinar atrophy in callitrichids. In this squirrel monkey, it is reasonable to assume that CP caused malabsorption/maldigestion associated with a primary and/or secondary deficiency of multiple antioxidants, that may have been involved in the pancreatic lesions, DM, dermatosis and testicular atrophy. PCM and antioxidant deficiencies should be considered in the differential diagnosis of reproductive failure in primates.

Initially, azotemia was mild and apparently prerenal; however, after rehydration there was a 2.8-fold rise in BUN and a lesser increase of creatinine that were attributed to myoglobinemic nephrosis. DM may have also increased creatinine per se. Hyperamylasemia and hyperlipasemia may correlate with azotemia and/or pancreatic and gastric lesions; however, on day 5 amylase and lipase values were 5.7 and 4.8 times greater than the upper limit of normal, respectively, which is unlikely to result from renal dysfunction alone.

Both candidiasis and bacterial necrotizing glossitis strongly suggest a poor immunologic status and should not be considered unusual complications in debilitated or hospitalized primates treated with antibiotics or corticosteroids or otherwise immunocompromised. Antioxidants play a major role in immunity; vitamin E levels correlate positively with positive delayed-type hypersensitivity to Candida, the candidacidal activity by neutrophils is reduced during selenium deficiency, and reduced macrophage phagocytosis of Candida occurs in zinc deficiency. Thus, candidiasis (and perhaps bacterial glossitis) were probably the result of a breakdown in the antioxidant system of this squirrel monkey.

ACKNOWLEDGMENTS

ISIS blood reference values for squirrel monkeys were gently provided by Dr. Roberto Aguilar, Audobon Zoological Gardens, New Orleans, LA. This report is part of a prospective study on wasting syndromes and antioxidant deficiencies of New World primates that is supported by Laboratorio de Diagnóstico General, Universitat Autònoma de Barcelona, and Clínica Exòtics (Barcelona, Spain).

LITERATURE CITED


PREGESTATIONAL, GESTATIONAL, AND POSTGESTATIONAL SERUM PROGESTERONE, β-HUMAN CHORIONIC GONADOTROPIN, AND ESTRADIOL LEVELS IN A BORNEAN ORANGUTAN (Pongo pygmaeus pygmaeus)

Wm. Kirk Suedmeyer, DVM and Daniel Stewart, MD

1Kansas City Zoological Gardens, 6700 Zoo Drive, Kansas City, Missouri 64132 USA; 2Shawnee Mission Medical Center, 9100 West 74th Street, Shawnee Mission, Kansas 66201 USA

Abstract

Urinary hormonal values of estrogen, pregnanediol, estrone, estradiol and androsterone during pregnancy and menstrual cycles of orangutans have been studied by several investigators.4-7,9 Serum values for estradiol, progesterone, testosterone and luteinizing hormone (LH) have been evaluated during the menstrual cycles of orangutans and macaques.10,12

In this study, serum estradiol-17β, progesterone, and β-human chorionic gonadotropin (β-HCG) were monitored before, during and after a pregnancy in a 22-yr-old female Bornean orangutan (Pongo pygmaeus pygmaeus). Results were compared to a non-pregnant 33-yr-old female Bornean orangutan in the same facility and human reference normals. Blood samples were obtained during weekly to biweekly conditioning programs without sedatives or tranquilizers. Trans-abdominal ultrasound images were obtained during the later stages of pregnancy.

Serum progesterone (4-prenen-3,20-dione), β-HCG, and estradiol [1,3,5 (10)-estratrien-3,17β-diol] values were determined using microparticle enzyme immunoassays (AxSYM®, Abbott Laboratories, Abbott Park, Illinois 60064 USA) performed on samples obtained from the orangutan through a stationary conditioning program. These samples were frozen and analyzed at a later date.

Values for serum progesterone, β-HCG, and estradiol are presented in Figs. 1-3. Results of pregestational, gestational and post-gestational values closely parallel human normals.2,13,14 In humans progesterone is produced primarily by the corpus luteum of the ovary.1 Progesterone prepares the uterus for implantation and maintains the uterus during pregnancy. During the menstrual cycle, serum progesterone values elevate to 10-20 ng/ml 5-7 days after ovulation. During the 6th wk of pregnancy, the placenta becomes the major source of progesterone.8 Values during this time elevate from 10-50 ng/ml during the first trimester to 50-2809 ng/ml during the third trimester. In this case, progesterone levels were between 10-15ng/ml during the first trimester, and elevated to 30-40 ng/ml during the second trimester. However, progesterone levels decreased to levels of 10-15 ng/ml during the third trimester (Fig. 1). Serum levels began to elevate to previous levels, but never fully recovered. Reasons for this are unknown. Although this pregnancy proceeded to term, the infant was stillborn. An underlying cause was not determined.
β-HCG appears to maintain the corpus luteum during early pregnancy in humans, allowing continued secretion of progesterone, which supports the endometrium. Values for serum β-HCG in humans demonstrate a maximal peak in the late first trimester, with a decline to a fairly constant level by mid gestation. In our study, a similar peak and return to lower, constant levels was observed (Fig. 2).

Estradiol levels in human studies demonstrate a constant rise until parturition. Levels of 100-500 pg/mL occur during the first trimester, 5,000-15,000 during the second trimester, and 10,000-40,000 pg/mL during the third trimester. A similar curve was seen in this group of data for the orangutan (Fig. 3).

To our knowledge, this is the first report of serial serum levels of progesterone, β-HCG, and estradiol before, during and after gestation in an orangutan. A similar sampling is being obtained in the same animal during its current gestation. Results are being correlated with weekly trans-abdominal ultrasound and daily urinary levels of progesterone, β-HCG, and estrogen. It is hoped that these values can be used to provide a basis for comparing the reproductive hormones of orangutans during pregnancy.

ACKNOWLEDGMENTS

The authors would like to thank the orangutan keeper staff, which has worked with the veterinary staff for the past 6 yr perfecting the conditioning programs with the orangutans at the Kansas City Zoological Gardens.

LITERATURE CITED


Figure 1. Serum progesterone values (ng/ml).
Figure 2. Serum β-Human chorionic gonadotropin values (mIU/ml).

Figure 3. Serum estradiol values (pg/ml).
FIELD ANESTHESIA AND CAPTURE TECHNIQUES OF FREE-RANGING MANTLED HOWLING MONKEYS (Alouatta paliatta) IN COSTA RICA

R. Scott Larsen, DVM,1†* Anneke Moresco, MS, DVM,1 and Kenneth E. Glander, PhD2

1College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523 USA, †Present address: College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 USA; 2Duke University Primate Center, Durham, NC 27705-5000 USA

Abstract

In July of 1998, 83 anesthetic procedures were performed on 68 free-ranging mantled howling monkeys (Alouatta paliatta). Animals were safely anesthetized using 42 ± 8 mg/kg of Telazol® administered with a Pneu-dart® system. Morbidity was associated with inappropriate dart placement and injection of infants and juveniles with adult dosages. All animals recovered with supportive care and there were no mortalities.

Introduction

Long-term behavioral and demographic studies of free-living primates require the ability to recognize individual animals.2 Such recognition is particularly difficult in arboreal primates as there is always a substantial distance between observer and subjects and because foliage often makes visual contact difficult. Marking of individuals greatly enhances the ability of field researchers to accurately identify animals. However this marking requires safe capture that minimizes morbidity, mortality and makes minimal changes in the animals’ behavior and environment.

Comprehensive health assessments of free-ranging primates have been infrequently reported.3 Such information enhances modern conservation efforts by providing baseline data for evaluation of environmental impacts on the health and well being of primate species.3 In July of 1998, veterinarians and primatologists collaborated to combine health evaluations with behavioral and demographic studies of free-ranging mantled howling monkeys. The results of the health assessments are still being compiled and will be reported elsewhere. This report documents the field techniques used to safely anesthetize and capture free-ranging mantled howling monkeys in Costa Rica. Using these techniques, researchers were able to safely and accurately perform morphometric measurements, create dental impressions, collect blood and feces, perform gastric lavage, insert transponder chips, and apply tattoos, identifying collars, and bracelets.

Materials and Methods

Eighty-three anesthetic procedures were performed on 68 individual mantled howling monkeys. Of these 83 procedures, 66 were accomplished under “routine circumstances,” while 17 occurred with “special circumstances.” Criteria for “routine anesthesia” included: 1) adult animal; 2) one dart-delivered anesthetic injection for capture; and 3) injection in target area (hindleg, tail or tailhead).

Monkeys were anesthetized before 1100 hr and after 1400 hr to minimize temperature stress and maximize visibility. Anesthetic was delivered using a carbon dioxide powered, modified Pneu-Dart™ system (Pneu-Dart, Inc., Williamsport, PA USA). Pneu-darts with 9-mm needles were used. For adult
monkeys, each dart was filled with 0.8 ml (200 mg) of Telazol® (tiletamine HCl and zolazepam HCl,
Fort Dodge Laboratories, Fort Dodge, IA USA; 250 mg/ml) in order to administer a dose of 42 ± 8
mg/kg (range = 29-69 mg/kg). Animal and shot selection were based on the animal's size, location, and
presentation. Shots were only attempted at animals that were not moving and that were not facing the
shooter. Clear visualization of the hindquarters was essential; females holding infants were not shot if
the infant was on or around the targeted area on the mother. Shots were avoided if the animal could
move into trees with branches over a body of water. Animals were darted from distances of 10-30 m.

Induction times were 6.0 ± 4.4 min (range = 1-18 min). As animals fell from the trees, they were caught
in nylon mesh nets (camper’s hammocks) that were held by two to three people. Occasionally animals
were caught “by hand”, however it is felt that this technique often provides unnecessary risk to monkeys
and to personnel. A few animals were not caught by personnel and fell to the ground. Falling distances
ranged from 5-20 m. Animals showed complete muscle relaxation during and after such falls; no
obvious injuries were associated with impact.

Several darted animals became anesthetized but did not fall from the tree. In these cases, the tree had
to be climbed or the branch on which the animal was resting had to be shaken with a saw attached to
the end of an aluminum pole (Azel Corp., Costa Mesa, CA, USA). The aluminum poles were composed
of 1.75 m sections that were bolted together until long enough to reach the darted animal. This pole
system has been used to reach animals up to 30 m off of the ground – however considerable amounts
of practice and patience are necessary to manipulate this length of pole.

Times from dart injection to being on the ground were 9.0 ± 6.2 min (range = 1-24 min). Once an
animal was on the ground, physical parameters were measured. Heart rate, respiration, and body
temperature were immediately measured and then monitored intermittently during the remainder of the
anesthesia. Animals were transported by van to a central, shaded processing area. Forty-three monkeys
that were insufficiently anesthetized for weighing, marking, or blood collection were given
supplementary injections of Telazol® (3.5-8.5 mg/kg). Supplemental injections were necessary
approximately 1 hr (x = 62 min; range = 28-145 min) after the first injection. Only two of these 66
animals required a third injection of Telazol® (3.5-8.5 mg/kg).

Atropine (0.014-0.022 mg/kg) was immediately administered to monkeys with heart rates less than 100
beats/min and to all monkeys receiving supplemental injections of Telazol®. Temperature was closely
monitored in all animals. Any rectal temperature greater than 39.0°C was treated by applying water or
rubbing alcohol under the arms and legs; any rectal temperature greater than 40.0°C was treated by
immersing the caudal half of the animal in a bucket of cool water. The body temperature of most
monkeys gradually decreased (from 38.1 ± 1.0°C to 37.3 ± 1.0°C) over the course of anesthesia.
Variability in heart rate (140 ± 25 bpm; range = 88 - 220 bpm) was noted, but increased (153 ± 29 bpm)
in response to atropine administration. Respiratory rate remained constant (27 ± 7.5 breaths/min; range
= 16-64 breaths/min) for most animals.

After all procedures had been completed, the animals were allowed to partially recover in a relatively
cool, shaded spot where they were constantly observed. All animals maintained a swallow reflex
throughout anesthesia and, once sternal, were allowed to sip small amounts of water given in a syringe. Once the monkeys began showing signs of deliberate limb movement, they were placed in burlap bags located in a relatively cool, shaded place. Here they were monitored until they had recovered enough to climb a tree a height of 2 m. The burlap bags confined the monkeys and reduced the amount of visual stimulation they received, but did not restrict their ability to breathe. Recovery times varied between 30 min and 4 hr and were dependent on Telazol® dosage. Once animals had recovered, they were transported to the sight of their capture and released. Animals that had not recovered by 1 hr before dusk, were kept confined in the bags overnight and released the following morning.

The 17 animals that were anesthetized under “special circumstances” included: 1) Five juveniles and infants that were hand-injected; 2) four animals that required more than one dart-delivered injection; 3) three adults that received intra-abdominal injections; and 4) four juveniles and infants that received dart-delivered injections of adult dosages.

Adequate anesthesia of juveniles and infants was accomplished with hand injection of 2.5-20 mg/kg of Telazol®. Of all the adults that were darted, three required a second dart-administered injection of Telazol® (200 mg), while one monkey required a total of three injections. Vital parameters of these animals did not differ from those anesthetized with one injection and their recovery times were not prolonged (30 min-2 hr). All three animals that received intra-abdominal injections had somewhat prolonged recovery times (3-4 hr). One of these animals appeared pale when first examined, but its mucus membranes gradually became pink. No other problems were noted.

The greatest amount of morbidity was associated with the three juveniles and two infants that were accidentally darted with adult dosages of Telazol® (77, 80, 133, 274 and 600 mg/kg respectively). These animals experienced varying degrees of bradycardia and respiratory depression. Bradycardia was responsive to treatment with atropine (0.01-0.03 mg/kg); respiratory depression was responsive to treatment with Dopram® (doxapram HCl, A.H. Robins Co., Richmond, VA, USA; 2-4 mg/kg). The three juveniles recovered approximately 4 hr post-injection, while the two infants required supportive care overnight. It is unknown whether the smallest infant (0.33 kg) received the full contents of the dart; if this did occur, it received a dose of 600 mg/kg Telazol®. Over the course of 8 hr, this infant required four doses of atropine (0.04 mg/kg) and two doses of Dopram® (4 mg/kg). It was also given balanced electrolyte and dextrose solutions administered subcutaneously. It was kept warm and monitored overnight. The following morning it appeared fully conscious, was moving vigorously, made contact vocalizations, and was observed to suckle from its mother. Contact vocalization was a critical parameter in evaluating the readiness of an infant for release, as howler mothers will not respond to infants that do not emit these calls.

Discussion

The dosages used to anesthetize these monkeys were much higher than those reported for captive animals. Doses of 2.6-4.4 mg/kg Telazol® have been reported to be effective on captive howling monkeys; however, field experience has suggested that higher dosages are necessary in free-ranging animals. These differences are most likely due to the conditions of field anesthesia. During this study,
anesthetized howling monkeys almost always became unconscious while still in a tree. The last part of the body to relax was the tail, and they often kept a firm tail grip on a branch while they were unconscious. This muscle tone provides a problem in field conditions and higher doses of anesthetic are often needed in order to allow for tail relaxation. Higher dosages were also used to decrease induction times. Variations in foliage thickness and understory terrain provide challenges to safely tracking monkeys that have been darted. During this study, visual contact was lost with only one animal after it had been darted. Other darted monkeys had quick induction times which prevented escapes and minimized the risk that monkeys would become anesthetized once visual contact with them had been lost. Other investigations have reported the successful use of lower dosages of Telazol® for the field anesthesia of arboreal primates.1,3,5 One such study in howler monkeys, using 15-30 mg/kg Telazol®, expressly excluded the results of animals that required more than one dart.1 Another investigation used lower dosages (10 mg/kg) on wild howlers that had already been captured.5 Yet another study used dosages of 12-19 mg/kg of Telazol® in spider monkeys, but only reported on eight animals; the investigators of this report also used a Telinject® darting system, which, with lighter darts, may provide problems when darting through heavy foliage.3

Telazol® is an appropriate anesthetic as it has a wide safety margin, provides effective anesthesia and analgesia, and provides adequate muscle relaxation in most animals.4 One of the authors (KEG) has previously used other anesthetics in arboreal primates with less desirable results. The use of ketamine HCl (alone or in combination with xylazine HCl, acepromazine HCl or diazepam) has not provided adequate relaxation, resulting in monkeys that are more difficult to get out of trees and in broken limbs when the animals fall. Sernylan (phencyclidine hydrochloride) has been successfully used, but has been removed from the commercial market.2 The lack of mortality during this capture event suggests a wide safety margin for Telazol® in this species. The most notable precautions that should be taken in its use include: proper dart placement, careful selection of the areas in which animals are darted, and special care to avoid darting juvenile or infant animals with adult dosages.

ACKNOWLEDGMENTS

We thank Dr. Marco Herrero and Dr. Mo Salman for support and assistance for this project. We thank participating students for their excellence in animal care and for assisting with animal monitoring and handling.

LITERATURE CITED

INTESTINAL ADENOCARCINOMA IN MACAQUES

Celia R. Valverde, DVM,1* Ross Tarara, DVM, PhD,1 and Stephen M. Griffin, DVM2

1California Regional Primate Research Center, University of California – Davis, Davis CA 95616 USA; 2Animal Resources Services, University of California – Davis, Davis CA 95616 USA

Abstract

Introduction

Colorectal carcinoma accounts for approximately 15% of all cancers in human beings and it is the second leading cause of cancer-related death in the United States. In the nonhuman primate, intestinal cancer appears to be the most common malignant neoplasm, and it is a significant cause of morbidity and mortality in the geriatric population.

A retrospective review of intestinal carcinoma in macaques between (1982-1999) at the California Regional Primate Research Center was undertaken to identify the incidence, clinicopathologic features and survival rate.

Material and Methods

The medical records of 34 macaques with intestinal adenocarcinomas were reviewed retrospectively. Thirty-two monkeys were selected for inclusion in the study based on the following criteria: histopathologic diagnosis, follow-up period of > 6 mo, and complete necropsy performed at time of death.

Clinical evaluation

In addition of physical examination, a complete blood count, serum biochemistry and urinalysis. Clinical evaluation involved a variety of diagnostic tests including upper gastrointestinal contrast series, ultrasonography, jejunoscopy, colonoscopy, and exploratory celiotomy.

Results

Of the 32 cases reviewed; 30 were rhesus macaques (Macaca mulatta) and 2 were crab-eating macaques (Macaca fascicularis). The median age of macaques diagnosed with intestinal adenocarcinoma was 23.3 yr (range, 4-29 yr). There were 15 males and 17 females.

Clinical presentation included severe weight loss, inappetence, or anorexia. A palpable cranial epigastric abdominal mass was present in the majority of the cases. Other signs noted included emesis, hematochezia, diarrhea, partial intestinal obstruction with scant or no stool, and episodes of bloating.
Marked microcytic, hypochromic anemia, fecal occult blood positive, hypoproteinemia, hypoalbuminemia, hypocalcemia and decreased albumin/globulin ratio were the predominant clinical laboratory findings. Pre and postoperative human carcinoembryogenic antigen (CEA) serology proved to be of no significant clinical value.

The most common sites of occurrence of adenocarcinoma were the colon (25%; \( n = 8 \)), ileo-cecal (ICC) junction (21.8%; \( n = 7 \)), ICC–cecum-colon (18.75%; \( n = 6 \)), jejunum (9%; \( n = 3 \)), ICC–cecum (3%; \( n = 1 \)), ICC- ileum-cecum (3%; \( n = 1 \)), and jejunum-ileum (3%; \( n = 1 \)).

**Pathology**

Grossly, the mass appeared as a circumferential to nodular focal thickening of the intestinal wall. Lesions typically grew as bulky, fungating, ulcerated masses causing a luminal stricture and partial obstruction. Multiple synchronous neoplasias in the intestinal tract were found in 12.5% (\( n = 4 \)) of the cases and included one of each renal adenocarcinoma, hepatic cholangiocarcinoma, and gastric adenocarcinoma.

Metachronous lesions, a second primary neoplasia, was noted in a case that had hepatic hemangioma and squamous cell carcinoma of the cheek pouch (3%; \( n = 1 \)).

Local recurrence was noted in a single animal 50 mo post-surgical excision (3%). Metastases were evident in 25% (\( n = 8 \)) of the cases and involved regional nodes (\( n = 4 \)), liver (\( n = 4 \)), lungs (\( n = 3 \)), pancreas (\( n = 1 \)) and adrenal (\( n = 1 \)) in order of occurrence.

**DNA analysis**

In 10 of 13 cases DNA was successfully extracted from archived paraffin blocks. PCR-SSCP (Single Strand Conformational Polymorphism) analysis of these 10 cases were analyzed to determine the presence of K-ras mutations. However, it showed no evidence of K-ras mutations in Exon 1 or 2.

**Surgical Excision**

Exploratory celiotomy was performed in 56% (\( n = 18 \)) of the animals; 27% (\( n = 5 \)) of the animals were euthanatized on the surgical table due to a perceived poor prognosis or the presence of gross metastasis. The remaining cases (40%) had surgical excision with intestinal resection and anastomosis. One animal was lost to follow-up, two animals died during the immediate postoperative, five animals died as a resulted of dissemination of the adenocarcinoma (median survival rate [MST] 18 mo; \( x = 22.2 ± 16 \) mo; and five animals are currently alive [MST] 25 mo postoperatively).

**Survival Analysis**

Disease free interval was defined to be the interval from date of surgical excision to date of death due to recurrence. There was an 11% mortality rate during the postoperative period (3-17 days).
Adenocarcinoma dissemination lead to death or euthanasia of 15.5% \( (n = 5) \) of the surgical excision cases. Median survival time was 18 mo (range 11-50 mo).

Currently, five animals are still alive. Median survival time is 25 mo postoperatively (range 8-30 mo).

The results of intestinal resection and anastomosis in 12 macaques indicated an overall survival of 83% at 6 mo, 58% at 1 yr (in addition 16%; 2 cases are currently alive at 8 mo postoperatively), 50% at 1.5 yr, 33% at 2 yr and 8% at 4 yr.

**Discussion**

Intestinal adenocarcinomas are typically slow growing and result in gradual and progressive weight loss, microcytic anemia and partial intestinal obstruction.

Most macaques with intestinal adenocarcinomas responded well to surgical excision alone. Immediate postoperative mortality was 11% and was associated with dehiscence of anastomosis and consequently peritonitis. The preliminary results suggest surgical excision of intestinal adenocarcinomas in geriatric macaques is associated with low perioperative mortality and greater than 50% survival 1 yr postoperatively. Survivability could be potentially improved by the use of adjuvant therapies.
SURGICAL TREATMENT OF A RECTAL PROLAPSE IN A FREE RANGING MOUNTAIN GORILLA, *Gorilla gorilla beringei*, IN BWINDI IMPENETRABLE NATIONAL PARK, UGANDA

_Gladys Kalema, BVetMed, MRCVS_

_Uganda Wildlife Authority, PO Box 3530, Kampala, Uganda_

Abstract

A female juvenile mountain gorilla, *Kahara*, of Mubare tourist group in Bwindi Impenetrable National Park, Uganda, developed a third degree rectal prolapse. The rectum did not return to the body and the tissue became increasingly necrotic and affected by fly strike over the course of 1 wk. Previous rectal prolapses seen before in gorillas of this group had resolved spontaneously. Kahara became very weak and lethargic. A decision was made to carry out surgery as the only option to save the gorilla’s life.

On clinical examination most of the rectum was found to be both necrotic and with myiasis. The affected 10-cm portion of rectum was removed by amputation of both serosal and mucosal layers which had fly strike, then the viable portion was sutured back to the body wall using simple interrupted cat gut absorbable suture material. Antibiotics and anthelmintics were administered systemically. The gorilla gained full recovery after 3 wk. Histopathology of the resected rectal tissue confirmed that there was intense inflammation and necrosis with myiasis. Possible aetiologies for the rectal prolapse are constipation, enteritis, dietary, weak connective tissue in the rectum or a combination of these.

This was the first rectal prolapse operation to be carried out on a free ranging mountain gorilla and raises the question of interfering with genetics and natural selection if this condition was found to be due to a genetic predisposition. Saving the gorilla’s life could have both a positive and negative effect on the conservation of this highly endangered species.
AN OUTBREAK OF Klebsiella pneumoniae TYPHLITIS IN A CAPTIVE GROUP OF SILVERY MARMOSETS (Callithrix argentata)

Michael J. Linn, DVM,1* Bonnie L. Raphael, DVM, ACZM,2 and Patrick L. McDonough, MS, PhD3

1Department of Pathology, Wildlife Health Sciences, Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, NY 10460 USA; 2Department of Clinical Studies, Wildlife Health Sciences, Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, NY 10460 USA; 3Diagnostic Laboratory, Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Upper Tower Road, Ithaca, NY 14853 USA

Abstract

Over the course of 7 mo (August 1997 to February 1998), 10 of 26 silvery marmosets (Callithrix argentata) in the WCS collection died. Of these, 7/10 died peracutely with severe typhlitis or typhlocolitis with ulceration and transmural abscess with variable involvement of the cecal lymph nodes, serosal cavities, and other organs. Both males and females were affected. The ages ranged from 3 mo-9 yr. Klebsiella pneumoniae was the predominant isolate from a variety of sites (Table 1). Cultures were negative for bacteria commonly associated with intestinal disease in primates (Campylobacter sp., Salmonella sp., Yersinia sp., and Shigella sp.). In most cases, the marmosets died with no antemortem clinical signs. Two cases presented semi-comatose and subsequently died. One animal had a palpable mass in the caudal abdominal cavity, which corresponded to an abscessed cecal lymph node. Blood work was available on two animals. One animal had a regenerative leukocytosis with 29% band cells and both animals had pre-renal azotemia, hypergammaglobulinemia and anemia. Group treatments of surviving animals were attempted based on sensitivities from cultures taken at necropsy, however sporadic deaths continued during the 7-mo period.

This presentation was unusual in that the most common manifestation of Klebsiella pneumoniae in humans and other primates is pneumonia with subsequent bacteremia and localization in distant sites. There is only one similar report of Klebsiella pneumoniae related enteric disease in a group of primates at a research facility in Iquitos, Peru. In the Peruvian outbreak, Saguinus, Aotus and one Saimiri were affected. In the Saguinus, the primary lesion was purulent peritonitis with no intestinal involvement. In the Aotus, the most common lesion was typhlitis with lymph node abscess and peritonitis.1 During the outbreak at WCS, the infection started in the cecum with local spread (colon, cecal lymph node and peritoneum) and subsequent bacteremia (intravascular bacteria) with spread to distant sites (pleural cavity, pericardium, and meninges). Bacteria were readily identified in smears and histopathology sections. In those cases where Klebsiella pneumoniae was isolated, bacteria identified in the lesions were gram-negative rods surrounded by a thick clear capsule, which supports the belief that Klebsiella pneumoniae was the cause of the disease. In all cases, even in cases with multiple aerobic and anaerobic bacterial isolates from the abdominal cavity, Klebsiella pneumoniae was isolated in pure culture from at least one site.
After the first few deaths, an intensive survey of the building and historic necropsy records was undertaken in an effort to determine the prevalence, extent, and source of the *Klebsiella pneumoniae* outbreak. *Klebsiella pneumoniae* was isolated from some of the cultures done of group feces, exhibit substrates, food, insects and food handling areas. Evaluation of the fatty acid composition of the bacterial capsule was done to try to determine the relationship between the different isolates of *Klebsiella pneumoniae*. The fatty acid composition of the majority of the isolates from the monkeys was the same as that of the isolates from primate feces and the cockroaches but differed from the isolates of the cage branches and bark substrate.

*Klebsiella pneumoniae* occurs in the environment and as an intestinal commensal. *Klebsiella pneumoniae* is associated with timber products and wood shavings have been associated with outbreaks of mastitis in cattle. It is also a significant cause of nosocomial infections in hospitals. The unusual disease presentation in this outbreak, may be due to a highly pathogenic strain of *Klebsiella pneumoniae* that spontaneously arose in the silvery marmosets or may be due to an undetermined stressor allowing a commensal strain to cause pathology.

**LITERATURE CITED**

Table 1. Culture results from a captive group of silvery marmosets (*Callithrix argentata*).

<table>
<thead>
<tr>
<th>Animal</th>
<th>Date</th>
<th>Peritoneal Cavity</th>
<th>Pleural Cavity</th>
<th>Liver</th>
<th>Cecum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8/24/97</td>
<td>many and pure <em>Klebsiella pneumoniae</em></td>
<td>many and pure <em>Klebsiella pneumoniae</em></td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>9/4/97</td>
<td><em>Klebsiella pneumoniae</em>, <em>Proteus mirabilis</em> (pericardial swab)</td>
<td><em>Klebsiella pneumoniae</em>, <em>Proteus mirabilis</em>, <em>Clostridium perfringens</em>, <em>Fusobacterium mortiferum</em></td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>9/5/97</td>
<td>many <em>Klebsiella pneumoniae</em></td>
<td>many <em>Klebsiella pneumoniae</em></td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>10/30/97</td>
<td>many <em>Klebsiella pneumoniae</em></td>
<td>many and pure <em>Klebsiella pneumoniae</em></td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>11/12/97</td>
<td>many and pure <em>Klebsiella pneumoniae</em></td>
<td>many and pure <em>Klebsiella pneumoniae</em></td>
<td>many <em>Klebsiella pneumoniae</em> (rectal swab)</td>
<td>many and pure <em>Klebsiella pneumoniae</em> (cecal abscess), <em>Clostridium perfringens</em> (intestinal swab)</td>
</tr>
<tr>
<td>7</td>
<td>2/7/98</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>moderate and pure <em>Klebsiella pneumoniae</em></td>
</tr>
</tbody>
</table>
EXPERIMENTAL TUBERCULOSIS IN RHESUS MONKEYS

James L. Blanchard, DVM, PhD,* Peter Didier, DVM, PhD, Kimberly Scamardo, BS, and Rickey Burkhalter  
Tulane Regional Primate Research Center, Covington, LA 70433 USA

Abstract

Tuberculosis (TB) continues to be a world wide problem in the human population despite the use of vaccines and the development of several drug therapies. It has also been a disease of major concern in animal populations, especially in captive nonhuman primates in zoos and research facilities. With very few exceptions, TB research utilizing nonhuman primates has not been conducted since the mid 70’s. Tulane Regional Primate Research Center (TRPRC) has initiated several TB studies using macaques. In studying the pathogenesis of TB using modern immunologic and molecular biologic techniques, our goals are to establish the rhesus monkey as a model of human TB, and to provide a mechanism to develop and test vaccines and therapeutic agents in an animal more closely related to humans than the small animal models currently used. We will also have an excellent source of material to develop and test new, reliable means of diagnosing TB in nonhuman primates.

We have completed preliminary studies using rhesus and cynomolgus monkeys. Studies were performed to develop techniques for inoculating monkeys with TB, to determine an infectious dose that would cause disease but not rapid death, and to test the efficacy of BCG and subunit vaccines. Tests using different doses and strains of TB provided us with ample material to study the immune response and demonstrated the unreliability of the existing skin test reagents for diagnosis of disease in experimentally infected monkeys. Some of the high dose animals became anergic very rapidly, and two of the low dose monkeys died before showing a positive skin test.

An ongoing study will be the focus of this presentation and will include results of several types of diagnostic measures and some immunologic parameters. We are testing two skin test reagents (old tuberculin and 250 unit PPD) and a commercially available colorimetric test kit. In addition, clinical parameters such as body wt, chest films, clinical pathology data, and bronchoalveolar lavage (BAL) will be correlated with laboratory results of BAL fluid cultures, blood cultures, and BAL cell populations.

A brief presentation of the techniques and safety considerations of performing TB research will be included.
NEUROLOGIC COMPLICATIONS OF FELINE IMMUNODEFICIENCY VIRUS (FIV) IN LIONS

Michael Podell, MSc, DVM, Dipl ACVIM (Neurology),1,2* Raymund Wack, MSc, DVM, Dipl ACZM,1,3 and Sue VandeWoude, DVM4

1Department of Veterinary Clinical Sciences, College of Veterinary Medicine; 2Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210 USA; 3Columbus Zoo, Columbus, OH 43065 USA; 4Department of Pathology, Colorado State University, Fort Collins, CO 80523 USA

Abstract

Purpose

The purpose of this project was to study in-depth three feline immunodeficiency virus (FIV) infected lions at the Columbus Zoo. Three uninfected lions served as a control, uninfected group. Detailed immunologic, virologic, biochemical, serologic, neurologic, and histologic evaluations were performed to determine the presence of clinical disease, viral infectivity, and the association of neurologic functional changes with that of immune status and infectivity. Further studies determined gene sequences from virus isolated from FIV positive lions as a way to evaluate commonalities of the source of the virus.

Study Design

Two adult female (DJ and EL) and one adult male (TT) FIV infected lions were evaluated (approximate ages of 17, 19, and 15 yr, respectively) at the Columbus Zoo between 1996 and 1998. Three mature, uninfected lions served as a control, uninfected group (two males and one female; approximate ages were 18, 21 and 23 mo). All FIV infected lions were confirmed positive and control lions confirmed negative by detection of plasma FIV antibody with Western blot analysis using lion specific antigens. All lions tested negative for feline leukemia virus antigen by ELISA plasma screening.

The specific objectives of the study and associated methodology were to:

1. Determine the presence of opportunistic infections in relation to the overall health consequences and immunologic deterioration in chronically FIV infected lions. General health testing consisted of a serum chemistry profile, complete blood count with differential analysis and platelet count, urinalysis, hemostasis screening (OSPT, APTT, fibrinogen, fibrin degradation products), and thoracic radiographs. Diagnostic testing for infectious agents included detection of serum and cerebrospinal antibodies to canine distemper virus, feline infectious peritonitis, *Toxoplasma gondii*, *Neospora caninum* and cryptococcosis. Immune status was monitored by lymphocyte subtype analysis and total granulocyte counts at three time points for infected and once for control lions. Lymphocyte sub-populations were enumerated by immunostain and flow cytometry. Results, expressed as numbers of Pan T, CD4 and...
CD8 lymphocytes, were obtained by multiplying percent positive populations from immunostain with blood count lymphocyte values.

2. **Determine the in vivo neuropathogenecity of chronic FIV infection lions through neurologic functional testing, cerebrospinal fluid analyses, and magnetic resonance imaging (MRI) scans of the brain.** Neurologic function was assessed at two levels: observational changes in behavior and quantitative neurophysiologic changes. Observational changes in behavior and activity was assessed on a monthly basis by trained animal handlers, scored and analyzed for two FIV infected lions (EL and TT). Selected elapsed time video analysis was reviewed by an independent observer. Frequency of behavior changes were scored on a discrete scale of 0 to 3 (0 normal; 3 = agitation, stereotypic movements, or “staring-gazing” activity. Quantitative neurophysiologic changes were analyzed using auditory evoked potential (AEP) and electroencephalography (QEEG) tests. A MRI scan of the brain was performed in one FIV infected female (EL) on a 0.3 Tesla open field Hitachi AIRIS magnet.

3. **Compare the genetic sequence subtype of the feline immunodeficiency virus (FIV) isolate from FIV-infected lions as compared to FIV-infected snow leopards (2) and a tiger housed at the Columbus Zoo.** The DNA was probed from the two snow leopards and the tiger with consensus lion virus primers and ones made specifically from the male FIV infected lion (TT) virus.

**Results**

*Examination and observation:* Infected lions developed progressive weight loss and mild lymphadenopathy over the study period. All FIV infected lions were observed to display periodic unusual “star-gazing” activity of variable duration that consisted of motionless staring into the sky with unresponsiveness. Other behavioral changes included dysphagia, abnormal gait and missing benches when jumping from one perch to another. Intermittent periods of lethargy were also seen. A progressive increase in the percentage of abnormal daily observed activity was scored for EL and TT. Both female infected lions were euthanatized due to progressive physical deterioration, while the male lion died of progressive, severe anemia.

*Virology:* Virus was not amplified in vitro from any of the animals in either primary cells or feline-derived cell lines using standard culture techniques. A 325-base pair sequence was amplified from the pol region of the male infected lion (TT) and sequence analysis indicated that it fell within the "A" clade of lion viruses, yet was distinct and divergent from other isolates that have been analyzed. Primer pairs were designed from this sequence and used in permissive PCR reactions on DNA from the remaining animals. Sequence derived from EL was identical to that amplified from TT using TT primers. No fragments were amplified from the snow leopards or the seropositive tiger using either consensus lion or puma lentivirus primers, or with primers derived from the TT sequence.

*Immunology:* High titers of serum antibodies ranging from 1:800 to 1:1600 were detected in the three infected lions. All FIV-infected lions demonstrated persistent lymphopenia, and specifically, depletion of CD4 lymphocytes, increase in CD8 counts, and inversion of the CD4:CD8 ratio (Table 1).
Neurodiagnostic testing:

Electrodiagnostic examination: All FIV infected cats demonstrated abnormalities in AEP and QEEG testing. Prolonged peak onset latencies of waves III and V, and interpeak latencies as compared to the 95% confidence of control cats was seen for the AEP for all FIV-infected cats, indicating altered brain stem pathway function. Infected lions also demonstrated an increase in diffuse slow-wave activity as compared to control lions.

Neuroimaging: The most significant abnormalities associated with the MRI study done on EL were the presence of cerebrocortical atrophy, demonstrated by sulcal widening and secondary ventriculomegaly (hydrocephalus ex vacuo), and symmetric disruption of the deep white matter in the parietal lobe.

Pathology: Complete histopathologic evaluation was performed on all FIV infected lions with neuropathologic abnormalities detected in all cases. Common to all cases were cerebrocortical atrophy, astrogliosis, and suspected microglial satellitosis. The most severe focal lesions were complete loss of normal white matter of the corpus callosum and adjacent cingulate gyrus with replacement by fibrous connective tissue, presumably of vascular adventitial origin (DJ). Observation of hemosiderin-laden macrophages supports a histopathologic lesion of a cerebral vascular accident in this region. No evidence of opportunistic infections was found in any animal.

Conclusions

The results of this study support the following conclusions:

The general health deterioration observed in these FIV-infected lions were not the result of opportunistic infections.

Phylogenetic analysis of the pol region of the virus from infected lions indicated that this virus was distinct from other lion lentiviruses that have been examined but was most closely related to the "A" clade of lion lentiviruses.

Feline immunodeficiency infection in these zoo lions did result in functional neurologic changes that paralleled progressive decline in CD4 lymphocytes.

1. Diffuse and focal neuropathologic changes were present in FIV-infected lions that resemble similar changes reported in FIV-infected domestic cats, and HIV-1 infected people.

2. Overall, this study demonstrates that certain FIV isolates have potential neuropathogenecity with significant health consequences in zoo lions.

Table 1. Comparison of lymphocyte phenotypes in FIV and normal lions (x and range).

<table>
<thead>
<tr>
<th>LION (sample #)</th>
<th>CD4 lymphocyte</th>
<th>CD8 lymphocyte</th>
<th>CD4:C8 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DJ (n = 1)</td>
<td>162</td>
<td>131</td>
<td>1.2</td>
</tr>
<tr>
<td>EL (n = 4)</td>
<td>983 (270-1284)</td>
<td>525 (427-624)</td>
<td>1.9 (0.5-2.1)</td>
</tr>
<tr>
<td>TT (n = 2)</td>
<td>105 (41-170)</td>
<td>470 (115-826)</td>
<td>0.3 (0.2-0.4)</td>
</tr>
<tr>
<td>CONTROL (n = 3)</td>
<td>905 (693-1198)</td>
<td>223 (182-278)</td>
<td>4 (3.8-4.2)</td>
</tr>
</tbody>
</table>
CHANGES IN T LYMPHOCYTE SUBSETS IN AFRICAN LIONS (*Panthera leo*) SEROPOSITIVE FOR FELINE IMMUNODEFICIENCY VIRUS (FIV) AND CORRELATION WITH LENGTH OF INFECTION

*Suzanne Kennedy-Stoskopf, DVM, PhD, Dipl ACZM and Marta E. Bull*

*North Carolina State University, College of Veterinary Medicine, Department of Microbiology, Pathology, and Parasitology, 4700 Hillsborough Street, Raleigh, NC 27606 USA*

Abstract

Feline immunodeficiency virus is a lentivirus of felids that causes a persistent infection of cells in the immune system.1 Onset of recognizable disease in domestic cats ranges from months to years. Clinical signs are often non-specific and include wasting, chronic gingivitis, anorexia and behavioral changes. During the asymptomatic period, CD4:CD8 ratios are low or inverted either due to a decline in the helper T lymphocyte population, CD4, or an increase in the cytotoxic/suppressor T lymphocyte population, CD8. With time, the changes in CD4 and CD8 lymphocyte subsets impair the ability of the infected host to mount appropriate immune responses to FIV and other infectious agents.

Controversy exists about whether FIV has an adverse impact on the health of African lions. Recent experiences with captive, FIV seropositive lions in several institutions suggests that it can. This study uses three-color flow cytometric analysis to examine CD4 and CD8 lymphocytes in cryopreserved peripheral blood mononuclear cells from captive and free-ranging lions to determine if changes in T lymphocyte subsets analogous to the domestic cat occur. Antibody status was confirmed by ELISA and western blot for all lions. Seropositive, free-ranging lions (*n* = 17) were collected from Kruger National Park and seronegative lions from Etosha and Hluhlule-Umfolozi National Parks (*n* = 27). Captive lions (*n* = 5) took up to 2 yr to seroconvert following introduction of a FIV seropositive male in 1990. Flow cytometry was performed on these animals from 1994-1999.

Overall, free-ranging, FIV seropositive lions have lower CD4:CD8 ratios than their seronegative counterparts. When examined by age class, there is no significant difference in CD4:CD8 ratios between seropositive and seronegative lions ≤ 4 yr of age. However, there is a significant difference for the 5-8 yr age classes and all FIV seropositive lions older than 8 yr have inverted CD4:CD8 ratios. Decreasing and inverted ratios are the result of an increase in CD8 lymphocytes which possess a suppressor phenotype observed in FIV infected domestic cats.2 This suppressor cell population is thought to inhibit virus replication thereby contributing to the prolonged asymptomatic period. When the host’s immune system no longer contains virus replication, then clinical decline begins.

Inverted CD4:CD8 ratios are first observed in captive, FIV seropositive lions 4-6 yr following exposure to FIV. Although the CD8 suppressor phenotype is elevated in these lions compared to their negative captive counterparts, CD4 lymphocyte numbers are significantly decreased throughout the study period and contribute to the inverted ratios. All the captive, FIV seropositive lions have experienced a severe...
decline in total T lymphocytes. Three of the five lions have been euthanatized during the past 3 yr because of deterioration in condition.

To summarize, FIV does have an impact on T lymphocyte subsets of both free-ranging and captive African lions similar to that observed in the domestic cat. Assuming that free-ranging lions are infected early in life and based on the length of post-exposure to FIV in the captive animals, CD4 and CD8 lymphocyte subsets alter over time with significant changes occurring at least 4 yr post-infection. Lions are consequently at risk for developing complications associated with FIV.

ACKNOWLEDGMENTS

This work was supported in part by a grant from Ralston Purina Big Cat Survival Fund. Numerous individuals were instrumental in providing samples. We wish to thank the veterinarians and staff at the North Carolina Zoological Park for annual sampling of their FIV seropositive populations and Dr. Rebecca Yates and the Wildlife Waystation Health Center for supplying samples from seronegative lions. Drs. Mitch Bush and Cobus Raath made it possible to collect samples from lions in Kruger National Park; Dr. Mike Briggs and the Brookfield Zoological Society made it possible to collect samples from lions in Etosha; and Drs. Craig Packer, Woody Meltzer, and Dave Cooper made it possible to collect samples from lions in Hluhluwe-Umfolozi.

LITERATURE CITED

IDENTIFICATION OF A RETROVIRUS IN BENNETT'S (*Macropus rufogriseus frutica*) AND DAMA (TAMMAR) (*Macropus eugenii*) WALLABIES

Nikolay Kapustin, DVM,1* Charles Kanitz, DVM, PhD,2 and Timothy Muench, DVM, MS3

1Relief Veterinary Services, 8331 Gallant Fox Drive, Indianapolis, IN 46217 USA;  2Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907 USA;  3Animal Health Diagnostic Laboratory, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824 USA

Abstract

Mortalities associated with toxoplasmosis, histoplasmosis, aspergillosis, and a variety of cutaneous and systemic bacterial infections occurred in a captive population of Bennett's and Dama (Tammar) wallabies. Individuals of both species demonstrated a rough hair coat and poor body condition. The clinical impression was that of an immunosuppressive disorder as seen in retroviral infections and diagnostics were directed toward that etiology.

Leukocytes were isolated from heparinized blood from both wallaby species at the Indiana Animal Disease Diagnostic Laboratory. Using co-cultivation techniques, a virus was isolated and replicated in three cell lines: a low passage of Dama wallaby fetal cells, a scrub wallaby kidney cell line (QK2K) and a potoroo kidney cell line (PTK2). The best growth was demonstrated in the potoroo cells and multiple passages have been made. Infected cultures show marked cytopathic effects (CPE) with the formation of very large syncytia. If allowed to incubate long enough, the entire cell sheet becomes fused into massive syncytia. This type of CPE is similar to that seen in cultures infected with caprine arthritis encephalitis (CAE) virus and ovine progressive pneumonia (OPP) virus, both retroviruses in the genus lentivirus. Reverse transcriptase (RT), an enzyme associated with retroviruses, was evaluated in infected and control potoroo kidney cell cultures. Both magnesium-dependent and manganese-dependent RT activity were detected in the supernatants from infected cultures. The presence of RT activity is consistent with that of a retrovirus but characterization of the virus to genus based on RT activity is not possible at this point. Viral particles consistent with retrovirus size and ultrastructure have also been observed on EM of leukocyte cultures from individuals of both wallaby species of the affected mob (Michael Worley, personal communication).

An indirect immunofluorescent antibody (IFA) assay was developed using wallaby retrovirus-infected PTK2 cells grown on Teflon matted, ten-well dot slides. Sera from individuals of both species in this retrovirus culture-positive population were tested and found to have antibodies against their virus. An ongoing serosurvey of captive macropods from other facilities has identified five seropositive individuals out of 44 wallabies of mixed species tested. Twenty-six individuals of other macropod species (e.g., tree kangaroo, red kangaroo, etc.) tested negative. Currently, a PCR-based test is being developed to detect the presence of the virus.
In summary, a retrovirus has been isolated from a population of Bennett’s and Dama wallabies which experienced mortalities from opportunistic infections. Further work is needed to characterize the virus to its genus. Additional RT activity studies are warranted. The availability of an IFA and the development of a PCR assay will allow screening of the population to determine prevalence and will permit monitoring to determine if infected animals are at greater risk for acquired immunodeficiency syndromes.
CLINICAL AND PATHOLOGIC ASPECTS OF A FATAL HERPESVIRUS DISEASE IN ASIAN (Elephas maximus) AND AFRICAN (Loxodonta africana) ELEPHANTS

Laura K. Richman, DVM,1* Richard J. Montali, DVM,2 Richard C. Cambre, DVM,2 Dennis Schmitt, DVM, PhD,3 Douglas Hardy, DVM,4 Richard L Garber, PhD,5 Thomas Hildbrandt, DVM,6 Joerns Fickel, PhD,6 Willem Schaftenaar, DVM,7 and Gary S. Hayward, PhD1

1Johns Hopkins School of Medicine, Baltimore, MD 21205 USA; 2Smithsonian Institution, National Zoological Park, Washington, DC 20008 USA; 3Southwest Missouri State University, Springfield, MO 65804 USA; 4Dickerson Park Zoo, Springfield, MO 65803 USA; 5PathoGenesis Corporation, Seattle, WA 98119 USA; 6Institute for Zoo Biology and Wildlife Research, Berlin, Germany; 7Rotterdam Zoo, Rotterdam NL-3000 AM, Netherlands

Abstract

A detailed account of the virologic and clinicopathologic features of a recently recognized fatal herpesviral disease of Asian (Elephas maximus) and African (Loxodonta africana) elephants has been described by our group.5,6 We have determined that there are two closely related herpesviruses that infect elephants, one virus is lethal for Asian elephants and the other for African elephants. The predominant clinical signs for both species included lethargy, edematous swellings of the head and thoracic limbs, oral ulceration, cyanosis of the tongue and death in most elephants in 1-7 days. Pertinent laboratory findings in several of the clinically evaluated animals included lymphocytopenia and thrombocytopenia. Necropsy findings in the fatal cases included pericardial effusion and extensive petechial hemorrhages in the heart and throughout the peritoneal cavity, hepatomegaly, cyanosis, of the tongue, and intestinal hemorrhage and ulceration. Histologically there was extensive microhemorrhages and edema throughout the myocardium and a mild degree of myocarditis. Similar hemorrhagic lesions with inflammation were evident in the tongue, liver and large intestine. Lesions in these target organs were accompanied by amphophilic to basophilic, intranuclear viral inclusion bodies in capillary endothelial cells and transmission electron microscopy of the endothelial inclusion bodies revealed 80-92 nm diameter viral capsids consistent with herpesvirus morphology. Prior to the identification and description of this highly fatal herpesvirus disease in captive elephants,4-6 there existed only several reports of herpesviruses occurring in skin papillomas1 and pulmonary nodules2 of African elephants by light and electron microscopy, but no references to herpesvirus isolation. Polymerase chain reaction (PCR), followed by sequencing of DNA extracted from African elephant skin papillomas has identical sequence in the terminase gene region as the virus lethal for Asian elephants.5 Similarly, a nearly identical viral DNA sequence was identified by PCR in biopsies of the lymphoid patches from the distal vaginal tract (vestibulum) of a wild African elephant which did not contain viral inclusion bodies histologically.3,5

Morphologic evidence of herpesviruses, in pulmonary nodules of wild African elephants has been documented.2 These lung nodules are composed of lymphoid follicles which surround epithelial cells.
that contain intranuclear inclusion bodies and herpesvirus-like particles by electron microscopy. PCR followed by sequencing of DNA extracted from these lesions suggests that the herpesvirus present in African elephant lung nodules is the same virus that was lethal for the two African elephants with disseminated endotheliotropic disease.\textsuperscript{6} This may indicate that African elephants can latently harbor the two novel herpesviruses, one that can cause fatal endotheliotropic disease in Asian elephants and the other in African elephants. Three young Asian elephants recovered after a 3–4-wk course of therapy with the anti-herpesvirus drug famciclovir.\textsuperscript{7} One of these treated elephants showed a rapid abatement of clinical signs and return of hemogram values to normal which coincided with a decrease in the concentration of herpesvirus in the blood as detected by temporal semi-quantitative PCR assays.\textsuperscript{6} Although we have documented an apparent carrier state for these herpesviruses in African elephants, the mode of transmission has not been proven. Assays to detect previous exposure and possible non-viremic carrier elephants are in the development stages.

ACKNOWLEDGMENTS

Funded by NIH grant No. 1 K08 AI01526-01, the Smithsonian Scholarly Studies Program, the Kumari Elephant Conservation Fund and Friends of the National Zoo. The authors thank the following individuals and institutions for their contributions to our study: J. Cohen, National Institutes of Health; S. Feldman, Ahmed/Biosafe Inc.; S. Mikota, The Audubon Institute; R. Mirkovic, Southwest Foundation for Biomedical Research; J. d'Offay and R. Eberle, Oklahoma State University; G. Letchworth, University of Wisconsin-Madison; K. E. Steele, B. Connolly and P. Jahrling, United States Army Medical Research Institute of Infectious Diseases; A. Ruebel, Zoo Zuerich; P. Ossent, University of Zuerich; F. Osorio, University of Nebraska, Lincoln; S. Kania and M. Kennedy, University of Tennessee, Knoxville; E. Dierenfeld, The New York Wildlife Conservation Society; J. Trupkiewicz, L. Munson and D. Taylor, University of California-Davis; D. Nichols, V. Bonshock, D. Fischer, A. Brathauer, N. Spangler, K. Clark, J. Sutton, N. Pratt, M. Bush, J. Block and the elephant keeper staff, National Zoological Park; J. Jenkins and R. V. Ferris, Armed Forces Institute of Pathology; J. Gaskin, University of Florida; D. Olson, African elephant Species Survival Plan (SSP) coordinator; M. Keele, Asian elephant SSP coordinator; A. Schanberger, Houston Zoological Gardens; Marine World Africa-USA, Vallejo, CA; L. Bingaman-Lackey, AZA; N. Kriek, Univ. of Pretoria, S. Africa; R. Bengis, Kruger National Park, S. Africa; San Diego Zoo and Wild Animal Park; Center for Reproduction of Endangered Species (CRES); New York Wildlife Conservation Society; Lincoln Park Zoo; Dickerson Park Zoo; African Lion Safari; Tulsa Zoological Park; Fort Worth Zoo; Indianapolis Zoo; Dallas Zoo and the Oakland Zoo.

LITERATURE CITED

THE MANAGEMENT OF SHEEP-ASSOCIATED MALIGNANT CATARRHAL FEVER IN A ZOOLOGICAL RUMINANT COLLECTION

Nancy P. Lung, VMD, MS, Suzan Murray, DVM, and Janet Warg, MS

1 Fort Worth Zoo, 1989 Colonial Parkway, Fort Worth, TX 76110 USA; 2 Diagnostic Virology Laboratory, National Veterinary Services Laboratories, VS, APHIS, USDA

Abstract

Between February 1994 and February 1998, seven nondomestic ruminants of five species in the Fort Worth’s Zoo’s collection died with strong histopathologic evidence of malignant catarrhal fever (MCF). This paper reviews the epidemiology of the disease in this collection and the use of multiple diagnostic assays to manage and attempt to eradicate MCF from the collection.

Losses to MCF occurred in axis deer (Cervus axis) (two), hog deer (Cervus porcinus) (two), tufted deer (Elaphodus cephalophus) (one), white tailed deer (Odocoileus virginianus) (one), and dama gazelle (Gazella dama) (one). Clinical signs ranged from acute onset, rapidly progressing weight loss, and death within 5 days, to chronic weight loss, bluing of the corneas, ocular and nasal discharge with death several weeks after onset of clinical signs. All deaths occurred as isolated events within single species herds.

Blood and/or tissue samples from 116 animals representing 16 species in the Zoo’s ruminant collection were screened by polymerase chain reaction (PCR) for the WC-11 sequence from wildebeest-associated MCF. All were negative. This same group was tested by PCR for a purported sequence of OHV-2, sheep-associated MCF. Fifty-seven tested positive, including five clinically ill animals that subsequently died of MCF. Fifty-two positive animals consisted of clinically healthy mouflon (41), aoudad (9), domestic sheep (1) and goats (1). Samples for PCR testing were not available from two of the seven dead animals (one axis deer and the white tailed deer.) At no time did a clinically healthy nondomestic ruminant (excluding aoudad and mouflon) test positive for OHV-2 (48 animals representing 11 species).

Of the 116 animals screened by PCR, 72 animals representing 14 species were also screened retrospectively by competitive ELISA. Although the date of the two assays did not always coincide, there was exceptional agreement of PCR and cELISA results. Excluding domestic goats, 64 of 65 animals tested by both methods had complete agreement of results. Validation of the cELISA for domestic goats is ongoing (pers comm, T. Crawford). This population of ruminants was also screened by the Immunoperoxidase test (IPT) and Serum Neutralization (SN) methods. Positive titers on the IPT were common, with 119 of 146 animals testing positive at dilutions of 1:100 or 1:20. Six of six animals that died of MCF were tested and were all positive. The presence of neutralizing antibodies in this population of ruminants was rare, with only nine animals positive by SN of 96 tested. Six of the seven
animals that died of MCF were tested and were negative for neutralizing antibodies. Evaluation of the specificity and sensitivity of IPT and SN in this population by statistical analysis is pending.

Mouflon and aoudad are suggested to be the point source of sheep-associated MCF at the Fort Worth Zoo. The herds were 93 and 82% positive by PCR, respectively. At no time did individuals of either species show clinical disease. They were housed adjacent to each other and directly uphill, approximately 30 yd from the pens of six of the seven animals that died of sheep-associated MCF. The white tailed deer that died of MCF was housed at the opposite end of the zoo from the mouflon and aoudad, but in close proximity to the domestic sheep and goats, which might represent another point source of OHV-2 in the collection. There is no overlap of staff, traffic, or equipment between the white tail deer herd and the mouflon and aoudad herds.

PCR and cELISA appear to be sensitive screening tools for the presence of OHV-2 in nondomestic ruminants. However, the existence of a carrier state of OHV-2 in nondomestic ruminants has not been confirmed or refuted by this study. Mouflon and aoudad were eliminated from the Zoo’s collection in 1996. A tufted deer died of MCF in March 1997. A hogdeer that tested negative in April 1997 tested positive and died of clinical disease in early 98. It is not known whether these animals harbored latent virus undetectable by PCR, or if an unknown source of infection existed in the collection after removal of the aoudad and mouflon. Herd mates of the affected hogdeer were euthanatized and screened by PCR and cELISA. Both animals were negative. No animals have been lost to MCF since February 1998, including herd mates of the dama gazelle and tufted deer that had intimate exposure to clinically ill animals.

ACKNOWLEDGMENTS

The authors thank Mr. Rick Tucker for his assistance with retrospective data collection and Dr. Tim Crawford of WADDL for his consultation on the cELISA assay.
ADENOVIRUS INFECTION IN CAPTIVE MOOSE (*Alces alces*)

Dale A. Smith, DVM, DVSc,1 Cathy M. Shilton, BSc, DVM,1,* and Leslie W. Woods, DVM, PhD2

1Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, N1G 2W1; 2California Veterinary Diagnostic Laboratory System, University of California, Davis, CA 95617 USA

Abstract

On 9 August 1998, a 1-mo-old moose calf, born at the Toronto Zoo, became acutely and severely ill. Omphalophlebitis and abscess were identified and surgical debridement of the lesion was attempted, but the animal died. On gross post-mortem, widespread hemorrhages and severe hemorrhagic typhlocolitis were present as well as a hepatic abscess associated with the infected umbilicus. Microscopically, there was widespread vasculitis and thrombosis, particularly in the gastrointestinal tract. Amphophilic, intranuclear inclusions were present in many endothelial cells, suggestive of adenoviral infection. On 14 August 1998, a 2-mo-old moose calf, also born at the Toronto Zoo, became acutely sick and died. Major post-mortem findings were severe hemorrhagic typhlocolitis and moderate chronic bronchopneumonia. Microscopic vascular lesions were similar to findings in the calf, which had died 5 days earlier.

Immunoperoxidase staining for bovine adenovirus type 5 was positive on tissues from both calves. Transmission electron microscopy revealed adenovirus particles within endothelial cell nuclei of both calves. Virus isolation using tissues from both calves was attempted in primary bovine spleen, equine ovary and rabbit kidney cell lines. No virus was isolated from either case following five passages over a period of 5 wk.

Three additional cases in moose calves were identified upon review of the post-mortem results of 29 moose which had died at the Toronto Zoo since 1974 (13 calves, 3 juveniles, and 13 adults). These calves, approximately 2 wk of age, had died over a 10-day period in 1985. Two of the calves were orphaned wild moose; the third was born at the Toronto Zoo but had been orphaned at 6 days of age when its mother died of severe necrotizing typhlocolitis. The major post-mortem finding in all three calves was severe multisystemic vasculitis and thrombosis, with amphophilic intranuclear inclusions in endothelial cells.
Adenoviruses are non-enveloped, highly host specific, DNA viruses. Infection is usually systemic, with strains of the virus having tropisms for the respiratory and alimentary tracts, vascular endothelial cells or hepatocytes. Typically, adenoviruses cause mild or subclinical respiratory or enteric infections, with clinical disease only in young or immunosuppressed individuals. In domestic cattle there are 10 serotypes of the virus. Many strains can be isolated from normal cattle. Enteric infections have been described sporadically in 1-8-wk-old calves and feedlot animals. Post-mortem findings in affected calves include vasculitis, endothelial intranuclear inclusions, and widespread thrombosis.¹

In 1996, an outbreak of adenoviral disease occurred in wild mule and black-tailed deer in Northern California.² The main post-mortem lesions included severe pulmonary edema and hemorrhagic enteritis, with widespread vasculitis and thrombosis. Intranuclear inclusions were prominent in endothelial cells. A diagnosis was made based on electron microscopic demonstration of adenoviral particles in endothelial cells, fluorescent antibody and immunohistochemistry using antibody to bovine adenovirus type 5 and virus isolation in deer pulmonary artery endothelial cells.

This is the first report of the infection of moose by an adenovirus, and of the presence of adenoviral disease in a cervid in Canada.

LITERATURE CITED

A MEDICAL SURVEY OF TOURISTS VISITING KIBALE NATIONAL PARK, UGANDA, TO DETERMINE THE POTENTIAL RISK FOR DISEASE TRANSMISSION TO CHIMPANZEES (Pan troglodytes) FROM ECOTOURISM

Hayley R. Adams, BS,1* Jonathan Sleeman, MA, VetMB, MRCVS,2 and John C. New, DVM, MPH1

1College of Veterinary Medicine, The University of Tennessee, Knoxville, TN 37901 USA; 2Colorado State University, Veterinary Teaching Hospital, Fort Collins, CO 80523 USA

Abstract

There are an increasing number of free-ranging chimpanzee groups in Uganda habituated for tourism. Due to the close taxonomic relationship between humans and great apes, there exists the potential for disease transmission to chimpanzees from visiting tourists. In order to ensure the development of successful ecotourism programs with minimal effect on the health of endangered primates, measures must be established to prevent the transmission of disease. The aim of this study was to determine the potential risk for anthropozoonotic disease transmission by surveying the medical histories of human visitors to chimpanzees at Kibale National Park, Uganda. Medical histories were taken by using a consent form, to ensure confidentiality, and a questionnaire, to gather information on vaccination and disease histories as well as any recent occurrence of disease symptoms. Approval for this study was obtained from the human subjects review committee of the University of Tennessee.

A total of 43 surveys were completed from July to November, 1998, with a predominance of European tourists 30/43 (70%), followed by tourists from Australia 6/43 (14%), the United States of America 4/43 (9%), and Africa 3/43 (7%). Although many individuals had been vaccinated against the diseases included in the questionnaire, not all were current on their vaccines. For example, 84% of those surveyed were vaccinated against hepatitis A, of which only 56% were current for the vaccination. Of those individuals diagnosed with a disease listed in the questionnaire, five cases of herpes virus infection, four cases of influenza, one case of chicken pox, and two cases of tuberculosis were still considered to be infectious at the time of visitation. When asked if they had been tested for tuberculosis, 44% answered no, and 10% were unsure about whether or not they had been tested. Of the 46% who answered yes to being tested, two individuals (11%) tested positive for tuberculosis.

The most common symptoms of disease or illness reported by foreign tourists during their stay in Africa were diarrhea (53%), coughing (23%), and vomiting (13%). If these symptoms were in fact due to some infectious agent, and were ongoing during the visit to Kibale National Park, the possibility of disease transmission to the chimpanzees is evident. Seventy percent of those surveyed indicated that they had either already visited another group of habituated chimps or gorillas prior to the Kibale visit, or they intended to upon leaving Kibale. Therefore, humans should be considered as potential sources of disease, both as carriers of infectious agents as well as fomites or vehicles of disease. These infectious diseases could then be transmitted from habituated animals to more isolated populations, further endangering the species. Of those individuals informed of the viewing regulations prior to their visit...
to the chimpanzees, 100% replied that they understood the need and rationale for these regulations. This is encouraging and suggests that proper education of all visitors to Kibale is an effective means of ensuring visitor compliance to regulations.

Results from these surveys should assist the Uganda Wildlife Authority to develop appropriate regulations to prevent disease transmission. This will contribute to the health management of the chimpanzees as well as to help develop a sustainable primate ecotourism program. For example, current park regulations for viewing chimpanzees include a maximum number of six tourists per visit, with a minimum distance of 5 m between the chimpanzees and the human visitors. Based on these results, regulations could be extended to include the use of face masks to reduce or prevent the transmission of airborne disease agents, and the enforcement of the right to refuse a visitor if he/she is ill. Other possible regulations to consider include the mandatory washing of hands and the use of disinfectant foot baths for all tourists prior to the visit. Further measures to minimize the potential for disease transmission could involve the provision of adequate pit latrines to ensure proper disposal of human waste. These facilities should remain at a safe distance from areas occupied by chimpanzee groups. Provision of regular health examinations and vaccination of park staff and field researchers would minimize disease risk from those in frequent contact with the chimpanzees.

The risk for disease transmission warrants further investigation. Future surveys of tourists could include serologic surveys for common infectious diseases, throat culture swabs to isolate potential aerosolized pathogens, and testing for tuberculosis. Lastly, development of non-invasive monitoring of chimpanzee health status via opportunistic blood, skin, urine, or fecal sampling, and necropsies, would further document the impacts of disease on chimpanzee populations, as well as indicate to which human diseases the chimpanzees are susceptible.

ACKNOWLEDGMENTS

We thank the Uganda Wildlife Authority, particularly Mr. Samson Werikhe, Research Coordinator, for granting permission to conduct this study. Valuable assistance was provided by the Chief Warden of Kibale National Park, Mr. Keith Musana, the Warden of Tourism at Kibale, Mr. Aggrey Rwetsiba, the Veterinary Officer, Dr. Gladys Kalema, and Debby Cox of the Jane Goodall Institute. Special thanks to Julia Lloyd and Daniela Pezzato of The Kibale Primate Habituation Project for assistance with the administration of the surveys, and to Dr. Antoine Mudakikwa and Dr. Juergen Schumacher for language translation of the questionnaire and consent form. This project was partially funded by the Center for Conservation Medicine, Tufts University.
NEURAL ANGIOSTRONGYLOSIS IN NONHUMAN PRIMATES: DIAGNOSIS, TREATMENT AND CONTROL OF AN OUTBREAK IN SOUTHERN LOUISIANA

Roberto F. Aguilar, DVM,1* Kathy Topham, DVM,1 J. Jill Heatley, DVM,2 Don Nichols, DVM, Dipl ACVP,3 John Cross, PhD,4 Rudy Bauer, DVM, PhD, Dipl ACVP,5 and Michael Garner, DVM, MS, Dipl ACVP6

1Audubon Zoo, 6500 Magazine Street, New Orleans, LA 70118 USA; 2Veterinary Teaching Hospital, Louisiana State University, North Stadium Drive, Baton Rouge, LA 70803 USA; 3Department of Pathology, National Zoo, Washington DC 20008 USA; 4Uniformed Services University of the Health Sciences, F.E. Hebert School of Medicine, Bethesda, MD 20814 USA; 5Louisiana Veterinary Medical Diagnostic Laboratory, PO Box 15070, Baton Rouge, LA 70894 USA; 6Northwest Zoopath, 18210 Waverly Drive, Snohomish, WA 98296-4815 USA

Abstract

Six cases of angiostrongylosis were seen in nonhuman primates at the Audubon Zoo over a 6-yr period. Affected animals included two howler monkeys (Alouatta caraya), three black and white ruffed lemurs (Lemur variegatus), and a talapoin monkey (Cercopithecus talapoin). All manifested with varying degrees of neurologic signs. In general, antihelmintic treatment was contraindicated, as clinical signs were attributed to host inflammatory responses, which increase with the death of the migrating nematode larvae. Two of the lemurs were euthanatized and one howler monkey died as a result of the infection. Angiostrongylus cantonensis was confirmed on histopathologic sections of the brains and spinal cords. The absence of cases since 1992 is attributed to the physical removal of the intermediate hosts, slugs and snails, from exhibit areas. The life cycle, clinical signs, diagnosis, treatment, and prevention of A. cantonensis in humans, nonhuman primates, and other species are reviewed.

Introduction

The nematode, Angiostrongylus cantonensis, is the lungworm of rats. Humans and other mammals can be aberrant hosts of the parasite. In humans, Angiostrongylus cantonensis infection is most often characterized by eosinophilic meningoencephalitis, although an ocular form and pulmonary involvement have been described. Natural occurring neural infections have been described in a variety of nonhuman primates (tamarin, cynomolgous monkey, howler monkey, white handed gibbon), wallaby, bettong, horse, and dog. The pathogenesis of the disease process is produced by motile larvae and young adults migrating through the CNS, and by the host s granulomatous reaction. Eosinophilic meningoencephalitis occurs widely in the tropics and has recently been reported in North America. Causes for the spread of this host parasite system are many and include cultural and economic factors. Infection occurs via the ingestion of the intermediate host (snails or slugs) or paratenic host (freshwater prawns, land crabs, frogs, or lizards) in an uncooked or undercooked state. In humans, antemortem diagnosis is dependent on serologic testing, although peripheral absolute eosinophilia is highly suggestive. Radiographs, computerized tomography or magnetic resonance imaging may also provide results consistent with Angiostrongylus infection; however, a negative result cannot rule out presence of the organism. Treatment is palliative and should be aimed at preventing damage caused by inflammation associated with parasite migration and secondary infection. Use of anthelmintics is not
recommended. Prevention is paramount and should involve preventing ingestion of the intermediate or paratenic hosts. Although prognosis is generally considered good in humans, prognosis in nonhuman primates and other mammalian hosts, especially those exhibiting signs of lower motor neuron dysfunction, is guarded.

**Life Cycle**

The definitive host of *A. cantonensis* is a variety of rodents of the genus *Rattus* and *Bandicota*. In one study, 21.4% of 94 rats caught in New Orleans, Louisiana were found to be infected. Adults of *A. cantonensis* inhabit the pulmonary arteries, where eggs are laid and hatch. The first stage larvae enter the alveolar space, migrate up the trachea and then down the alimentary tract, and are excreted in the feces. Terrestrial snails, slugs, and aquatic snails serve as intermediate hosts, with infection occurring by percutaneous or oral (ingestion) routes. Larvae develop to third stage within the mollusk. Third stage larvae were recovered from slugs but not snails found at the Audubon Zoo. Rats then become infected by ingestion of infected slugs or snails. The larvae migrate from the intestine to the central nervous system where development through two more stages occurs in 2-3 wk. Larvae then migrate to the subarachnoid space, enter the venous system, and make their way to the pulmonary arteries for maturation and reproduction.

In human and other aberrant hosts, infection occurs secondary to ingestion of raw or undercooked snails or slugs (intermediate hosts), contaminated vegetables, or paratenic hosts (freshwater prawns, land crabs, frogs, lizards) which feed on the intermediate host. In humans, larvae migrate to the CNS, where development stops. Humans and nonhuman primates are considered dead end hosts. Pulmonary involvement has been reported but is considered rare. The incubation period ranges from 1-5 wk with an average of 2 wk.

**Pathogenesis/Clinical Signs**

In humans, headache, stiffness, vomiting and myalgia in the arms and shoulders are complaints in mild cases. Paresthesias involving the trunk, lower extremities, bowel and bladder dysfunction, weakness and hyporeflexia of the legs have been reported with involvement of the brain spinal cord and nerve roots. Pathogenesis depends on the damage caused by the larvae and young adults, as well as the host’s inflammatory response. Cranial nerve palsies (III, IV, VI, VII) are also common clinical signs. In a study of Taiwanese children, common additional initial signs were fever, cough, rhinorrhea, marked abdominal distension, and hepatomegaly.

At the Audubon Zoo, clinical signs in nonhuman primates have invariably been restricted to the nervous system and have included posterior paresis progressing to paralysis, loss of balance, nystagmus, facial hemiparesis, head tremors, ataxia, abnormal forelimb extension, and progressive hemi or tetraparesis. Based on clinical experience, animals showing upper motor neuron signs tend to recover with supportive care, while animals showing progressive lower motor neuron signs evolve into deeper states of paresis until they become unsalvageable. Similar patterns have been noted in affected dogs.
Primates surviving initial onset are occasionally left with permanent sequelae, such as hypermetria and head tilt.

Previous cases of Angiostrongylus infections in nonhuman primates manifested constitutional signs such as weakness, lethargy, anorexia, loss of body condition and vomiting. Neurologic signs have included progressive tetraparesis, incoordination, an inability to walk, sit, stand, climb, or eat, and paralysis of extremities. In addition, diarrhea, urinary incontinence, tail chewing, ptyalism, tremors, and disorientation were seen in tamarins. In dogs, clinical signs were primarily limited to tail and ascending paresis, bladder paresis, lumbar hyperalgesia, and muscle wasting. In the horse, tetraparesis, hyporeflexia of all limbs, urinary incontinence, and an inability to rise were common presenting signs in two foals. In three bettongs (Aepyprymnus rufescens), progressive hindlimb dysfunction from paresis to paralysis, deterioration of proprioception and superficial sensation, along with bladder dysfunction and flank lesions caused by excessive grooming were noted. In a single Bennet's wallaby (Macropus rufogriseus), poor body condition and incoordination progressed to posterior paresis, followed by recumbency, bilateral mydriasis, nystagmus, and opisthotonic spasms.

**Diagnostics**

In humans, laboratory diagnosis is based on the findings of eosinophilic CSF pleocytosis (500-5,000 cell/mm³ with 20-90% eosinophils), elevated CSF proteins, and normal to slightly decreased CSF glucose. Charcot-Leyden crystals may be observed in the CSF. Blood leukocytosis (>10,000) with eosinophilia (>10%) is uncommon in adults; however, in children this appears to be a frequent finding, accounting for 80% of the cases documented in Thailand. In humans, diagnosis may be confirmed by serologic testing by means of IF or EIA.

Signs of peripheral eosinophilia and leukocytosis are noticeable in most affected mammals that become aberrant hosts. In bettongs, consistent elevations were noted in cerebrospinal leukocytes and eosinophils. In all nonhuman primate cases seen at Audubon Zoo, peripheral eosinophilia and leukocytosis were observed consistently. However, eosinophilia did not always correlate with onset of clinical disease and was seen as late as 2 mo after clinical signs ceased.

Serology was used successfully in nonhuman primates in the initial cases diagnosed at the Audubon Zoo (J. Cross, personal communication). A reagent was changed in 1991, and cross-reactivity with nonhuman primate serum ceased. The samples taken from a male black and white lemur euthanatized in 1992 were sent to Taiwan, where cross-reaction was not observed, in spite of known infection. Magnetic resonance imaging was attempted in a suspected positive female howler monkey, but the size of the animal produced artifactual images that were not diagnostic. At present, there is no effective serologic means for diagnostic confirmation in nonhuman primates.

**Treatment and Prognosis**

In humans, the disease is usually benign and self-limiting, with symptoms persisting for only 2-4 wk. Death is rare. Treatment is aimed at control of pain and inflammation with analgesics and sedatives,
while corticosteroids are reserved for cases with neurologic deficit or severe inflammation. No treatment has been recognized as effective. Anthelmintics are generally not recommended as the death of the parasites is associated with a worsening of clinical signs or death due to an increased reaction to the dead or dying worms. Nevertheless, in a study in affected Taiwanese children, albendazole and levamisole were used with good results.

In the nonhuman primate cases at Audubon Zoo, 4/6 were treated with anthelmintics, 6/6 were placed on at least one form of antibiotic, 5/6 were placed on steroids, and 1/6 was placed on nonsteroidal anti-inflammatories. A female howler (age 6 yr) and a female talapoin monkey (age 3 yr) experienced complete resolution of clinical signs. Two male black and white ruffed lemurs (ages 4 and 5 yr) had to be euthanatized due to irreversible neurologic damage, while a female black and white ruffed lemur (age 4 yr) recovered with a permanent head tilt. A male howler monkey (age 9 yr) was found dead, while a talapoin monkey recovered without visible sequelae.

Other affected mammals reported to be infected showed similar patterns. Two foals reported with angiostrongylosis were euthanatized. In macropods reported to have suffered visceral larva migrans secondary to *Angiostrongylus cantonensis*, a wallaby died and bettongs were euthanatized. All five of the tamarins (*Saguinus* sp.) reported to be infected died. In a study of 55 cases of canine neurostrongylosis, only puppies with the most severe neurologic signs consisting of complete hind limb paralysis, urinary overflow incontinence, flaccid tail paresis, and hind limb paralysis were euthanatized. This followed 2 wk of supportive care and corticosteroids with no clinical improvement noted. Most other puppies in the study recovered uneventfully. There is a single report of infection in a reptile, a yellow tree monitor (*Varanus bengalensis*).

The poor prognosis in mammals other than humans remains unexplained, but may be due in part to the large dose of parasites received relative to body size, or an inability to treat as early due to common clinical signs such as headache and myalgia, which are difficult to evaluate in animals.

**Prevention in Zoos**

Successful resolution of angiostrongylosis in the primate collection at the Audubon Zoo depended on repeated physical removal of slugs and snails from animal and public contact areas. Attempts at organic and safe baiting were unsuccessful. Treatment of the area with copper based compounds was deemed to be too risky due to the possibility of secondary toxicity to collection mammals. Beer traps, citrus rind and other low toxicity natural methods had no appreciable results. Initial removals were bi-annual, until low total slug and snail counts were achieved in exhibits considered to be high risk. The now annual Slugathon has between 30-60 volunteer participants, with first prize and recognition going to the Slugmaster, the person who collects the most slugs and snails from a single enclosure. Slugs and snails collected were initially submitted to the Tulane School of Tropical Medicine for analysis. A total infection rate of 1-2% was estimated from 200 animals dissected and examined in 1994 (Little, personal communication). Instituting an aggressive and effective pest control program has reduced the total population of rodents on zoo grounds, so the cycle has fewer possibilities of perpetuating itself. Snail and slug removal has been subjectively deemed to be effective. The first Slugmaster collected over
400 slugs and snails (over 500 g in weight) from a single outdoor howler exhibit, while the runner up had over 200 gastropods from the same enclosure. The last contest, only 40 gastropods could be removed from the entire area around the exhibit. There has not been a suspected case of angiostrongylosis since the inception of the removal program.

LITERATURE CITED

EPIZOOTIC HOCK OSTEOARTHritis IN CAPTIVE SIBERIAN CRANES (Grus leucogeranus)

Julia A. Langenberg, VMD* and Nancy K. Businga, RVT, MS

International Crane Foundation, Box 447, Baraboo, WI 53913-0447 USA

Abstract

Arthritis involving the joints of the legs is a common problem in captive cranes.1,4 Trauma is the most common etiologic factor; infectious arthritis and arthritis secondary to congenital or developmental deformities have also been documented.1 Here, we report on bilateral progressive degenerative osteoarthritis involving the intertarsal (hock) joints and seen uniquely in one species of cranes, the Siberian crane (Grus leucogeranus). The International Crane Foundation (ICF) maintains a breeding population of 15 Siberian cranes to produce eggs and chicks for release programs in Asia. The Siberian crane is the third rarest species of crane, and there is intensive international work to conserve the endangered wild populations.3 Siberian cranes are relatively rare in captivity.

Lameness was first noted in two of ICF’s adult Siberian cranes at 15 yr and 21 yr of age, respectively. In both cases, the lameness was associated with bilateral firm enlargement of the intertarsal joints. Radiographically, there was both significant soft tissue swelling and periarticular bone proliferation. No bacteria were found on aerobic culture of synovial fluid aspirated from the joints; cytology of joint fluid was compatible with mild chronic inflammation. In both cases, the severity of the joint changes and the lameness progressed over the next 5 yr. Non-steroidal anti-inflammatory drugs (Table 1) initially appeared to help maintain the birds’ mobility, but later in the course of the disease these drugs were no longer palliative. Inter-articular and i.m. injections of polysulfated glycosaminoglycans (Adequan®) had no observable effect. One bird was euthanatized when it became so debilitated that it was considered inhumane to prolong its life; it was still producing eggs that year. Gross and histopathologic examination of its hock joints showed replacement of the articular cartilage by dense fibrocartilaginous tissue with many areas of erosion. The joint capsule was thickened with chondroid metaplasia, but little inflammation. There was marked periarticular bony reorganization. In the second case, replacement of the more severely affected hock with a human digit artificial joint was attempted; this surgical treatment will be reported by Bennett et al. at this meeting.

The other Siberian cranes in the ICF flock were screened radiographically for evidence of osteoarthritis in the hock joints. All the cranes 13 yr and older (seven birds) had degenerative changes in at least one hock. The consistent early change was a small osteophyte (“chip”) anterior to the hock joint, apparently associated with the joint capsule. A survey requesting information on occurrence of hock arthritis and husbandry practices was mailed to the 17 zoos world-wide known to house Siberian cranes. Affected Siberian cranes were identified at two other institutions.
Because this degenerative osteoarthritis is bilateral, not known to be associated with trauma, occurs regularly in young adults, and is seen in Siberian cranes but not the other 14 crane species at ICF, we investigated species-specific biology and husbandry practices as possible causal factors. Siberian cranes are the most aquatic of the cranes, exclusively using wetlands for roosting and feeding in both their breeding and wintering ranges. It is probable that their leg joint anatomy is adapted to “mushy” substrates. At ICF, and at both of the two other centers that have affected Siberian cranes, the cranes have generally been housed in dirt pens without ponds, with access to concrete-floored buildings. We hypothesize that the chronic joint stress associated with standing and walking on packed dirt and concrete may precipitate the osteoarthritis seen in this species. ICF is working on modifying its husbandry of Siberian cranes, primarily through seasonally providing ponds with mud substrates. The Siberian cranes will be monitored radiographically to see if these husbandry changes reduce the incidence and progression of progressive degenerative hock arthritis in the flock.

LITERATURE CITED


Table 1. Non-steroidal anti-inflammatory drugs used orally in arthritic cranes.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carprofen</td>
<td>5-7 mg/kg b.i.d.-t.i.d.</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>0.3-0.5 mg/kg b.i.d.</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>5-10 mg/kg b.i.d.</td>
</tr>
<tr>
<td>Flunixin meglumine</td>
<td>1-2 mg/kg s.i.d.</td>
</tr>
</tbody>
</table>

*Flunixin meglumine has been shown to be nephrotoxic, even at very low doses, in several species of birds, including cranes. It is no longer used at ICF.*
USE OF A FINGER JOINT PROSTHESIS FOR THE MANAGEMENT OF TARSAL ARTHRITIS IN A SIBERIAN CRANE (Grus leucogeranus)

R. Avery Bennett, DVM, MS, Dipl ACVS* and Eugene T. O’Brien, MD

1University of Florida, College of Veterinary Medicine, Department of Small Animal Clinical Sciences, PO Box 100126, Gainesville, FL 32610 USA; 2The Hand Center, 9150 Huebner Rd., Suite 290, San Antonio, TX 78240 USA

Abstract

Introduction

Degenerative joint disease (arthritis) occurs secondary to various disease processes such as trauma, infection, immune mediated disease, and congenital abnormalities. Primary arthritis is uncommon in animals. Medical management of arthritis centers around the use of antinflammatory medications to help decrease the pain associated with arthritis. Surgical management involves either an excision arthroplasty (such as femoral head and neck excision arthroplasty for hip dysplasia) or joint replacement using prosthetic implants. In human medicine artificial joints are available for most joints including the digits. In veterinary medicine, joint replacement surgery has primarily involved the use of total hip replacement for the management of arthritis secondary to hip dysplasia in dogs. Other joint replacements have been used with limited success.

This report describes the use of a silastic human finger joint prosthesis for the management of severe degenerative joint disease in the hock of a Siberian crane (Grus leucogeranus).

Case Report

A 20-yr-old, 4-kg male Siberian crane was evaluated for severe degenerative joint disease of both tarsal joints. The bird was hatched from an imported egg at the International Crane Foundation in Baraboo, WI. The arthritis was progressive over a period of approximately 5 yr involving first the tarsus of the right leg and later the left tarsus was noted to be affected. Both joints were enlarged and firm on digital palpation. There was a 2 × 3 cm scab on the plantar surface of the right hock apparently related to the bird hock sitting for extended periods of time. For 3 mo, the bird had been barely able to rise and stand requiring its wings to balance itself while rising from a sternal position. As a result, the skin over both carpal joints was eroded. Treatment had consisted of 2 mg/kg methylprednisolone i.m. as needed for several weeks prior to presentation. Because of concerns regarding the potential effects of the glucocorticoid on wound healing, the methylprednisolone was discontinued 2 wk prior to presentation. The arthritis pain was then managed using butorphanol at 1.25 mg/kg i.m. as needed.

A preoperative CBC and plasma chemistry profile revealed an anemia (PVC = 25%). Other values were within ISIS normal ranges. Radiographs of both tarsi showed severe degenerative joint disease with
the right being slightly more severely affected than the left. A total hock joint replacement using a human silastic finger joint prosthesis (Wright Medical Technology, Arlington TN 901-867-9971) was performed on the right tarsus because it was clinically and radiographically most severely affected.

The crane was anesthetized with isoflurane in oxygen through an endotracheal tube and given 20 mg/kg cefazolin i.v. every 2 hr during the procedure. A 20-cm incision was made along the cranial aspect of the distal tibiotarsus, across the tarsal joint and down the proximal tarsometatarsus. Care was taken to avoid major blood vessels. There was concern that significant vascular injury might result in avascular necrosis of the distal extremity. The joint capsule was very thick (approximately 1 cm) and numerous osteophytes were encountered. Large osteophytes were removed with rongeurs to allow access to the joint. Both medial and lateral collateral ligaments were elevated off their attachments to the tarsometatarsus to allow the joint to be opened enough to insert the bone saw. Extensor tendons were retracted rather than being transected. Stay sutures were placed in the common digital extensor tendon and it was incised. This tendon courses along the cranial aspect of the tarsus interfering with the saw used for the osteotomies. It passes through an osseous tunnel which prevents it from being retracted enough to expose the joint. At this point, the joint could be opened in a direction opposite to the normal flexion of the tarsus. This hyperextension allowed access to the distal tibiotarsus and proximal tarsometatarsus. An oscillating bone saw was used to cut the articular surfaces off the distal tibiotarsus and proximal tarsometatarsus leaving a 1-cm gap between the cut ends to accommodate the prosthesis.

The medullary canals of the proximal tarsometatarsus and the distal tibiotarsus were reamed with a broach manufactured to create a hole to precisely accept the stems of the prosthesis. The prosthesis was inserted and the joint flexed into a normal position. The tarsus was put through several flexion-extension cycles to assure the prosthesis fit and functioned well. A Kirschner wire was used to create a small hole from cranial to caudal in both the lateral and medial aspects of the proximal tarsometatarsus. The collateral ligaments were secured to the proximal tarsometatarsus by passing 2-0 polypropylene suture through the collateral, through the hole, then exiting out the collateral ligament. The joint capsule was closed using 3-0 polydioxanone suture in a simple interrupted pattern. The common digital extensor tendon was repaired using 2-0 polypropylene in a three loop pulley pattern. Subcutaneous tissue and skin were closed routinely. During recovery a lateral thermoplastic splint was applied from the proximal tibiotarsus to the distal tarsometatarsus. A Doppler flow probe confirmed the presence of arterial perfusion of the digits. Recovery was uneventful.

The bird was given butorphanol at 1.25 mg/kg every 4-6 hr postoperative for pain. The day after surgery the foot was warm to the touch; however, the bird was not able to stand. A sling was created to keep the bird off the ground but keep it from bearing weight. Physical therapy consisted of taking the bird out of the sling and encouraging controlled weight-bearing. On the third postoperative day it was noted that there was external rotation at the tarsus. Ten days after surgery the splint was removed and it was determined that the external rotation was a result of medial collateral instability. The splint was replaced in a manner to decrease tension on the medial aspect of the joint in an effort to encourage the insertion of the ligament to attach to the proximal tarsometatarsus. Eighteen days later there was still medial instability and it was decided that surgical intervention was necessary.
An incision was made through the scar of the previous incision. The skin was elevated exposing the medial aspect of the joint. Tissues were bluntly dissected to expose the medial aspects of the distal tibiotarsus and proximal tarsometatarsus. A 3.5-mm bone screw was placed in the medial cortex of each bone. The screws were not placed through to the lateral cortex as they would have damaged the stems of the prosthesis. Nylon (60# test sterile fishing line) was passed in a figure eight pattern around the screws to reconstruct the medial collateral. The incision was closed routinely and the leg placed in a lateral splint again. Prior to closure the site was cultured and the bird was placed on enrofloxacin at 10 mg/kg every 12 hr. Aerobic and anaerobic cultures were negative.

One week after the second surgery upper respiratory noises were observed and a CBC and plasma chemistry profile were evaluated. The crane had a WBC count of 35,200/mm³ with a mature heterophilia, lymphopenia, and monocytosis. Radiographs of the body were made and were within normal limits. A tracheal wash yielded no bacterial or fungal growth and normal cytology. Blood cultures were positive for an *Enterococcus* sp. During the examination it was determined that the medial joint compartment was again unstable. The bird was started on amoxicillin+clavulanic acid at 150 mg/kg and itraconazole at 10 mg/kg orally. One week later a third surgery was performed in an effort to reconstruct the medial collateral ligament.

The screws placed during the previous surgery had failed resulting in laxity of the medial joint compartment. A Kirschner wire was used to create a hole from cranial to caudal in both the distal tibiotarsus and proximal tarsometatarsus. Nylon (60# test sterile fishing line) was passed through these holes in a figure eight pattern and tightened to stabilize the medial compartment. A transarticular external skeletal fixation device was placed to immobilize the hock joint allowing the medial collateral to heal without stress. Two pins were placed in both the tibiotarsus and tarsometatarsus. The site was cultured again and this time was positive for *Enterococcus* sp. which was sensitive to amoxicillin+clavulanic acid.

The bird was still maintained in a sling but was taken out three times daily for physical therapy consisting of walking with the body supported in a sling. After the fixator was applied, the crane was able to bear weight on the leg with the prosthesis but was no longer able to bear weight on the left (unoperated) leg. Because of the bird’s inability to use the left leg and the protracted course of the recuperation a poor prognosis was offered for regaining the ability to stand and walk again. Euthanasia was being considered when the crane was found dead in his enclosure.

Gross and microscopic evaluation fail to reveal a specific cause of death for this Siberian crane. He had moderate to severe muscle atrophy as well as hepatic lipidosis; however, his weight had maintained between 4-5 kg for the duration of his hospitalization.

**Discussion**

In the management of arthritis, surgery is indicated to eliminate pain by removing the articular surfaces of the bones involved and either allowing a pseudoarthrosis to form or implanting a prosthetic joint for a better functional outcome. Because of the endangered status of Siberian cranes, the long life span (up
to 70 yr), and the value of this bird as a breeding animal, a joint replacement was considered. The advanced state of the degenerative joint disease was considered to be a contraindication for surgery, but a decision had been made to manage the bird medically until it was refractory to medical management. It is likely the outcome would have been better if the surgery were performed before both legs were severely arthritic. Following the surgery, this crane did not have a good leg on which to stand; consequently, the morbidity associated with managing a crane in a sling for an extended period of time became an issue.

Another factor in this case was the failure of the medial collateral repair. This resulted in severe external rotation at the tarsal joint and two additional surgeries were required to attempt to repair the medial collateral ligament. It appears the lateral splint did not provide adequate stability to allow the ligaments to heal appropriately. Based on this case it would seem more appropriate to place a transarticular external fixation device during the initial joint replacement surgery as there does not appear to be a way to avoid damaging the collateral ligaments during the approach.

The prosthetic joint appeared to function well during range of motion physical therapy. Unlike the tarsal joint of mammals, that of birds is a simple hinge joint between the distal tibiotarsus and the proximal tarsometatarsus similar in size and function to a human interphalangeal joint. The collateral ligaments provide medial and lateral support. The digital extensor tendons course along the cranial aspect of the joint and the digital flexor tendons travel along the plantar surface of the joint. Blood supply is provided by a single artery (dorsal metatarsal artery) and a single vein (medial metatarsal vein). Damage to one or both of these vessels can result in avascular necrosis of the distal extremity. It would be ideal to be able to implant the prosthesis without damaging any ligaments, tendon, or blood vessels. Based on preoperative dissections using Siberian crane cadaver legs the surgical approach used in this case appears to be the best for allowing the articular surfaces of the bones to be cut off with minimal damage to support structures. The prosthesis used in this crane was the largest finger joint prosthesis made.

The cause of the degenerative joint disease in this crane was not definitively determined. It was suspected to be due to a less aquatic captive environment than they live in naturally. It is thought that their buoyancy in water results in less impact on the tarsal joints, and when they are in a more dry environment the impact associated with walking results in cartilage damage over time and subsequent degenerative joint disease. This syndrome appears to be unique to Siberian cranes which are more aquatic in their natural habitat than most other species of cranes.
USE OF PHOTODYNAMIC THERAPY AGAINST SQUAMOUS CELL CARCINOMA IN THE CASQUE OF A GREAT INDIAN HORNBILL (Buceros bicornis)

Wm. Kirk Suedmeyer, DVM,1* Dudley McCaw, DVM,2 and Susan Turnquist, DVM, MS, PhD3

1Kansas City Zoological Gardens, 6700 Zoo Drive, Kansas City, MO 64132 USA; 2University of Missouri-Columbia, College of Veterinary Medicine, Clydesdale Hall, 1600 East Rollins, Columbia, MO 65211 USA; 3University of Missouri Veterinary Medical Disease Laboratory, University of Missouri-Columbia, College of Veterinary Medicine, 1600 East Rollins, Columbia, MO 65211 USA

Abstract

Squamous cell carcinomas are uncommonly encountered neoplasms in avian species.2,4,8,9 Squamous cell carcinomas of the mandibular and maxillary areas have been infrequently reported.5,8 Treatment, when initiated, has generally been unsuccessful.8 Photodynamic therapy has been used with success in humans and dogs with squamous cell carcinomas,10 and was elected in this case. Photodynamic therapy involves the use of an i.v. photosensitizing agent that is subsequently activated by a light source. Upon activation, photochemical generation of cytotoxic oxygen radicals destroys neoplastic cells. Ease of application, minimal side effects, and post-operative care were prime considerations in electing this form of therapy as opposed to standard treatment modalities such as chemotherapy, cryosurgery and radiation therapy.

A 33-yr-old male great Indian hornbill (Buceros bicornis) weighing 3.1 kg was presented for having a roughened rostral aspect to his casque. A pervasive “yeast-like” odor was noted when staff entered the bird’s holding stall. Physical examination revealed a softened rostral casque. Aerobic and anaerobic cultures revealed Candida albicans, Staphylococcus epidermidis and Proteus mirabilis. A CBC and select serum profile revealed no abnormalities. A biopsy was obtained from the keratinized layers of the casque and placed in 10% neutral buffered formalin. The initial biopsy contained only stratum corneum, and there was marked orthokeratotic hyperkeratosis and heavy pigmentation. Based on culture results, treatment was initiated with 5 mg/kg enrofloxacin (Baytril®, Bayer Corp., Shawnee Mission, KS 66201 USA) and 250 mg/kg flucytosine (Ancobon, Rouche Pharmaceuticals, Paramus, NJ 07652 USA) p.o., b.i.d. After 20 days of therapy, a noticeable decrease in odor was noted. A second biopsy was performed and a squamous cell carcinoma was diagnosed. Histologically, the tumor was composed of anastomosing cords and fronds of neoplastic squamous epithelial cells. The cells were polygonal to spindle-shaped with moderately anisokaryotic, round to oval, hypochromic nuclei, one to three prominent nucleoli that varied in size and shape and moderate amounts of eosinophilic cytoplasm with distinct cell boundaries. Desmosomes were prominent. There were up to 16 mitoses per 400x field, and mitotic atypia was frequent. There were rare dyskeratotic cells. Survey radiographs of the head and casque demonstrated radiopaque material within the rostral aspect of the casque with a slight osteolysis of the crista7 on the left side.

One injection of 0.3 mg/kg hexylether pyropheophorbide-a (Photochlor, Roswell Park Cancer Institute, Buffalo, New York, USA) warmed to the bird’s body temperature was administered in the basilic vein.
The next day, the bird was anesthetized by preoxygenation with 100% oxygen for 2 min, followed by incremental increases in isoflurane (Aeranne, Anaquest, Madison, WI 53713 USA) until a surgical plane of anesthesia was achieved. The bird was placed on a heated surgery table, intubated and maintained on 2.5% isoflurane during the procedure. A 20-ga intraosseous spinal catheter was placed in the right ulna, and 35cc of warmed physiologic fluids (LRS, Abbott laboratories, Chicago, IL 60064 USA) was administered every 30 min during therapy. Respiratory excursions, cardiac rate and rhythm were constantly monitored during therapy.

Portions of the casque were mechanically removed to provide better visualization and access to the tumor. Necrotic dermal papillae were also removed. A diode laser (AOC Medical Systems, South Plainfield, NJ 08670 USA) fitted with a 400 µm microlens optic fiber (PDT Systems Inc., Santa Barbara CA 98117 USA) was used to deliver 665 nm of light to the tissue. The tissue dose was 100 joules cm². To prevent thermal effects, the fiber output was maintained at 100mW/cm². The treatment time was 16 min, 49 sec for each 3-cm focal spot. After each period of therapy, the laser was adjusted to treat adjacent neoplasia. After 3.5 hr, therapy was complete. The bird was placed in an incubator, recovered uneventfully, and began eating the same day. Butorphanol tartrate (Torbugesic, Fort Dodge Laboratories, Overland Park, KS 66210 USA) at 3 mg/kg was administered i.m. daily for 2 days. The bird was kept indoors for 3 wk, as severe skin photosensitization from direct sunlight has been noted in human patients with similar photosensitizing agents.1

Within 72 hr, a firm, dark eschar was noted over the treated tissue. Over the course of the next 5 wk, 20 biopsies were obtained from various areas of the treated tissues. Initial histopathologic evaluations were encouraging, as necrosis of the neoplastic tissue was noted. However later biopsies confirmed the presence of a viable, well-differentiated squamous cell carcinoma. Rapid growth of the tumor was noted clinically, with a concomitant massive increase in the bird’s appetite observed.

A second therapy session was performed 8 wk after the first therapy. The bird had lost 0.5 kg in weight, and was not as active as before, according to the keeper. The anesthetic protocol was the same. During the second session, aggressive debulking of the dermal papillae and tumor was performed. Laser therapy was performed as before. The bird went into respiratory arrest at the culmination of the procedure, but responded to one injection of 5 mg/kg doxapram hydrochloride (Dopram, Fort Dodge Laboratories, Overland Park, KS 66210 USA) i.v., positive pressure ventilation, and discontinuance of anesthesia. Paralysis of the left leg was noted upon recovery. Survey radiographs of the leg and acetabular area revealed no abnormalities. The foot was splinted in a natural position. Butorphanol tartrate was administered as before and appeared to alleviate pain associated with the vasoconstrictive effects of therapy on the neoplasia.1 The bird was ataxic for 4 days but gradually improved in attitude and strength over the course of the next several days. One week after surgery, the splint was removed, with a full return to normal strength and mobility noted. A very firm, blackened eschar was noted at the treatment site. Biopsy at that time revealed destruction of a substantial amount of the squamous cell carcinoma. However, 1 wk later, an additional biopsy revealed recurrence of the neoplasm. Upon further assessment and discussion, additional treatment options were not pursued. Upon failing appetite and lethargy of several days’ duration, the bird was euthanatized. Survey radiographs demonstrated progression of the
neoplasia to the deeper layers of the rhamphotheca, destruction of cancellous bone and keratin layers of the maxilla.

Conclusions

Photodynamic therapy is currently being used in experimental trials to treat various neoplastic processes in people and animals, including esophageal squamous cell carcinomas.\textsuperscript{1,3,6} Photodynamic therapy has been used infrequently in exotic animals, with varying results.\textsuperscript{10} As a precaution, the bird was kept indoors for 3 wk, even though this newer class of photosensitizing agents has not demonstrated the severe skin sensitization observed with other agents. In this case the large size and depth of the initial tumor, combined with accessibility and a paucity of information on normal anatomy may have hampered efforts to eliminate the neoplasia. The apparent high occurrence of this specific type of neoplasia in a specific location of great Indian hornbill casques (personal communications) warrants additional investigation.

ACKNOWLEDGMENTS

The authors would like to thank the Kansas City Zoological Gardens keeper staff, and Mrs. Debbie Tate, oncology technician, for their efforts in caring for the hornbill.

LITERATURE CITED

PHARMACODYNAMICS OF FLUNIXIN MEGLUMINE AND KETOPROFEN IN MALLARD DUCKS, (Anas platyrhynchos)

Karen L. Machin, DVM, MSc,* Lise A. Tellier, BSc, and Alexander Livingston, BSc, PhD, FRCVS

Department of Veterinary Physiological Sciences, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, University of Saskatchewan, Saskatoon, SK, S7N 5B4, Canada

Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) such as flunixin meglumine (FLX) and ketoprofen (KET) have been used in both small and large animals as anti-inflammatories and analgesics. Plasma levels of NSAIDs do not reflect physiologic or pharmacologic activity. Thromboxane (TBX), an inflammatory mediator whose production is inhibited by NSAIDs, plasma measurements can be used to predict length of drug action. Plasma TBX levels were measured following i.m. administration of 5 mg/kg KET or FLX to mallard ducks (Anas platyrhynchos). Compared to baseline, TBX was suppressed significantly for 4 hr for both drugs. Decline of plasma TBX levels, over 48 hr in the control group, may indicate that stress plays a role TBX suppression. The NSAIDs, FLX and KET appear to suppress the inflammatory process but cannot be directly correlated to analgesia.

Introduction

Flunixin meglumine (FLX) and ketoprofen (KET) are potent non-steroidal anti-inflammatory drugs (NSAIDs) used therapeutically in human and veterinary medicine to alleviate pain and decrease inflammation.5,9,10 In avian species, FLX has been recommended for pain relief11 but use of KET has not been reported. The pharmacokinetics and pharmacodynamics of these NSAIDs in birds are unknown. In dogs and cats, both FLX and KET provide appropriate analgesia for surgical pain with a half life of 3-4 hr and 2-3 hr, respectively.9 Anti-inflammatory, analgesic, antipyretic, and antithrombotic actions of NSAIDs are brought about by inhibition of prostaglandin production. More specifically, NSAIDs block the access of arachidonic acid to its binding site on the cyclo-oxygenase enzyme, preventing its conversion to prostaglandin and thromboxane B2 (TBX).5 NSAIDs accumulate at sites of inflammation as they are weak acids10 and possibly because of increases in vascular permeability, blood flow, and protein passage to these sites.4 Plasma levels of NSAIDs do not reflect physiologic or pharmacologic activity, therefore, plasma TBX levels are more accurate indicators of length of drug action.5,13 The purpose of this study was to investigate the pharmacodynamics of the two NSAIDs, FLX and KET, in mallard ducks (Anas platyrhynchos) by measuring plasma TBX levels.

Materials and methods

Sixteen captive raised adult mallard ducks were used for the study. The ducks were of equal sex ratio (eight male, eight female), with average body wt of 1055 ± 114 g and 943 ± 85 g, respectively. Ducks, in an equal sex ratio, were randomly assigned to three treatment groups: (1) control (n = 4), (2) flunixin
meglumine (FLX, 5 mg/kg, n = 6, Banamine, Schering-Plough Animal Health, Point-Claire, Quebec, Canada) or (3) ketoprofen (KET, 5 mg/kg, n = 6, Anafen, Rhône Mérieux Inc. Athens, Georgia). Two ducks in the control group had a jugular catheter placed during isoflurane (IsoFlo, Abbot Laboratories Limited, Saint-Laurent, Québec, Canada) anesthesia and bupivacaine (2 mg/kg, Marcaine, Sanofi Winthrop, Chatham, Ontario, Canada) local anesthetic and two ducks were anesthetized but received neither a jugular catheter nor bupivacaine. Ducks in the later group had blood samples taken from either the jugular or brachial veins. All ducks in the FLX and KET groups had a jugular catheter placed during isoflurane anesthesia but bupivacaine was not given. During isoflurane anesthesia, the right jugular vein was isolated surgically and a 20-ga, 5.0-cm catheter was inserted for the purpose of repeated blood sampling.

Blood samples of 0.3 ml were drawn into heparinized syringes at -1 hr (60 min prior to catheter placement) and 0 hr (after isoflurane induction) from the brachial vein. Immediately after induction, either FLX or KET was administered i.m. into the left pectoral muscle. Equal volumes (5.1 ml) and concentrations of NSAID were given by diluting with sterile saline. Ducks in the control group received an equal volume of saline i.m. in the left pectoral muscle. Blood samples of 0.5 ml were taken at 15 min, 30 min, and 1, 2, 4, 6, 12, 24, 36, and 48 hr. Time 0 and 15 min samples were taken during anesthesia. Blood samples were placed in integrated plasma separation tubes with lithium heparin (Sherwood Davis and Geck Medical, St Louis, MO). Samples were centrifuged and frozen at -20°C until analysis were performed. Duplicate samples from each bird were analyzed using a Thromboxane B2 Enzyme Immunoassay Kit (Cayman Chemicals, Arkansas, USA). The coefficient of variation for this study was 5.44 ± 4.71 (x ± SD). Feces were tested at 0, 24, and 48 hr for the presence of hemoglobin using Haematest tablets (Miles Canada Inc., Etobicoke, Canada) to evaluate for NSAID associated gastrointestinal bleeding. All ducks were maintained individually in cages (75 cm³) with a 20 × 40 cm pool and fed duck and goose grower ad libitum. Ducks were euthanatized for complete necropsy at the end of the study. Histologic analysis of muscle, heart, kidney, liver, spleen, and gastrointestinal tract are pending.

Data are reported as mean ± SEM. Wilcoxon signed-rank tests were used to determine if there were significant differences between time -1 and 0 hr before combining results. Plasma TBX data were analyzed using a repeated measures analysis of variance (ANOVA) for the average value derived from duplicate samples where the same individuals were sampled at each time point. Data was log transformed to avoid violating the normality assumption of the ANOVA. Where significant differences occurred, a multiple comparison using a contrast statement for repeated measures was used to compare results with baseline (average of time 0 and -1 hr). Results were considered significant when P < 0.05.

Results

Results from one male duck in the FLX group were excluded when necropsy and histologic findings consistent with systemic mycobacterial disease were found. As there was no difference in TBX suppression between males and females or between control groups, results from both sexes and control groups were combined. TBX was suppressed in all birds following administration of either FLX or KET (Figs. 1 and 2). Maximal TBX suppression occurred at 30 min and was still significantly suppressed at
4 hr (F = 30.3, df = 10, 90, P = 0.0001, Fig. 1). There was no significant difference between drugs (F = 1.05, df = 10,90, P = 0.392) but TBX suppression by KET did not appear to be as long lasting as with FLX (Fig. 2).

Ducks in the control group had a gradual decline in TBX from the start of the experiment (time -1 and 0 hr) to 48 hr. The decline was not related to any obvious circadian rhythm, the highest mean values (4299 ± 958 pg/ml) were recorded at baseline (times -1 hr and 0 hr) and the lowest value (361 ± 180 pg/ml) occurred at 24 hr. At 36 (787 ± 394 pg/ml) and 48 hr (530 ± 265 pg/ml), TBX values were marginally higher than at 24 hr but not as high as at baseline (Fig. 1).

At necropsy, all birds given FLX had severe, focally extensive muscle necrosis of approximately 1-2 cm³ at the injection site. No other gross abnormalities were found on postmortem and histology results are pending. None of the ducks had evidence of gastrointestinal bleeding.

**Discussion**

In this study, plasma TBX was significantly suppressed by both drugs for at least 4 hr. Similar studies of these two NSAIDs has shown that TBX suppression in mammals is longer. Significant suppression may be short as 4 hr or as long as 24 hr, with levels returning to normal by 12 hr.7,12 or 48 hr, respectively.7

Compared to baseline, maximum TBX suppression, was achieved by 30 min, which may indicate that uptake and distribution is more rapid in mallard ducks than reported in mammalian species. Significant suppression (73 to 96 %) was maintained for only 4 hr. The short inhibition of TBX may be related to the higher metabolic rate of birds. Nonpasserine birds have a minimal energy requirement that averages almost 10% more than that of placental animals.11 The increase in TBX production seen after suppression in this study is similar to that reported in other studies following but the cause is unknown.3,8

Return of TBX to baseline values was difficult to estimate in this study. The gradual decline in control TBX concentration over 48 hr, as seen in the control ducks, has not been reported in similar studies. The stress of being confined, isolated, and multiple blood sampling that may have resulted in high levels of circulating corticosterone, which, through lipocortin, production indirectly can limit the activation of the inflammatory cascade.1,2

It is unknown if the degree of TBX inhibition can be correlated with the degree of analgesia provided by the NSAID. A possible mechanism for the peripheral analgesic effects of NSAIDs is through suppression of the cyclo-oxygenase pathway.4,5 As FLX and KET appear to cause significant suppression of TBX by 30 min and continue for at least 4 hr, they may be useful during the surgical procedures and may also reduce altered behavior post-operatively. Further studies are required to determine the effectiveness of pre-emptive NSAID administration as analgesics in birds.
Muscle necrosis seen in the ducks, associated with FLX injections have been observed in northern bobwhite. However, a dose of 32.0 mg/kg/day in quail over the course of 7 days was necessary to induce the lesions. Mallard ducks may be more susceptible to these detrimental effects of FLX but further studies are required to establish their susceptibility to lesions.

Flunixin meglumine and KET can produce significant inhibition of TBX and therefore the inflammatory cascade for at least 4 hr. Administration of KET may benefit waterfowl research if it can provide post operative analgesia and thus reduce altered behavior and weight loss in birds after surgical implantation of radio transmitters. Flunixin meglumine may not be a suitable NSAID for use in ducks because of the muscle necrosis produced at the injection site. More research is needed to determine if pre-emptive administration of NSAIDs can reduce deleterious effects of post-operative pain.

ACKNOWLEDGMENTS

This research was supported by the Canadian Wildlife Service, Delta Waterfowl Foundation, Ducks Unlimited’s Institute for Wetland and Waterfowl Research, Interprovincial Undergraduate Student Summer Research Award, and the Wildlife Health Fund, University of Saskatchewan. We thank Susan Cook for assistance in sample analysis and Dr. Robert Brua for aid in statistical analysis and writing of this abstract.

LITERATURE CITED

Figure 1. Comparison of mean (SEM) plasma thromboxane (TBX, [pg/ml]) in mallard ducks following i.m. injection of flunixin meglumine (FLX, [5 mg/kg]), ketoprofen (KET, [5 mg/kg]), and saline controls.

*Significantly different from baseline values, $P < 0.05$.

Figure 2. Percent thromboxane (TBX) suppression compared to baseline in mallard ducks following i.m. injection of flunixin meglumine (FLX, [5 mg/kg]) and ketoprofen (KET, [5 mg/kg]).

$n = 5$ for FLX and 6 for KET at 0.25, 0.5, 1, and 2 hr.

$n = 4$ for FLX and 5 for KET at 4 hr.

$n = 4$ for FLX and 3 for KET at 6 hr.
URETEROLITHIASIS IN A DOUBLE YELLOWHEADED AMAZON PARROT (Amazona ochrocephala)

Patricia M. Dennis, MSL, DVM* and R. Avery Bennett, DVM, MS

Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 USA

Abstract

Ureterolithiasis has not been reported in avian medicine literature. Diagnosis of this disease in other species by the use of palpation, radiography, or ultrasonography has been reported.²⁻⁴ The purpose of this case report is to discuss the diagnosis and surgical treatment of ureterolithiasis in a double yellowheaded Amazon parrot (Amazona ochrocephala) that presented to the Veterinary Medical Teaching Hospital (VMTH), University of Florida.

The bird presented to the VMTH with a 3-yr history of straining to urinate and defecate. The bird had a recent onset of depression and inappetence. Radiographs made by the referring veterinarian 3 yr prior to presentation showed mineral-dense opacities in the dorsal caudal coelomic cavity. Radiographs made 1 yr prior to presentation showed enlargement of the kidneys and more caudal location of the mineralized opacities.

On physical examination the bird was quiet and alert. Skin was dry, flaky and feathers were dull. The cloaca was surrounded by pasted urates. The area around the cloaca was featherless and inflamed. No other abnormalities were noted on physical examination. Complete blood count showed a leukocytosis (WBC 20,000 cells/µl) with a heterophilia (15,600 cells/µl) and a lymphopenia (2,800 cells/µl). The biochemical profile showed an elevation in aspartate transaminase (AST 706 u/L).

A left lateral celiotomy was performed to remove the mineralized material. A prominent, white, fluid-filled tubular structure in the region of the left kidney and ureter was identified. The structure, based on biopsy and histologic examination, was the left ureter. The ureter contained a firm 2 cm × 1 cm × 1.5 cm yellowish-white mineralized mass which was removed. The ureter was explored but no other masses were located. Following surgery, a ventrodorsal radiograph was made to determine whether all masses had been removed. One calculus could be seen in the caudodorsal lateral coelomic cavity. Four days following the initial surgery, the remaining ureterolith was removed by a cloacotomy and ventral midline celiotomy.

At a follow-up examination 10 days after the second surgery, an excretory urogram was made. There was evidence of normal peristaltic movement of the right ureter on several radiographic views. On the 10-min post-injection radiographs, the left ureter was homogenously opacified and measured approximately three times the width of the right ureter.
Surgical removal of the ureteroliths was complicated by the anatomy of the bird. Removal of the ureteroliths was not possible through a single surgical approach. Use of other techniques to remove ureteroliths, such as extracorporeal shock wave lithotripsy,\textsuperscript{1} is not possible due to the presence of air sacs.

The underlying cause of ureteroliths is unknown, though vitamin A deficiency has been suggested as a possible etiology.\textsuperscript{5} The ureter, being an epithelial lined structure, is a possible site for the development of squamous metaplasia in response to vitamin A deficiency. In this case, no epithelium was seen on histologic examination of the ureteral tissue, so the presence of squamous metaplasia could not be determined. Other possible causes of the ureteroliths include chronic dehydration or bacterial infection.

LITERATURE CITED

THE USE OF MEDETOMIDINE AS AN ORAL SEDATIVE IN GALLIFORMES

Stephanie B. James, DVM,1* Christine Sheppard, PhD,2 Marcia Arland, MS,2 and Bonnie L. Raphael, DVM, Dipl ACZM 1

1Wildlife Health Sciences, Wildlife Conservation Society, 2300 Southern Blvd, Bronx, NY 10460-1099 USA; 2Department of Ornithology, Wildlife Conservation Society, 2300 Southern Blvd, Bronx, NY 10460-1099 USA

Abstract

The purposes of this study were to investigate medetomidine as an oral sedative in galliformes, to determine an effective dose range, and to determine if the effects of this alpha-2 agonist were reversible.

Six male and six female 9-mo-old domestic chickens (Gallus gallus) were used in this study with the males housed separately from the females. An ethogram, to document the effects of sedation, was developed prior to the onset of the study. Behaviors that were common to both sexes included standing, sitting, walking, perching, eating, eyes closed, and head down. Male behaviors also included aggression and crowing, while female behaviors included nesting.

The males and females were observed at 2-min intervals for 30 min at the end of which each animal was hand caught and the males were given 0.6 mg medetomidine (0.19-0.2 mg/kg) (Domitor, 1.0 mg/ml, Pfizer Animal Health, Exton, Pennsylvania 19341 USA) and the females were given 0.4 mg medetomidine (0.21-0.31 mg/kg) p.o. via a 1-ml syringe (Becton-Dickinson & Co., Franklin Lakes, New Jersey 07417 USA). After administration of the medetomidine the birds were returned to their enclosures and behaviors recorded for an additional 45 min. The animals were then divided into two groups. Group A (three males and three females) received i.v. atipamezole (Antisedan, 5.0 mg/ml. Pfizer Animal Health) (3 mg and 2 mg, respectively), in a wing vein. Group B (three males and three females) were not reversed and were bled for plasma biochemical analysis. Behaviors were recorded for an additional 2.5 hr. Data for the two groups were compared.

The results of time spent at each behavior before and after medetomidine are presented in Fig.1. The initial effects of the medetomidine were seen within 4 min, the average time for sedation was 6.2 min, and all animals were fully sedated by 10 min. Subjectively, after treatment, all animals had decreased activity, were minimally rousable, and were easy to catch and manually restrain. As evident in Fig. 1, walking and eating decreased substantially after the medetomidine was given, whereas eyes closed and head down behaviors increased. Before the medetomidine was administered, the males vocalized an average of 5.6 times in 2 min and the females vocalized constantly. Ten minutes after the medetomidine, there were no vocalizations by either sex.

Adverse effects of the medetomidine included one male and one female which were tachypneic at 12 min (respiratory rates approximately 60 bpm). The tachypnea resolved without intervention between 24 and 26 min. The plasma biochemistry results were within normal limits for the species.
After atipamezole was given, initial reversal effects were observed within 30 sec. Fig. 2 depicts the behaviors of both groups. This figure demonstrates that group A (animals that received atipamezole i.v.) spent less time with their eyes closed and heads down. Subjectively, they were also more alert, moved more, and were more easily rousable than group B. Animals in group B remained stationary and were minimally rousable. Approximately 50 min after the administration of the atipamezole the animals in group A appeared to resedate even though they remained more easily rousable than those animals in group B. There were no adverse effects noted of i.v. atipamezole treatment and both reversed and non-reversed birds were normal approximately 2.5 hr after the administration of the medetomidine.

In conclusion, medetomidine (0.25-0.34 mg/kg p.o.) appears to be a good sedative for chickens when administered orally into the crop in fasted animals. The induced sedation was reversible with atipamezole (1.3-1.6 mg/kg i.v.). More research is needed in order to determine if medetomidine can be used effectively in food as an oral sedative.

![Figure 1. Behaviors of chickens (Gallus gallus) before and after the administration of medetomidine.](image1)

![Figure 2. Behaviors comparing groups A and B.](image2)
MEDICAL MANAGEMENT OF CURASSOWS

Maryanne E. Tocidlowski, DVM,1* Terry M. Norton, DVM,2 and Lee A. Young, DVM3

1Houston Zoological Gardens, 1513 North MacGregor, Houston, TX 77030 USA; 2St. Catherine’s Wildlife Survival Center, 182 Camellia Road, Midway, GA 31320 USA; 3San Diego Zoo, PO Box 551, San Diego, CA 92112 USA

Abstract

To provide better medical care and improve husbandry techniques, a brief review of curassow ecology,1 husbandry,2 and screening of curassow medical and necropsy records from the Houston Zoological Gardens (HZG) was conducted. The Houston Zoo has housed over 230 curassows of 10 various species since 1973 from which the records were reviewed.

Curassows are in the Family Cracidae, a primitive bird group in the Order Galliformes. It is a long lived (20+ yr), arboreal gallinaceous bird group found in the Central and South American tropics and subtropics. The Family Cracidae contains approximately 13 curassow species (Table 1), Chachalacas, and Guans. Curassows are fowl-like with strong legs and feet, ample tail and wings, and a well developed hind toe used to grasp branches. They are primarily vegetarians with a muscular gizzard which can grind hard seeds and nuts as well as fruit, greens, insects, and invertebrates. Due to the muscularity of the ventriculus, curassows tend to swallow large amounts of small pebbles or gravel. Curassows are the only group of the Cracidae Family that have a developed crop. Male birds are usually larger than the females and both sexes are vocal with a well developed syrinx. Certain species of male curassows: Nothocrax urumutum, Crax globulosa, C. pauxi, and C. mitu have an elongated trachea, used in vocalization for increased loudness or low pitch sounds. The trachea extends under the skin, and in some species overlays the abdomen, then curves back around and enters the thoracic inlet. Some curassow species have a feathered crest on the top of the head. Curassows natural enemies include predatory birds, mammals, and man.

Curassows are generally monogamous and occur in pairs, although trios (cock and two hens) or family groups can be found. Males have an intromittent organ, which can be used to sex young birds. Adults birds are sexually dimorphic, with the males being larger in size than females and in many species the males have a large knob or wattle on the cere. The female lays and broods two eggs, incubation lasts approximately 29-32 days. Females can produce four clutches per year if the eggs are pulled for artificial incubation or domestic chicken brooding after the clutch is laid. Chicks are precocial, grasping and perching as soon as they are hatched, thus smaller perching should be provided. They are fed by both parents by offering foods in the beak, curassow parents do not regurgitate for their young.

Certain normal mannerisms of the curassow, if one is not aware of them, can lead to misdiagnosis of neurologic disease. Curassows have a tendency to flick their heads and present with a head tilt when

1999 PROCEEDINGS AMERICAN ASSOCIATION OF ZOO VETERINARIANS 295
anxious or nervous. They also will flick their tails up and down as well as pass the head over the shoulders and back.

Curassow pens should be fairly large and contain several perches for roosting due to the birds body size and their arboreal nature. It is thought that curassows spend approximately one-half of their time perching above the ground. An enclosed section for protection from the cold and frostbite should be included. Males are territorial and two or more housed together tend to fight. The birds can be excessively aggressive during the breeding season and may even attack zoo visitors. Curassows may also be aggressive towards and can kill smaller birds and generally do not make a good species for free-flight pens.

In general, curassows are hardy birds and are not prone to disease. Because they are classified in the Order Galliformes, it is felt that curassows are susceptible to most of the diseases affecting poultry such as reticuloendothelial virus, Salmonella spp., Mycoplasma spp., and Chlamydia. Very few infectious diseases have been diagnosed in live curassows at the HZG. Bacterial pododermatitis has affected some birds. Low numbers of endoparasites have been found and included ascarids, Capillaria, strongyles, strongyloides, dispharynx, heterakis, and coccidia. Feather lice and mites have also been found on several birds.

Non-infectious diseases predominated in curassows presented for medical attention at the HZG. The birds often are found to have general clinical signs such as debilitation, emaciation, weight loss, abnormal behavior, lethargy, lameness, and occasionally moribund. Trauma was the medical problem most commonly diagnosed for the following reasons; cage mate aggression, parental trauma to young, self mutilation (rubbing), restraint, and incompatible neighboring species. Fractures, of toes, legs, and wings, and integument lacerations and tears were common. Other integument problems consisted of overgrown beak, toe nail trauma, and uropygeal impaction. Curassows are susceptible to frostbite when temperatures approach the low 40’s (Fahrenheit) or lower. Curassows also have a tendency to pick objects off the ground, thus there have been several cases of zinc toxicosis in captive zoo birds but only rare instances of intestinal obstruction. Reproduction problems were also commonly found in curassow hens due to their large egg size and included egg shell retention, egg binding, and cloacal prolapse. Intromittent organ prolapse and infection was seen in one adult male. Curassow chicks greater than 1 day of age generally have few problems at HZG but rotational leg deformities were found in chicks that did not have good perching material supplied after hatching. Many chicks had problems with poor or abnormal hatching.

Diagnoses of necropsies performed at the HZG reflected the clinical signs and diagnoses found in live birds. Forty-two complete necropsy reports of approximately 60 available were reviewed and diagnoses recorded. Necropsy results not included were those that were incomplete or the diagnoses were not confirmed by histologic review. Diseases affecting multiple organs were peritonitis, primarily due to egg yolk contamination, and septicemia. Digestive tract diagnoses included enteritis, colitis, and hepatitis. Respiratory lesions found were pneumonia and aspiration (especially in newly hatched or pre-hatched chicks), bronchitis, and aspergillosis. Reproductive diseases of hens were similar to those in the live birds such as egg binding, salpingitis, metritis, one ovarian granuloma was found. Histologic lesions often were not found in chicks, many appeared to die during hatching sometimes associated with
abnormal positioning. Histologically, omphalitis and yolk sacculitis predominated. The HZG has seen necropsy lesions in several curassows that are typically found in birds infected with reticuloendothelial virus (REV) such as lymphoid leukosis, lymphoma, and lymphoreticular disease. Reticuloendothelial virus infection was not confirmed in any of these cases, as most were diagnosed histologically before REV PCR testing was available. Other neoplasias found were intestinal carcinoma and adenocarcinoma. Non-infectious, gross necropsy lesions from birds that were euthanatized due to poor prognosis or had died included; hypothermia, frostbite, trauma, and musculoskeletal deformities and malformations (rotational deformities, fractures, scoliosis, slipped tendons, bumblefoot, myopathy).

In conclusion, curassows are large, unusual tropical gallinaceous birds that are not hard to keep and maintain in a zoological setting. The species is susceptible to many diseases but with good quarantine, disease screening protocols and husbandry procedures, most diseases found can be prevented or treated.

**LITERATURE CITED**


**Table 1.** Curassow taxonomy (genus and species vary slightly with different authorities).³

<table>
<thead>
<tr>
<th>Genus / Species</th>
<th>Common name</th>
<th>Genus / Species</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Crax alberti</em></td>
<td>Blue-knobbed</td>
<td><em>Crax pauxi</em></td>
<td>Northern-helmented</td>
</tr>
<tr>
<td><em>Crax alector</em></td>
<td>Black</td>
<td><em>Crax rubra</em></td>
<td>Great</td>
</tr>
<tr>
<td><em>Crax blumenbachii</em></td>
<td>Red-billed</td>
<td><em>Crax salvini</em></td>
<td>Salvin's</td>
</tr>
<tr>
<td><em>Crax daubentoni</em></td>
<td>Yellow-knobbed</td>
<td><em>Crax tormentosa</em></td>
<td>Crestless Razor-billed</td>
</tr>
<tr>
<td><em>Crax fasciolata</em></td>
<td>Bare-faced</td>
<td><em>Crax unicornis</em></td>
<td>Southern-helmented</td>
</tr>
<tr>
<td><em>Crax globulosa</em></td>
<td>Wattled</td>
<td><em>Nothocrax urumutum</em></td>
<td>Nocturnal</td>
</tr>
<tr>
<td><em>Crax mitu</em></td>
<td>Razor-billed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Representative hematology and plasma biochemistry values of the curassow (*Crax globulosa*) from International Species Information System (ISIS)\(^3\) and the Houston Zoological Gardens (HZG) in-house analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>ISIS</th>
<th></th>
<th>HZG</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(x)</td>
<td>1 S.D.</td>
<td>N</td>
<td>(x)</td>
</tr>
<tr>
<td>WBC</td>
<td>(10^3/\mu l)</td>
<td>22.4</td>
<td>13.8</td>
<td>48</td>
<td>20.9</td>
</tr>
<tr>
<td>RBC</td>
<td>(10^6/\mu l)</td>
<td>3.25</td>
<td>0.35</td>
<td>31</td>
<td>3.23</td>
</tr>
<tr>
<td>HGB</td>
<td>g/dl</td>
<td>15.5</td>
<td>2.5</td>
<td>34</td>
<td>15.2</td>
</tr>
<tr>
<td>HCT</td>
<td>%</td>
<td>43.5</td>
<td>5.5</td>
<td>47</td>
<td>42.0</td>
</tr>
<tr>
<td>MCV</td>
<td>fl</td>
<td>131.8</td>
<td>13.7</td>
<td>31</td>
<td>131.9</td>
</tr>
<tr>
<td>MCH</td>
<td>pg</td>
<td>47.3</td>
<td>5.2</td>
<td>30</td>
<td>47.4</td>
</tr>
<tr>
<td>MCHC</td>
<td>g/dl</td>
<td>36.6</td>
<td>4.6</td>
<td>33</td>
<td>36.0</td>
</tr>
<tr>
<td>Heterophils</td>
<td>(10^3/\mu l)</td>
<td>5.1</td>
<td>5.7</td>
<td>47</td>
<td>4.5</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>(10^3/\mu l)</td>
<td>14.5</td>
<td>12.2</td>
<td>47</td>
<td>12.6</td>
</tr>
<tr>
<td>Monocytes</td>
<td>(10^3/\mu l)</td>
<td>1.6</td>
<td>1.8</td>
<td>39</td>
<td>1.7</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>(10^3/\mu l)</td>
<td>0.7</td>
<td>0.7</td>
<td>37</td>
<td>0.5</td>
</tr>
<tr>
<td>Basophils</td>
<td>(10^3/\mu l)</td>
<td>0.9</td>
<td>0.6</td>
<td>41</td>
<td>1.0</td>
</tr>
<tr>
<td>Plasma protein</td>
<td>g/dl</td>
<td></td>
<td></td>
<td></td>
<td>4.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>mg/dl</td>
<td>306</td>
<td>45</td>
<td>46</td>
<td>309</td>
</tr>
<tr>
<td>BUN</td>
<td>mg/dl</td>
<td>3</td>
<td>1.0</td>
<td>40</td>
<td>3.5</td>
</tr>
<tr>
<td>Creatinine</td>
<td>mg/dl</td>
<td>0.3</td>
<td>0.1</td>
<td>9</td>
<td>0.3</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>mg/dl</td>
<td>9.8</td>
<td>3.2</td>
<td>46</td>
<td>10.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>mg/dl</td>
<td>11.6</td>
<td>1.2</td>
<td>46</td>
<td>11.8</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>mg/dl</td>
<td>6.9</td>
<td>1.6</td>
<td>14</td>
<td>9.0</td>
</tr>
<tr>
<td>Sodium</td>
<td>mEq/L</td>
<td>162</td>
<td>8</td>
<td>21</td>
<td>161</td>
</tr>
<tr>
<td>Potassium</td>
<td>mEq/L</td>
<td>4.0</td>
<td>1.2</td>
<td>21</td>
<td>4.3</td>
</tr>
<tr>
<td>Chloride</td>
<td>mEq/L</td>
<td>117</td>
<td>4</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>(\mu g/dl)</td>
<td>229</td>
<td>36</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>mg/dl</td>
<td>179</td>
<td>34</td>
<td>42</td>
<td>170</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>mg/dl</td>
<td>120</td>
<td>66</td>
<td>34</td>
<td>132</td>
</tr>
<tr>
<td>Total protein</td>
<td>g/dl</td>
<td>4.0</td>
<td>0.5</td>
<td>45</td>
<td>4.0</td>
</tr>
<tr>
<td>Albumin</td>
<td>g/dl</td>
<td>1.7</td>
<td>0.4</td>
<td>16</td>
<td>1.5</td>
</tr>
<tr>
<td>Globulin</td>
<td>g/dl</td>
<td>3.9</td>
<td>5.6</td>
<td>16</td>
<td>3.1</td>
</tr>
<tr>
<td>AST (SGOT)</td>
<td>IU/L</td>
<td>35</td>
<td>15</td>
<td>43</td>
<td>34</td>
</tr>
<tr>
<td>ALT (SGPT)</td>
<td>IU/L</td>
<td>13</td>
<td>7</td>
<td>41</td>
<td>14</td>
</tr>
<tr>
<td>T. bilirubin</td>
<td>mg/dl</td>
<td>0.3</td>
<td>0.2</td>
<td>25</td>
<td>0.3</td>
</tr>
<tr>
<td>Alk. Phos</td>
<td>IU/L</td>
<td>263</td>
<td>214</td>
<td>44</td>
<td>214</td>
</tr>
<tr>
<td>LDH</td>
<td>IU/L</td>
<td>378</td>
<td>158</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>CPK</td>
<td>IU/L</td>
<td>1718</td>
<td>694</td>
<td>14</td>
<td>1026</td>
</tr>
<tr>
<td>CO(_2)</td>
<td>mmol/L</td>
<td>13.7</td>
<td>3.2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>GGT</td>
<td>IU/L</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
EVALUATION OF AVIAN MYCOBACTERIOSIS DIAGNOSTIC ASSAYS: A COMPARISON OF CULTURE, ACID FAST STAINING, AND POLYMERASE CHAIN REACTION TESTING

Lisa Tell, DVM,1* Janet E. Foley, DVM, PhD,1,2 Richard Walker, DVM, PhD,3 Leslie Woods, DVM, PhD1 and Martha Needham, BA1

1VM: Department of Medicine and Epidemiology; 2Center for Companion Animal Health; 3California Veterinary Diagnostic Laboratory Systems (CVDLS); University of California, Davis, Davis, CA 95616 USA

Abstract

Mycobacteriosis is an insidious disease that causes mortality in pet birds and is of zoonotic concern. Mycobacterium avium and more recently, M. genavense have been the causative agents most commonly identified in companion avian species.5,6,8 Avian mycobacteriosis predominately affects the gastrointestinal and hepatic systems. Affected birds shed organisms in their feces, that may remain viable in the environment for months to years. This environmental contamination poses a threat to domestic animals, other birds, and human populations.7

Avian mycobacteriosis is a challenging disease to diagnose ante-mortem, particularly in the early stages of the infection. Ante-mortem clinical diagnostic procedures for screening avian patients for this disease include skin testing and hematologic, radiographic, and/or laparoscopic examinations.9 The tuberculin intradermal test has been used successfully for many years for identifying infected fowl and domestic poultry flocks, but this test has been of little use in exotic avian species.1 The disadvantages of hematologic, radiographic, and endoscopic testing are their low sensitivity, their inability to provide a definitive answer and their requirement of restraint and/or anesthesia. Reported laboratory tests for diagnosing mycobacteriosis in humans and animals include serologic tests, acid fast staining, histopathology, mycobacterial cultures, the radiometric BACTEC system, DNA probes, and nucleic acid amplification by polymerase chain reaction (PCR).2,9 The majority of these tests has been applied to birds, but their ability to detect the early stages of disease is poor. PCR has been used successfully in the identification of M. avium and M. genavense in tissues from necropsied birds,2,3 however obtaining adequate antemortem tissue samples is more difficult. Nucleic acid amplification via PCR and its application for detecting mycobacterial DNA in fecal samples has been minimally investigated in avian species.4 Although some diagnostic methods are considered more reliable than others,2 a direct comparison of ante-mortem diagnostic assays has not been reported.

The purpose of this study was to compare and contrast the efficiency and reliability of three fecal diagnostic assays throughout the course of experimentally induced mycobacterial infections in Japanese quail (Coturnix coturnix japonica). The three methods utilized were acid fast staining of fecal smears, fecal mycobacterial culture, and PCR evaluation of fecal samples to detect mycobacterial DNA. These methods were chosen based on their non-invasive nature and potential for ante-mortem diagnosis. The clinical advantage of acid fast staining and PCR testing is that the assay results can be obtained within several days, whereas culture of the organism may take as long as 8 wk.
Fecal samples for this study were obtained from Japanese quail after i.v. inoculation with $3.9 \times 10^7$ colony forming units of *M. avium*. The course of the infection was followed for 12 wk. Fecal samples were collected for a 24-hr period every other week for the first 6 wk post-inoculation. During weeks 7 through 12 post-inoculation, feces were collected for a 24-hr period three times per week. Fecal smears were analyzed utilizing the Ziehl-Neelsen and Truant's acid fast stains. In addition, samples were analyzed via fecal culture and PCR testing.

ACKNOWLEDGMENTS

This work was supported by a grant from the Center for Companion Animal Medicine, School of Veterinary Medicine, University of California, Davis.

LITERATURE CITED

STARTING A ZOO NUTRITION PROGRAM

Helena Marquès1* and Michael T. Maslanka, MS2

1Parc Zoologic de Barcelona, Parc de la Ciutadella s/n, 08003 Barcelona, Spain; 2Memphis Zoological Society, 2000 Galloway Ave., Memphis, TN 38112 USA

This publication is based on a paper presented at the First European Zoo Nutrition Meeting held in Rotterdam, Netherlands between 8–11 January 1999.

Abstract

The first nutritionists associated with North American zoos appeared in the mid-1970’s and in Europe in the 1980’s. Since that time, the number of nutritionists working at zoos has slowly increased. Captive exotic animal management has always included nutrition, but in the last 20 yr, several zoos have built nutrition programs and substantially developed those that already existed. There are basic needs for the foundation of a nutrition program including an interested and capable person or group, some rudimentary equipment, an understanding of the history of the animal collection and its management, and an organizational structure conducive for properly documenting the development. Beyond this, development of a nutrition program hinges on the stability of the initial structure and its direction is determined by reasons surrounding its inception. Properly developed, a nutrition program can contribute to the multi-disciplinary care and management of the animals in our charge.

Introduction

Although zoo nutrition is a relatively new field, its importance is growing quickly among the zoo’s community. All of us are aware that one of the roles of zoos is to preserve endangered species and take an active part in conservation. Beyond that, we are charged to take an active role in educating the visitors who enter our zoos in order to substantially increase the conservation effort outside of the zoo’s immediate boundaries. Nutrition represents one of the many inter-related parts that determine the well being of an animal.1,4 If any of the parts fail, animal health may be compromised, and, with it, our ability to educate the public and promote conservation of endangered species.

When starting to work on nutrition at a zoo, the last thing to be done actually may be the “nutrition” itself. A foundation and structure must be created first, upon which to build a nutrition program. That foundation can begin by demonstrating the need for a nutrition program. That “need” may take the form of evaluating current diet items for quality and/or price, the need to streamline food processing operations, or, most directly, the examination of nutritional/metabolic problems of the animals in the collection. If the zoo has already examined use of a “nutritional consultant” or similar person, many of the staff may already be aware of the “need” which exists. Regardless of how or why the “need” is expressed, institution-wide support of a nutrition program is imperative to its success. Often, that support is not present at the outset and develops as the benefits of such a service are displayed over time. Once the impetus to develop a nutrition program exists and receives the appropriate encouragement
(moral, financial, or both), there are several directions the program can take based on why it was developed (price, quality, or operational evaluation, diet evaluation and/or adjustment, etc.). Development of the program often proceeds based on the reasons it was conceived.

**Advantages of a Nutrition Program**

When building a nutrition program, presenting some of its advantages may be beneficial to gaining financial and moral support. The advantages of having a nutrition program at a zoo can be approached from two perspectives: from the animal’s point of view and from the institution’s point of view.

From the animal’s point of view, a nutritionist can provide the expertise to minimize the incidence of health problems and improve the animal’s quality of life. To that end, the nutritionist can assure that the food offered is of the best quality for the animals. A nutrition program can allow better control over the items, thus the nutrients, delivered to an individual animal. The nutritionist can contribute to better management of the entire diet – from its formulation to the spatial and temporal distribution of the diet items. This can allow for more active animals and can potentially minimize the incidence of stereotypic behaviors, aggression, and/or weight management issues. Additionally, if the diet more closely meets the nutrient requirements of the animal, it may allow for more successful reproductive efforts and/or increased longevity.

From the institution’s point of view, a nutritionist can provide adequate diets for their animals. Beyond that, a nutritionist can help insure that the diet formulated is the diet offered, and determine if the diet offered is the diet consumed. This can allow for better maintenance of the collection, which ultimately provides the experience for the zoo visitor and the revenue of the institution. Increased knowledge regarding what and how much the animals eat can allow for better control of expenses. A nutritionist can allow for better organization through fostering communication between the staff responsible for preparing diets and the animal management staff, thereby increasing efficiency and effectiveness. They also can contribute valuable information when special animal problems arise (in coordination with keepers, curators, veterinarians, etc.). A nutritionist also can provide the expertise to allow for exchanges among food items which may be similar in nutrient content but more affordable. Good nutritional status of animals ultimately leads to better health of the animals, potentially minimizing veterinary care and costs as well.

**Development**

*How to start – the foundation*

There are a few prerequisites to develop a nutrition program which begin at a very basic level: (1) a person (more or less qualified) willing to work and learn, with (2) lots of energy, enthusiasm, and patience, and (3) a salary or other financial aid. Beyond that, there are a few things which assist the endeavor immensely: (1) tables of requirements and food nutrient contents, (2) a scale (or scales), and (3) a computer.
A person can begin working part time on nutrition issues while filling another role at the zoo. This may allow the person to develop good working relationships with the curatorial and keeper staff prior to working intensively with nutrition. As the nutrition aspect of that person’s job becomes more prominent (slowly or quickly), the institution and the individual can continue to grow with the increased responsibilities and expectations.

Before doing anything, it is important to become familiar with the institution’s policies – their goal and mission statements, their organizational structure (formal and informal), how operations work in each area, and all of the aspects associated with diet preparation and distribution. From this, one can get an idea of what is working well, what can work well with a minor alteration, and what can work well with a more extensive change. It is also basic to listen to staff opinions and experiences. The keepers work with their animals on a daily basis, many have been in the zoo for years, and know not only their animals but also how the zoo functions. Some changes may have been tried previously, and did not work. Long-time keepers can provide this historic perspective, which can save time and wasted effort. This background information is pivotal in providing a foundation for a nutrition program and remains important as the program grows and develops.

The first steps – the framework

Once the initial background information is gathered and the foundation has been set, work can begin with some basic skills and knowledge. Good organizational skills are critical to document progression of diet formulations - basic communications, what worked, what did not work, why. The skills of the nutritionist and those of the diet preparation staff should be well coordinated. If utilized, the commissary must be well organized and trained with respect to sanitation - food handling and storage. Diet preparation also must be organized, sanitary, detailed, and dynamic (to account for changes that occur).

Again, it is critical that everyone understands the importance of good nutrition, especially the keeper staff. They are often responsible for diet preparation and observation of diet consumption, and they know the animals around the zoo better than anyone. Their understanding of the importance of adequate nutrition for their animals is crucial for the success of the nutrition program.

Diet evaluation itself may not fit into the first steps of developing the nutrition program. If the basic skills are lacking, it may be worthless to design diets that meet probable requirements because they may not be utilized correctly or at all. In the development of the program, it may be more important to concentrate on the basic first steps of gathering background information, communication of the purposes of the nutrition program, and development of organizational skills. It may be more important to concentrate on these basic first steps and develop a framework upon which to build later.

The next steps – filling the framework
When developing a nutrition program, financial support is necessary. At the outset, this may represent a salary from the zoo. In some cases, nutritionists have started as volunteers and slowly developed into their current salaried positions over a period of years. After a certain point, financial support over and above a salary becomes important. If that support does not come from within the zoo, external support can be sought. This support can come from manufacturers (food or equipment), private donors, grant or endowment programs, etc. and take the form of concrete financial support or the donation of equipment and/or services that may be of assistance to the nutritionist.

There are many ways to actually start working on diet evaluation, primarily based on the reasons why the program was created. A stepwise systematic approach (taking one area at a time) may be very successful to progress through diet evaluations of the entire zoo and improve animal husbandry over an extended period. However, if the program was created due to specific dietary evaluation needs stemming from specific nutrition-related disorders, a troubleshooting or problem-solving approach may be needed to get started. Each area and group of animals has unique nutritional issues, from how the diets are prepared and offered to the specific nutritional needs of the animals. Diets not only need to be formulated to meet the nutritional needs of the animals (a topic better discussed and more adequately addressed elsewhere), but attention must be paid to insure that the diets fit well into the daily routines of all the areas which will be preparing/handling the diets on a daily basis. In this way, the role of the nutrition program is not only to provide adequate nutrition to the animals in the zoo collection, but, additionally, to do so in a practical manner. Additionally, by considering the behavioral needs of the animals being fed, as well as their nutritional needs, the nutrition program can provide a well-rounded approach to diet formulation for the good of the animal collection.

As the nutrition program develops, the guidance and advice of other nutritionists and experts can be invaluable. These resources can be found at other zoos, zoo-related organizations (AAZK, etc.) universities, and private research facilities, to name only a few, on a worldwide scale. Keep in mind that “progress in [the field of nutrition] can be best made through the cooperative efforts of qualified individuals,” from the most basic to the most complex endeavors.

Potential problems - reinforcing the framework

Every institution is different, but there may be some common problems that arise. When trying to convey the need for a nutrition program, the benefits of such a program should be presented and evaluated. Nutrition problems are not always detected in a timely fashion (at post-mortem exam or after a long nutritional insult has occurred) and the advantages of a nutrition program similarly materialize over the long term. For this reason, it may be difficult for the zoo to immediately see the advantages of a nutritionist. The evaluation of the benefits of a nutrition program may be based on decreased veterinary costs, decreased incidence of health problems, decreased feed costs, and/or increased efficiency. Although the best initial approach may be to begin concentrating on the financial aspects, keep in mind that they may not materialize immediately.

Many keepers may have been working at an institution for a long time and may not have any experience with “formal” nutrition or a nutritionist. They may not understand that there is a difference in the
amount of nutrients provided in 2.5 g of egg vs. 3.0 g of egg (for example), and why this may be an important consideration for some species or individuals and not others. For these reasons, they need to be introduced to the concept of nutrition, educated, and motivated as to the goals of a nutrition program, how the program can assist them to achieve their own goals, and allowed time to adapt to the changes that may occur. In many zoos, this introduction/education may be necessary for other members of the multi-disciplinary team responsible for animal care as well.

If there is a lack of organization, changes can be made more complicated or even impossible. In some cases, it is up to the nutritionist to be inventive and find new ways to organize and present information so that it is clear. Being able to track changes, what worked, and what did not are imperative to avoid making the same mistake twice.

Zoos are not always ready to support a nutritionist financially and it may be important to get external support. However, this is not always easy and may take a considerable amount of time and effort. Remember that financial aid can be found in the form of a monetary donation or in the form of a piece of equipment. Both may equally satisfy the imminent need.

When animal nutrition is included as a curriculum at many universities, it examines and teaches about a broad range of domestic animals. There are few curricula that examine animal nutrition widely and, further, many students who might otherwise be interested in pursuing such a career do not know it exists as a viable option. For this reason, it is sometimes difficult to get help—specifically to assist with the aspects of the job which demand a nutrition background. This stresses the importance of collaborating with other nutritionists and similar resources. Local experts in universities can also provide advice, technical support, and analysis of food items (if needed).

Conclusion

Nutrition programs within zoos exist on a gradient from zoo nutrition services which have staffs of 10 or more people, formulate their own pelleted and gel diets, and have well equipped labs to analyze feeds to a single volunteer working to improve diets using tables with nutrient content information, a scale, and as many spare moments as can be found in their day. When starting out, zoos should focus on adapting a nutrition program to the resources of their institution, not a perceived “ideal.” Building a successful nutrition program takes a period of years, even decades. If the foundation is firm and development takes place in a stepwise fashion, an effective nutrition program can be created which is invaluable to successful maintenance of exotic animals in captivity.

LITERATURE CITED


EVERYTHING YOU NEVER WANTED TO KNOW, BUT SHOULD, ABOUT FEEDSTUFFS ‘OR’ THE IMPORTANCE OF CHEMICAL ANALYSIS IN FEED QUALITY CONTROL

Janet L. Dempsey, MS¹* and Joni B. Bernard, PhD²

¹Department of Animal Health/Nutrition, Saint Louis Zoological Park, One Government Drive, St. Louis, MO 63110 USA; ²Department of Zoology, Michigan State University, East Lansing, MI 48824 USA

Abstract

Chemical analysis is an integral part of a good quality control program to insure the nutritional value of feedstuffs. Establishing a quality control program may begin with identifying feeds to be analyzed based on their overall impact with the animal collection and instituting a regular schedule for sampling feeds for chemical analysis. The types of analyses to be performed are selected based on the type of feed to be analyzed and any nutritionally related problems. Specific protocols should be developed and followed for obtaining representative samples. A number of factors must be considered when choosing a laboratory to perform the specified analyses. Proper implementation of a quality control program, using chemical analysis as a tool, will produce results essential to maintaining high standards of nutritional care for captive zoo animals.

Purpose of Quality Control

If it looks good and the animals eat it, it must be okay, right? This may sound nonsensical, but all too often this type of criteria is applied when determining the quality of feeds and food items used in zoo animal diets. While the appearance and palatability of a feed or food item are important, the chemical analysis more accurately determines the value of these items to captive animals. In addition to chemical analysis, use of feed microscopy may be, on occasion, appropriate to evaluate feed quality.

Feeds should be systematically and regularly analyzed for a number of reasons. Manufactured feeds may not meet specifications due to inattention to detail during mixing or due to mechanical problems during the manufacturing process. While feeds may meet minimum nutrient level specifications, some nutrients may be included at higher inappropriate levels. Another reason for conducting routine feed analyses is to determine if unauthorized ingredient substitutions have been made. Often this is best accomplished by microscopic analysis. It is crucial to analyze foods such a frozen fish, to monitor the wide fluctuations in some nutrients (e.g., fat) which may occur on a seasonal, regional or species specific basis. Fluctuations in nutrient composition also occur in forages based on season, region produced and species of plant, and in whole prey items such as rodents and insects due to variation in developmental stage or differences in the diet fed to prey items.

Development and implementation of a quality control program is essential to insure the nutritional quality of feeds and food items used in captive animal diets. Chemical analysis is an integral part of
such a program. A schedule for sampling and analysis should be established for all feeds, especially those that represent a significant part of the zoo’s budget and those used in large quantities.

**Identifying Feeds for Analysis**

One of the first steps in instituting a quality control program is to identify primary feeds; that is feeds that are used in large quantity and fed to a number of different animals throughout the collection. For most zoos, these would include forages, fish, herbivore pellets, dog food and primate diets. Since the quality of the primary feeds will have a greater impact on a larger proportion of the collection, these feeds should be analyzed more often than the specialty feeds. Primary feeds should be analyzed at least four times per year. Specialty feeds, those that are fed to a small proportion of the collection, should be analyzed at least twice per year. If problems are encountered, they can be analyzed more frequently.

In general, it is not economically feasible or necessary to set up a regular schedule of sampling and chemical analysis for produce items (e.g., fresh fruits and vegetables), since produce inventory typically has a rapid turnover, usually 1 wk or less. In addition, produce items should contribute minimally to an individual animal diet, with the majority of nutrients supplied by a nutritionally complete feed. Therefore, the produce portion should not significantly impact the overall nutrient composition of the diet and slight variations in produce nutrient content are of less concern.

**Choosing the Types of Analyses Performed**

The analyses performed depend on the type of feed item and the reasons for sampling the item. Typically chemical analysis of feeds should include proximate analysis (dry matter, crude protein, ether extract, and ash), gross energy, and fiber fractions (neutral detergent fiber, acid detergent fiber, and acid lignin). Analysis for major minerals (calcium, phosphorus, sodium, potassium and magnesium) as well as trace minerals (iron, copper, manganese, selenium, and zinc) are also important. For some feeds, it may be important to analyze for various vitamins, but cost may be a consideration. Analyzing for other specific nutrients may be necessary if there is evidence of a specific health problem with a possible nutritional link. Routine analyses which should be performed, based on type of feed, are listed below in order of priority:

- **Forages** – proximate analysis, fiber fractions, gross energy, major and trace minerals.
- **Dry/semi-moist/moist feeds** – proximate analysis, gross energy, fiber fractions, major and trace minerals.
- **Fish** – proximate analysis, gross energy, fat soluble vitamins, major and trace minerals.
- **Meats** – proximate analysis, gross energy, major and trace minerals, vitamins A and E.
- **Whole prey** - proximate analysis, gross energy, major and trace minerals, vitamins A and E.

**Selecting the Representative Sample**

The goal when sampling feeds for analysis is to obtain a small portion that is representative of the entire lot (batch, catch, load, etc.) of a particular feed or food item. Obtaining a representative sample is critical because it is frequently the initial sampling step that introduces the greatest variability and most
effects the reliability of the analysis results. Ideally, every new lot should be sampled and sent for analysis. Also, the larger the sample size, the more reliable the results. However, the constraints of time, cost, and facilities available for collecting samples and analyzing data usually do not allow for the ideal situation. It is important to establish a plan for sampling and chemical analysis to improve the reliability of results.

**Protocol For Sampling Forages For Nutritional Analysis**

Hay should be sampled using a core forage sampler.
1. A composite of cores from 15-20 bales should be collected from each lot, of each hay type to be sampled.
2. The cores should be taken by drilling into the center of the end of the bale.
3. The complete length of the sampler should be drilled into the bale of hay.
4. The sample should be placed in a plastic bag, sealed and labeled with the type of hay, the date the sample was taken, and the name of the zoo.
5. The samples should be stored in a cool, dry place.

**Protocol For Sampling Dry/Semi-Moist/Moist Feeds For Nutritional Analysis**

Dry/semi-moist/moist feed samples should consist of at least 500 g.
1. Samples should be taken from at least 10 containers (bags, boxes, cans) of feed. Samples consisting of at least 100 g should be collected from the center of each container and combined. 2. A 500 g sample should be held for analysis and the remainder discarded.
3. The sample should be placed in a plastic bag, sealed and labeled with the type of feed, manufacturer’s name, date code or lot number, the date the sample was taken, and the name of the zoo.
4. Dry feed samples should be stored in a cool, dry place. Samples of semi-moist/moist feeds should be placed immediately into freezer storage.
5. All opened dry/semi-moist feed containers, which do not require refrigeration, or freezing after opening must be closed and sealed. Open containers of semi-moist/moist feeds, which require refrigeration or freezing, should be used immediately or discarded.
6. For dry feeds delivered in bulk and placed in hoppers, random sampling of feeds should take place on days when the hopper is being filled.

**Protocol For Sampling Fish And Other Frozen Feeds For Nutritional Analysis**

Fish
1. Frozen fish samples should consist of at least 1 kg for each species.
2. For IQF fish, samples should be taken from at least five, randomly selected cases, for each species of fish.
3. Bulk frozen fish should be sampled by cutting sections from blocks using a band saw. Samples should be taken from each of five randomly selected cases of fish. Cases should be opened and the block of fish cut into two approximately equal sections. One of the two sections should have a strip
(approximately 5 cm wide) cut from the original outer side and from the inner, newly cut side. Total sample size obtained should be at least 3 kg from the five cases, this should be thoroughly mixed, a 1-kg sample held for analysis and the remainder discarded.

4. Sampled fish should be placed immediately into large, thick plastic bags that have been pre-labeled with species of fish, lot number and date of catch, the date the sample was taken, and the name of the zoo. The bags should be placed immediately into freezer storage.

Other frozen feeds
1. Other frozen feeds, such as tubes of carnivore diets, should be sampled by cutting approximately ¼ kg off the end of the each of 5, randomly selected tubes, using a band saw.
2. Sampled feeds should be placed immediately into large, thick plastic bags that have been pre-labeled with type of feed, manufacturer’s name, date code or lot number, the date the sample was taken, and the name of the zoo.

*Protocol For Sampling Whole Prey For Nutritional Analysis*

Vertebrate prey
1. Frozen or fresh vertebrate prey samples should consist of a minimum of 10 animals not to exceed 1 kg total weight for each species.
2. Sampled prey should be placed into large, thick plastic bags that have been pre-labeled with species, the date of shipment, the date the sample was taken, and the name of the zoo. The bags should be placed immediately into freezer storage.

Invertebrate prey
1. Frozen or fresh invertebrate prey samples should consist of a minimum of 100 g for each species; protocol for sampling marine invertebrates is the same as fish.
2. Sampled prey should be placed into large, thick plastic bags that have been pre-labeled with species, the date of shipment, the date the sample was taken, and the name of the zoo. The bags should be placed immediately into freezer storage.

*Choosing a Laboratory*

Once appropriate samples have been collected and are ready for chemical analysis, there are numerous laboratories from which to choose, including commercial, university and hospital laboratories. There are even a few zoos with nutrition laboratories. However, all laboratories are not equal in their abilities or experience in analyzing different types of feeds and food items. For example, a lab specializing in hay analysis may not be familiar with procedures for sample preparation and analysis of fish or whole prey items. Cost is certainly a consideration when choosing a laboratory for performing analyses. It is important to note that inexpensive analyses are no bargain if the results produced are not reliable. Therefore, prospective laboratories should be critically evaluated before sending samples for analysis. Questions to ask include:
1. Does the laboratory have experience performing the type of analysis requested on the specific type of feed to be analyzed?
2. Do they use AOAC (Association of Official Analytical Chemists) approved methods or methods which are proven/accepted and referenced in current literature?
3. Are they willing to provide detailed references on the methods used?
4. Are they familiar with differences in method of sample preparation depending on the type of food sample being analyzed?
5. Do they regularly check the accuracy of results by running duplicate samples and/or do they participate in the National Institute of Laboratory Standards and have their own quality control programs?

Professionals in the nutrition field may be a resource to be consulted for recommendations on laboratories and accepted analytic methods. For more specific information on analytic and sampling techniques the following literature may also be referenced:

THE IMPLEMENTATION OF A HAZARD ANALYSIS CRITICAL CONTROL POINT (HACCP) PROGRAM IN AN ANIMAL FOOD OPERATION

Joseph E. Rindler

Disney’s Animal Kingdom, Walt Disney World Resort, PO Box 10000, Lake Buena Vista, FL 32830 USA

Abstract

Quality assurances are becoming an integral part of exotic animal facilities. The concept of Hazard Analysis Critical Control Point (HACCP) can be used to produce priorities and procedures for food safety and quality control, leading to the prevention of contamination and cross contamination of food products and equipment to reach higher hygiene standards. By raising these standards, the risks of possible illness related to food can be reduced.

Introduction

In the 1950s, Hazard Analysis and Critical Control Points (HACCP) was developed by The National Aeronautic and Space Administration to analyze the hazards of space flight. These principles were also used to produce food for the space program. The United States Department of Agriculture ruled in 1996 that all raw meat and poultry processing plants must implement HACCP programs. In 1997, Walt Disney World® Resort instituted HACCP in their Food and Beverage Department. The Disney Animal Kingdom Forage Warehouse with the Walt Disney World® Environmental Health developed a HACCP program for animal food preparation. This program was implemented in April 1999.


Methods

By following the seven food safety principles utilized for the Walt Disney World® Resort’s HACCP Program, the Forage Warehouse operation was analyzed.

Step 1: Conduct a Hazard Analysis - Hazard is defined as any microbiologic, chemical or physical property that may cause an unacceptable animal health risk. Examples: E. coli and Salmonella spp. on raw meats and eggs (microbiologic hazard), food exposed to a cleaning compound (chemical hazard), and metal in food (physical hazard). By evaluating each procedure the Forage Warehouse performs, hazards of significance and all associated preventive measures were identified and listed. The risk of encountering each hazard was then assessed.
Step 2: Identify Critical Control Points (CCP) - CCP are any point or procedure in a specific food process at which control can be applied and a food safety hazard can be prevented, eliminated or reduced to acceptable levels. Forage Warehouse CCPs included: contamination and cross contamination, cleaning of the equipment and facility, cooking and cooling, storage and handling, and thawing and handling raw animal products.

Step 3: Establish Critical Limits - Critical limits are one or more prescribed tolerances or criteria that must be met to ensure that a critical control point effectively eliminates or controls a microbiologic, chemical, or physical hazard. The Forage Warehouse plan identified critical limits with zero tolerances and criteria associated with time and temperature. The CCPs with a zero tolerance critical limit are: contamination and cross contamination, cleaning the equipment and facilities, handling raw animal products, and handling of food products. CCPs which use the criterion of time and temperature are: cooking and cooling, thawing raw animal products, and storage of food products.

Specific Time and Temperature Criteria:

- When cooking animal products center temperature must reach 165°F.
- When cooling, the item’s temperature must drop from 140°F to 70°F within 2 hr and from 70°F to 41°F within 4 hr.
- Animal products being thawed must maintain a temperature below 41°F. Raw animal products must be maintained below 41°F when preparing and proportioning animal product for diets.
- Storage temperatures were established at: 33-41°F for browse cooler, 33-41°F for produce cooler, 0-20°F for freezer, 55-70°F for grain room, and 33-41°F for refrigerators in animal holding facilities. Temperature for handling food products are set as 0-30°F for freezer trucks, 33-41°F for cooler trucks delivering animal products, and 33-45°F for coolers delivering non-animal products.

Step 4: Establish CCP Monitoring Requirements - Monitoring is defined as a planned sequence of observations or measurements of critical limits designed to produce an accurate record and intended to ensure that the critical limit or criteria maintain product safety.

Step 5: Establish Corrective Actions - Corrective action is defined as a planned action that is implemented when monitoring indicates that there is a deviation from an established critical limit or criteria. At the Forage Warehouse, deviation for CCP’s with zero tolerance is disciplinary action. The corrective action for cooking criterion is to continue to heat until the product reaches the correct temperature and for cooling and raw meat if the criteria are not met, the corrective action is to dispose of the product. If the storage temperature is not met then the action requires moving the product to a safer environment and immediately contacting maintenance. If the delivery temperature criteria are not met then action will be addressed with the product vendor.
Step 6: Establish Record-keeping System - The record keeping system consists of a series of daily and weekly checklists to monitor CCPs. These include HACCP temperature logs, and accountability lists. These records are required to be maintained at the supervisor’s office for a minimum of 2 wk.

Step 7: Establish Verification Procedures - The HACCP system in place at the Forage Warehouse will be reviewed by the Walt Disney World ® Environmental Health staff on a bi-annual bases. The verification process will include an in depth inspection of the facility and testing for microbes.

Upon completion of the step-by-step analysis, a manual was designed to train the Forage Warehouse staff. This manual includes an introduction to HACCP, Standard Operational Procedures for all processes at the Forage Warehouse with corrective actions if critical limits are not maintained, and a sample of the forms utilized for documentation of CCP. A 1-hr class was also developed to train the staff.

Results

By implementing a HACCP plan for an animal food operation, the risks of a possible hazard contamination during food handling and preparation can be reduced.

Discussion

Standards for production and handling of food fed to captive exotic animals are generally lower than those for humans. There are many reports of food hazards leading to the illness and/or deaths of captive exotic species. Accidental contamination of felid diets with ethylene glycol have been reported,10,11 scombroid poisoning from improper storage temperature of mackerel1,5 and mycotoxin poisoning in grains, grain by-products, and forage2 have all been documented in captive species. Most still need to be tested for contaminates during manufacturing. Even with the knowledge of possible contamination, the implementation of a HACCP plan will greatly reduce the risk of food-related illness.

The exact number of animals that are affected by food related illness in animal holding facilities is unclear. This is due to the fact that most food borne bacteria have several vectors of infection (e.g., feces and water), and in many cases the evidence (contaminated food) is consumed. In addition, many food borne bacteria cause mild illness which resolves spontaneously. Fatal septicemia caused by Streptococcus zooepidemicus has been reported in brindled bandicoots (Isodon macrourus), a tree shrew (Tupaia glis), and an elephant shrew (Elephantulus rufescens).9 Salmonellosis outbreaks in zoos have been reported especially among felids.12 Infant animals are particularly susceptible to salmonellosis as well as other food borne microbes.6 Proper food management can prevent illness.

In the Forage Warehouse operation, two of the most important critical control points are maintaining and handling meat products below 41°F and eliminating potential cross contamination points between animal and non-animal products. Salmonella, E. coli, Enterobacter freundii, Klebsiella pneumoniae, and Streptococci sp. have been all cultured from several horsemeat-based diets.7,3 Testing on raw chicken yielded E. coli and Serratia odorifera.7 By maintaining the core temperature of meat products...
below 41°F during thawing and handling, microbial growth can be slowed. The Forage Warehouse kitchen is large enough to allow a total separation between animal and nonanimal products including a separate preparation table and thawing cooler as well as separate staff. If the same equipment, prep table, and staff are utilized for meat and nonmeat product preparation, then a thorough cleaning and disinfesting plan should be developed and implemented, as well as proper hand washing practices. Food types should be separated during storage. Animal products should be placed in covered leak proof containers and on the bottom if stored on the same shelf with non-animal products to prevent contact with dripping juice and/or blood.

Another important critical point was cross contamination between departments. Whether an animal food operation delivers the food or it is picked up by the animal care staff, the food facility and its staff can be possible transmitters for disease between buildings. Foot baths should be utilized at all times and food prep staff should have minimal contact with animal areas. For facilities that utilize animal care staff for food preparation and delivery, this should be done prior to contact with the animals.

Implementing a HACCP plan is not expensive. The only resource needed is the time to analyze all possible hazards, to determine acceptable and unacceptable risks, to take corrective action, to ensure training, and verification of the plan.

ACKNOWLEDGMENTS

This paper was prepared with the help of Walt Disney World © Resort Environmental Health, Disney Animal Kingdom Veterinary Service, and Forage Warehouse staff.

LITERATURE CITED

THE DEVELOPMENT OF RAW MEAT-BASED CARNIVORE DIETS

Mary E. Allen, PhD,1* Duane E. Ullrey, PhD,2 and Mark S. Edwards, PhD3

1Smithsonian Institution, National Zoological Park Washington DC 20008 USA; 2Department of Animal Science, Michigan State University, East Lansing, MI 48864 USA ; 3The Zoological Society of San Diego, PO Box 551, San Diego, CA 92112-0551 USA

Abstract

Raw meat is highly perishable and requires safe and sanitary preparation, handling and storage. Wild cats may tolerate concentrations of microbes in prey that would be pathologic to captive felids. Just as zoo animal nutritionists have the knowledge to formulate many types of animal feeds and arrange for their manufacture, so do we have the ability to develop safer, raw meat-based diets according to a specific formula. We also can develop standards for more sanitary methods of manufacture, handling and storage. Meat based canned (heat processed) and baked diets have been tested in zoo felids (S. Crissey and M. Edwards, personal communications), but so far stool condition and acceptability are poor. However, Crissey, et al. demonstrated that sand cats (Felis margarita) with a body mass of about 2 kg, consumed an extruded diet with good digestibility and no deleterious effect on stool condition.1

Dry, extruded diets are also being tested in large cats (M. Edwards, personal communication). However, many species of larger cats do not seem to tolerate more than 50% of dry matter intake as dry food, as evidenced by food refusals or poor stool condition. Due to recent reports of quality and consistency problems with some frozen carnivore diets in 1997 and 1998, we began to consider a new formulation for raw meat-based diets. In response to a number of requests, ingredient and nutrient specifications for both horsemeat and beef-based raw diets have been developed (Table 1). In addition, microbiologic guidelines have also been established with the assistance of a professional food safety microbiologist (Table 2).

Unlike most products marketed in North America, the specifications call for horse or beef muscle tissue, a small amount of a carbohydrate “filler” and specific vitamins and minerals. Nutritionists at the Metro Toronto Zoo (MTZ) developed similar specifications some time ago. Milliken Meats in Toronto, Ontario, Canada, manufactures MTZ carnivore diets according to their specifications. These products are well accepted by MTZ carnivores. At present our specifications have been given to three potential manufacturers in the United States. Two of these companies are making test batches of both horse and beef-based diets. These have been fed to over 15 species of felids and have been well received by both keepers and captive felids. Acceptable manufacturing plants are required to process diets under conditions acceptable to U.S. Department of Agriculture (USDA) or to Agriculture Canada (AC) using only muscle meat that is fit for human consumption. No contaminated (3-D) meat (disabled, diseased and down) may be used. Processors must also use a metal detector that will detect metal that is 2 mm thick or greater. We will require any manufacturer to provide Safe Sanitary Operating Procedures
(SSOP’s), Material Safety Data Sheets (MSDS) and their HACCP (Hazard Analysis Critical Control Points) program.

The quantitative nutrient requirements of nondomestic cats are not known. However, we used the established requirements of the domestic cat as guidelines in formulating diets for zoo cats.2 Other considerations in feeding captive cats include attention to the integrity of teeth and gums. Oral heath can be improved by feeding bones with meat attached at least twice per week, or by feeding whole vertebrate prey. Such feed items will also extend the time spent feeding, which is desirable from a behavioral standpoint.

We believe that zoo animal nutritionists have an obligation to assure that not only nutritional needs are met, but that sanitation and food safety are equally important, particularly for highly perishable rations.

LITERATURE CITED

Table 1. Frozen horsemeat-based carnivore diet ingredient and nutrient specifications (11/14/98).

**Application.** This product is a frozen, fresh meat diet for use in the feeding of captive carnivores. It may be used as the sole diet for felids. In addition, provision of knuckle bones to chew on, two or more times per week, may aid in promoting oral health. With further testing, it is probable that this diet also will be suitable for carnivorous birds, reptiles and other carnivorous mammals.

**Ingredients.** Horsemeat or horsemeat trimmings, Solka Floc (wood cellulose), calcium phosphate tribasic, sodium chloride, carnivore trace element premix, carnivore vitamin premix, choline chloride, taurine, stabilized L-ascorbyl-2-polyphosphate.

Ingredient and Product Standards. All meat and meat products shall originate from animals slaughtered in plants subject to the Meat and Poultry Inspection Operations regulations of the USDA Food Safety and Inspection Service (FSIS), or under a system of inspection approved by FSIS. All bones, cartilage, heavy connective tissue, lymph glands, and central nervous system tissue shall be removed. Likewise, meat and meat products that originate from animals or carcasses designated as 3-D or 4-D shall not be used. Other (non-meat) ingredients shall conform to standards as defined by the Association of American Feed Control Officials (AAFCO).

The product shall be routinely monitored for specific microbial populations. The diet must test negative for the presence of *Salmonella* and *Listeria*, and within specified tolerance limits for total coliforms and *E. coli*.

**Nutrient Concentrations.** The product has been formulated to meet or exceed the minimum NRC nutrient concentrations required in purified diets for the growing domestic kitten and AAFCO nutrient profiles for growth and reproduction of cats fed practical diets. All values, except moisture, are expressed on a dry matter basis.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein, %</td>
<td>30</td>
</tr>
<tr>
<td>Crude Fat, %</td>
<td>3</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>4.3</td>
</tr>
<tr>
<td>Taurine, %</td>
<td>0.3</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>1.3</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>1.2</td>
</tr>
<tr>
<td>Magnesium, %</td>
<td>0.09</td>
</tr>
<tr>
<td>Vitamin A, IU/kg</td>
<td>14000</td>
</tr>
<tr>
<td>Vitamin E, IU/kg</td>
<td>470</td>
</tr>
</tbody>
</table>

Table 2. Microbiologic guidelines for raw meat-based diets.

<table>
<thead>
<tr>
<th>Test</th>
<th>Test</th>
<th>N</th>
<th>C</th>
<th>Acceptable (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stand plate count</td>
<td>5</td>
<td>3</td>
<td>&lt; 500,000</td>
</tr>
<tr>
<td>2</td>
<td>Total coliforms/g</td>
<td>5</td>
<td>3</td>
<td>&lt; 500</td>
</tr>
<tr>
<td>3</td>
<td><em>E. coli</em> /g</td>
<td>5</td>
<td>3</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>4</td>
<td>Staph Species (TSN+) /g</td>
<td>5</td>
<td>3</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>5</td>
<td><em>Salmonella</em>25 g</td>
<td>5</td>
<td>0</td>
<td>&lt; 100</td>
</tr>
</tbody>
</table>
TOO MUCH OR TOO LITTLE OF A GOOD THING: WEIGHT MANAGEMENT FROM THE ZOO NUTRITIONIST'S PERSPECTIVE

Ann M. Ward, MS,1# Barbara Lintzenich, MS,2 and Mike Maslanka, MS3

1Fort Worth Zoological Park, 1989 Colonial Parkway, Fort Worth, TX 76110 USA; 2Daniel F. and Ada L. Rice Conservation Biology and Research Center, Chicago Zoological Society, 3300 Golf Road, Brookfield, IL 60513 USA; 3Memphis Zoological Garden and Aquarium, 2000 Galloway Avenue, Memphis, TN 38112 USA

Abstract

The nutrient content of a diet can be altered to facilitate weight loss or gain. Sources of calories as well as the digestibility of these sources are important factors to consider when selecting food items. Utilizing on exhibit and off exhibit space as well as resources available through training and enrichment programs can help achieve consumption of the appropriate food items by the appropriate animals as well as increase their activity or energy expenditure. A successful program includes assessing body condition on a regular schedule which facilitates making changes in the diet or management of the animals in a timely fashion to achieve the desired weight goal in the desired time.

Introduction

Managing excess weight gain or loss is a challenging task. While it may not be difficult to formulate a diet to promote weight loss or gain, it can be very difficult to assure an individual animal receives the prescribed diet. In most situations it is not practical to separate an animal to facilitate consumption of a carefully calculated diet. A successful weight management program often must include making changes in the level of activity. More recent emphasis on training and enrichment programs assists with this goal. Often, assessing body condition and monitoring progress of weight changes can in itself make a weight management program difficult. In many situations it may not be feasible to have a scale resulting in subjective assessment that may vary from individual to individual. A coordinated effort between nutritionists, veterinarians, and animal management staff can result in a successful weight management program.

Manipulating the Diet

The goal of most weight management programs is to decrease or increase the calories available to the animal thus evincing the desired weight loss or gain. Consequently, it is important to consider the sources of calories in the diet and their different digestibilities. Protein, fat, and carbohydrate are sources of energy in the diet. The gross energy content (measured as the heat of combustion, expressed in calories) of these sources can be measured by bomb calorimetry. More energy is available from fat than from protein or carbohydrates. In general, using bomb calorimetry fat, protein, and carbohydrates have the energy values of 9.3 kcal/g, 5.4 kcal/g and 4.1 kcal/g respectively.3 When considering how much of the gross energy is actually utilized by the animal, digestibility must be examined. By increasing the fiber in the diet of an animal that cannot utilize fiber as a carbohydrate source, the diet...
becomes less calorie dense and may assist in promoting weight loss. Alternatively, the same diet offered to an animal that can utilize the fiber results in a different amount of available energy to that animal.

To alter calories available to the animal, a change can be made in the total quantity of food offered or in the composition of foods in the diet. A percentage increase or decrease of each food item (thus not changing the composition of the diet, rather changing the total amount of the diet) can be successful if an overweight animal does not have access to additional food items or if a thin animal is currently consuming all of its diet. Disadvantages to having less food available may include (1) a reduction in energy expenditure due to decreased foraging time and (2) increased competition if housed in a group situation. Regardless of whether the total amount of diet offered or the caloric density of the diet is reduced or increased, all diet manipulations should result in nutrient levels that are still within a target range established as most appropriate for the species.

Among fat, protein, and carbohydrate, fat contributes the most energy to a diet on a kcal/g basis, and is often the source of energy altered to provide a less or more calorie dense diet. A variety of nutritionally complete biscuits, canned diets, and whole prey items are available for use in zoos (Table 1). There are several reduced calorie (via decreased fat and/or increased fiber), nutritionally complete foods available specifically formulated for domestic dogs and cats that can be useful in omnivore and carnivore diets in a zoo setting (Table 1). Biscuits formulated specifically for primates vary in nutrient content based on their specific intended application and manufacturer. Fat (and protein) content of whole prey items can vary based on developmental state, reproductive condition, species, and season/stage of harvest. With this in mind it is possible to select lower or higher fat whole prey items for specific feeding situations. In some cases, it may be possible to incorporate a reduced calorie dog or cat food in place of a higher calorie whole prey item to reduce the total caloric content of the diet.

Overweight herbivores for which fiber is a caloric source also may benefit from lower calorie feeds or increased roughage, by providing the same or increased “bulk” while maintaining or reducing caloric density of the diet (Table 1). Lower energy pellets or lower energy hays substituted or incorporated into the diet have been successfully used to encourage weight loss in several herbivorous species.

For the overweight animal it is desirable to lose weight as fast as possible without sacrificing the overall health of the animal. As a result of a lack of measurable data on animal species, it may be appropriate to extrapolate successful approaches used for weight management in humans. Methods used by human nutritionists applied successfully to mammals achieve weight loss at a rate ranging from 2.6% of body weight per month (1 pound per week per 70 kg animal) to 4% of body wt per month. These guidelines have been used with a variety of species to achieve desired weight loss.

**Manipulating the Animal**

The ability to manipulate the animal is often the biggest obstacle to offering the most appropriate diet for weight management. In most situations it is not practical or possible to house animals individually to ensure consumption of a carefully calculated diet. Many times the dominant animal is the obese
animal in the group while the animals with specialized needs, such as growing or geriatric animals or the “poor doers,” are the subordinates. As a result of this social situation, getting the desired foods to the desired animals appears almost impossible. While animals may not be separated on exhibit, this may still be possible off exhibit. Keepers can utilize off exhibit time and space to work closely with the animals. This can include separating within holding and offering the concentrated sources of calories in the diet at this time, thus allowing the greatest control over the most calorie dense portions of the diet. When animals cannot be separated in holding, selecting favorite high or low calorie items from the diet and offering them at the cage front can be a successful method to facilitate consumption of the appropriate calories by each animal. On or off exhibit creep feeders also can be employed to make food items accessible to some but not all individuals within a group.

Activity

Changes in activity resulting in increases in energy expenditure also facilitate weight loss. Training and enrichment programs can be utilized to encourage more activity in almost every species. Even a sedentary individual can become active if it must forage for live food items. Changes in feeding schedules and food presentation, such as chopping into smaller sizes and scattering, hiding, or strategically placing food items can increase activity by increasing time spent foraging. It is important to remember any item used that is ingested (whether used for enrichment or not) is a source of energy for the animal and must be considered a part of the diet. Several nonfood items have been used successfully to increase animal activity. It is not possible within the scope of this paper to discuss all of the successful items used for this purpose. However, whenever possible, modifications should be made to exhibits and holding areas to encourage activity. Although potentially not aesthetically pleasing, exhibits or holding areas filled with climbing and swinging apparatus may promote great amounts of energy expenditure. Animals that may not want to use these structures can be encouraged to do so if the diet is offered in or on these structures. In addition to enrichment methods, target training can be utilized to encourage activity and get specific food items to specific individuals.

Assessing Body Condition/Monitoring Weight Changes

Assessing body condition and following changes in weight is crucial to a successful weight management program. Regularly weighing animals is the ideal method for following progress. Often it is difficult, if not impossible, to weigh large animals such as elephants, giraffes, and hippos. In the absence of a scale, body measurements have been shown to be helpful to assess body condition of Asian elephants, and may hold promise for other species. However, it may not be possible to handle an animal to allow for body measurements. Photographs taken overtime with an object or background as a common denominator may be useful. Condition scoring used to judge domestic animals may be applied in a zoo setting to score different individuals based on “species normals.”

Conclusions
A successful weight management program is a multi-faceted project. A weight loss or weight gain diet must be formulated to achieve a weight goal and meet all nutritional needs of the animal. Animal staff must be committed to developing methods to manipulate the animals to achieve the necessary consumption of the appropriate foods. Physical activity or energy expenditure facilitate weight loss and thus are important aspects of the program as well. Training and enrichment play an additional role in getting animals the appropriate diets and increasing activity. A schedule and protocol for assessing body condition should be established at the onset of any weight management project to (1) achieve consistency in assessment, (2) facilitate the necessary adjustments in the diet in a timely fashion, and (3) become firmly established as a part of the daily management of, not only over or underweight individuals but, all animals in the collection.

ACKNOWLEDGMENTS

The authors would like to acknowledge all of the Zoos that have contributed to the experiences related in this paper: the Brookfield Zoo (Chicago, IL), the Fort Worth Zoo (Fort Worth, TX), the North Carolina Zoo (Asheboro, NC), and the Memphis Zoo (Memphis, TN).

LITERATURE CITED

<table>
<thead>
<tr>
<th>Table 1. Nutrient composition of nutritionally complete foods, whole prey, fish, and hay.(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nutrient Analysis on a Dry Matter Basis</strong></td>
</tr>
<tr>
<td><strong>Protein, %</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><strong>Nutritionally Complete Foods</strong></td>
</tr>
<tr>
<td>Primate Biscuit</td>
</tr>
<tr>
<td>High-fiber Primate Biscuit</td>
</tr>
<tr>
<td>Dog Food</td>
</tr>
<tr>
<td>Canned Cat Food</td>
</tr>
<tr>
<td><strong>Whole Prey</strong></td>
</tr>
<tr>
<td>Rat adult, 280 g</td>
</tr>
<tr>
<td>Rat pup, 5.9 g</td>
</tr>
<tr>
<td>Mouse adult, 27.6 g</td>
</tr>
<tr>
<td>Mouse pup, 1.6 g</td>
</tr>
<tr>
<td>Mouse pup, 3.9 g</td>
</tr>
<tr>
<td>Mouse pup, 5.9 g</td>
</tr>
<tr>
<td>Chick, 34.4 g</td>
</tr>
<tr>
<td>House cricket</td>
</tr>
<tr>
<td>Mealworm larva</td>
</tr>
<tr>
<td>Waxmoth larva</td>
</tr>
<tr>
<td>Common earthworm</td>
</tr>
<tr>
<td><strong>Fish</strong></td>
</tr>
<tr>
<td>Atlantic herring</td>
</tr>
<tr>
<td>Spanish mackerel</td>
</tr>
<tr>
<td>Capelin</td>
</tr>
<tr>
<td>Great Lakes smelt</td>
</tr>
<tr>
<td>Squid</td>
</tr>
<tr>
<td><strong>Herbivore Pellets &amp; Hay</strong></td>
</tr>
<tr>
<td>Herbivore Pellets</td>
</tr>
<tr>
<td>Grass Hay</td>
</tr>
<tr>
<td>Legume Hay</td>
</tr>
</tbody>
</table>

\(^a\) Analyzed values.
\(^b\) Not determined.
NUTRACEUTICALS – A REGULATORY PARADOX?

Roger D. Hoestenbach, Jr.

Feed and Fertilizer Control Service, Texas Agricultural Experiment Station, Texas A & M University System, College Station, TX 77843-2114 USA

Abstract

The marketing of nutraceuticals is exceeding the science needed to insure safe marketing. While many may have beneficial uses, the processes to ensure safety and standards should not be circumvented. There is no compelling reason to hold these products to a lesser standard than that of other feed ingredients.

In the 1980’s, we began seeing the term “nutraceutical,” along with similar terms like “functional foods,” “designer foods,” “chemopreventive agents,” and “pharmafoods,” and although they have no official standing, they do have recognition with consumers and health care professionals alike. There exist several variations within the definitions that have been suggested for these products, such as provided by the North American Veterinary Nutraceutical Council, "a substance which is produced in a purified or extracted form and administered orally to patients to provide agents required for normal body structure and function and administered with the intent of improving the health and well-being of animals” or as described by Boothe, “a product that has characteristics of both nutrients and pharmaceuticals.” Boothe further suggests that the products are neither drugs nor food. Thus, no federal agency regulates nutraceuticals, and the products are not subject to premarket approval. The Food and Drug Administration (FDA) may not require premarket approval for dietary supplements; however, that does not mean the products do not fall under regulation.

The Dietary Supplements, Health and Education Act (DSHEA) of 1994 is generally considered the definitive legislation for dietary supplements and was an amendment of the Federal Food, Drug, and Cosmetic Act and, therefore, under the regulation authority of the FDA. The passage of DSHEA created considerable confusion over what and how the dietary supplements market is regulated. For example, it appears some have interpreted DSHEA as implied consent for animals since “if it is alright for humans, it has to be okay for animals.” But, DSHEA does not apply to animals (Federal Register, 4/22/96).

To best understand current interpretations regarding the regulations as they apply to animals, consider the following definitions:

(1) Federal Food, Drug, and Cosmetic Act §201 (f) The term “food” means (1) articles used for food or drink for man or other animals, (2) Chewing gum, and (3) articles used for components of any other such article. This definition does not differentiate between food and feed (for animals),
and it does not offer separate regulation for food producing animals from those non-food producing animals, as has been suggested by some.

(2) Federal Food, Drug, and Cosmetic Act §201 (g)(1) The term “drug” means (A) articles recognized in the official United States Pharmacopeia, official Homeopathic Pharmacopeia of the United States, or official National Formulary, or any supplement to any of them; and (B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and (C) articles (other than food) intended to affect the structure or any function of the body of man or other animals; and (D) articles intended for use as a component of any articles specified in clause (A), (B), or (C); but does not include devices or their components, parts, or accessories. A product that affects the structure or function of the body by providing nutrients is acceptable, but questions have arisen if the product is not usually recognized as a nutrient.

(3) Federal Food, Drug, and Cosmetic Act §201 (s) The term “food additive” means any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food,… While “food additives are generally considered non-nutritive, they may also include products that are not traditional sources of recognized essential nutrients.

(4) 21 C.F.R. §570.30 (a) Generally Recognized As Safe or GRAS may be established by either (1) scientific procedures or (2) use in food prior to January 1, 1958. GRAS is usually approved or affirmed for a specific use. The preponderance of evidence is the responsibility of the applicant and the requirement is the same as for a food additive petition, except a certain amount of the support is expected to come from the public domain.

The term “Commercial Feed,” as defined in the Model Bill1 promoted by the Association of American Feed Control Officials (AAFCO) means all materials or combination of materials which are distributed or intended for distribution for use as feed or for mixing in feed, unless such materials are specifically exempted. Unmixed whole seeds and physically altered entire unmixed seeds, when such whole or physically altered seeds are not chemically changed or are not adulterated within the meaning of section 7(a) of this act, are exempt. The (blank) by rule may exempt from this definition, or from specific provisions of this Act, commodities such as hay, straw, stover, silage, cobs, husks, hulls, and individual chemical compounds or substances when such commodities, compounds or substances are not inter-mixed with other materials, and are not adulterated within the meaning of section 7(a) of this Act. (Note: the only exempted product suggested by the AAFCO Model Regulations is loose salt.) This loosely interprets commercial feed to include essentially everything ingested orally, other than water, except what is exempted by rule.

A “dietary or nutritional supplement” could be defined as any product that provides essential nutrients that supplement the diet to meet physiologic needs and prevent nutritional deficiencies. However, any explicit or implicit claims that a product treats, cures, prevents, or mitigates a disease, or affects the structure or function of the body in a manner other than providing essential nutrients as in food identifies the product as a drug. This requires premarket approval via a new animal drug.
A number of the products being marketed as nutraceuticals (dietary or nutritional supplements) are offered by either implied or specific claims for the prevention or treatment of disease. This identifies them as a drug. Why would they then be identified and offered as “dietary supplements?” Many are not patentable and, since they do not have the profit potential of a patentable substance, it has been perceived that by offering them as dietary supplements, they do not have to meet premarket approvals. This has been speculated as most likely reason that drug clearance is not attempted on many of these products.

While some of these supplements may play a beneficial role in health as part of a varied diet, research regarding the health benefits should be supported. And, despite the excitement over potential health benefits, experts should remain cautious. Safety should be of primary concern, and clients should be encouraged not to neglect traditional therapies in lieu of nutraceuticals unless clinical evidence of efficacy exists. Safety is the critical issue. Optimum levels must be determined because a number of animal studies show that some of the same phytochemicals (e.g., allyl isothiocyanate) investigated for cancer preventing properties can be carcinogenic at high concentrations. Thus, the 15th century doctrine of Paracelsus, that “nutriment is both food and poison, its the dosage makes it either poison or remedy” receives additional support.

The veterinary profession should support the development of production standards. Many of these products, though represented by certificates of content, are not being tested or prepared by any established standards. Without proper testing methods and standards for production, neither concentrations nor quality can be assured either between products or even within product lines.

What about “natural products?” Natural does not mean safe—arsenic is natural. What about products used by ancient cultures? Surely hundreds of years of use indicates both safety and efficacy? Gossypol and ground rhinoceros horn are still being used. Established American companies may not always be trusted as they push for new or expanded markets. A prominent manufacturer was recently found to be promoting formulas not supported by research, but by anecdotal information and market trend analysis. Yank Coble, MD, AMA, said a lot when he stated, “In God we trust, all others need research.”

In conclusion, how can anyone argue that generally acceptable scientific principles* should be utilized to support the safety, utility, and efficacy of these products. There does not appear to be any valid reasons for holding these substances to a lesser standard than those used for other nutrients.

*Here are some good general guidelines7,8 for evaluating studies, particularly clinical trials, that have been adapted from User’s Guide to the Medical Literature:

Validity of the study?
Was the assignment of subjects randomized?
Were all the subjects entering into the study accounted for and attributed at its conclusion?
Were the study participants “blind” to the assigned treatments?
Were groups similar at the start of the study?
Aside from the intervention studied, were the groups treated equally?
Assessing study results?
How large was the treatment effect?
How precise was the estimate of treatment effect?

Claim supportable?
Can the results be applied to the target species?
Were all important outcomes considered?
Was the claim supported?

LITERATURE CITED

Abstract

Although clinical signs of vitamin E imbalances have not specifically been reported in chelonians, plasma concentrations of this nutrient have been shown to vary directly with dietary content in other species.\textsuperscript{1,2} Hence, blood samples can provide a basis of quantitative evaluation of vitamin E status. Conversely, vitamin A status and dietary adequacy cannot be as readily assessed by blood sampling.\textsuperscript{3} Vitamin A-responsive syndromes have been reported in numerous turtle and tortoise species. Toxicities and induced fat-soluble vitamin antagonisms should also be considered in evaluating the nutritional health of reptiles. Samples were opportunistically obtained from turtles and tortoises during normal zoo medical procedures, and during field work with free-ranging specimens. Plasma was separated by centrifugation, and samples were stored frozen (-20°C to –70°C, or liquid nitrogen) for no more than 6 mo prior to analysis using high performance liquid chromatography as per standardized laboratory methods that have been previously detailed.\textsuperscript{5} These survey data were compiled to contrast and compare “physiologically normal” circulating levels of tocopherol and retinol, as measures of vitamin E and A assessment, respectively, in non-aquatic chelonian species. A total of 547 blood samples from 24 species are summarized in Tables 1 and 2.

Comparative values among species which occupy differing ecologic habitats, and particularly differences between free-living and zoo-held species, need to be interpreted with caution. Variables that are known to affect concentrations (e.g., sex, age, seasonality, and diet)\textsuperscript{1-6} need to be better examined through controlled feeding trials with chelonians to allow adequate data interpretation. Nonetheless, some useful broad interpretations can be made. 1) In general, the more herbivorous tortoise species tended to display lower circulating levels of \(\alpha\)-tocopherol than more omnivorous chelonians. 2) Further, levels of \(\alpha\)-tocopherol measured were comparable to ranges reported for dietetically equivalent (herbivorous vs. omnivorous) mammalian species.\textsuperscript{1,2} 3) Additionally, a greater proportion of tortoise species examined displayed measurable concentrations of \(\gamma\)-tocopherol in blood samples compared with turtles. Gamma-tocopherol isomers are found almost exclusively in plant-based tissues, whereas \(\alpha\)-tocopherol is distributed in both animal and plant tissues. The presence/absence of specific tocopherol isomers may be useful to distinguish differences in primary dietary ingredients. 4) With some exceptions, retinol concentrations in turtles and tortoises were at the lower end of the expected normal range for domestic livestock species (0.2 to 0.8 \(\mu\)g/ml). 5) Where direct species comparisons were possible between free-living and captive populations, no statistically significant differences were noted.
LITERATURE CITED


**Table 1.** Circulating tocopherol and retinol concentrations measured in selected turtle species (x ± SD).a

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Sampling Location(s)</th>
<th>α-tocopherol µg/ml</th>
<th>γ-tocopherol µg/ml</th>
<th>retinol µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Callagur borneoensis</em></td>
<td>3</td>
<td>1 US zoo</td>
<td>2.65 ± 0.94</td>
<td>n.d.b</td>
<td>0.11 ± 0.06</td>
</tr>
<tr>
<td>Painted terrapin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chelydra serpentina</em></td>
<td>21</td>
<td>1 US zoo</td>
<td>3.28 ± 1.71</td>
<td>n.d.</td>
<td>0.35 ± 0.15</td>
</tr>
<tr>
<td>Snapping turtle</td>
<td>4</td>
<td>NY, USA</td>
<td>3.77 ± 2.85</td>
<td>n.d.</td>
<td>0.31 ± 0.25</td>
</tr>
<tr>
<td><em>Chrysemys picta</em></td>
<td>47</td>
<td>2 US zoos</td>
<td>5.21 ± 3.19</td>
<td>n.d.</td>
<td>0.16 ± 0.09</td>
</tr>
<tr>
<td>Painted turtle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clemmys guttata</em></td>
<td>22</td>
<td>NY, USA</td>
<td>6.93 ± 3.22</td>
<td>n.d.</td>
<td>0.22 ± 0.09</td>
</tr>
<tr>
<td>Spotted turtle</td>
<td>5</td>
<td>1 US zoo</td>
<td>2.45 ± 0.56</td>
<td>n.d.</td>
<td>0.08 ± 0.04</td>
</tr>
<tr>
<td><em>Clemmys mullenbergii</em></td>
<td>2</td>
<td>NY, USA</td>
<td>17.23 ± 0.03</td>
<td>n.d.</td>
<td>0.28 ± 0.05</td>
</tr>
<tr>
<td>Bog turtle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cuora amboinensis</em></td>
<td>2</td>
<td>1 US zoo</td>
<td>10.30 ± 5.35</td>
<td>n.d.</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Malayan box turtle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cuora galbinifrons</em></td>
<td>1</td>
<td>1 US zoo</td>
<td>6.96</td>
<td>n.d.</td>
<td>no data</td>
</tr>
<tr>
<td>Indochinese box turtle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mauremys caspica</em></td>
<td>1</td>
<td>1 US zoo</td>
<td>7.85</td>
<td>n.d.</td>
<td>0.06</td>
</tr>
<tr>
<td>Caspian turtle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Podocnemis erythrocephala</em></td>
<td>9</td>
<td>1 US zoo</td>
<td>6.82 ± 5.15</td>
<td>0.15 ± 0.05</td>
<td>0.10 ± 0.08</td>
</tr>
<tr>
<td>Red-headed Amazon river turtle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sternotherus odoratus</em></td>
<td>11</td>
<td>NY, USA</td>
<td>6.64 ± 2.10</td>
<td>n.d.</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>Common musk turtle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Terrapene carolina carolina</em></td>
<td>3527</td>
<td>2 US zoos</td>
<td>3.91 ± 6.42</td>
<td>n.d.</td>
<td>0.06 ± 0.06</td>
</tr>
<tr>
<td>Eastern box turtle</td>
<td></td>
<td>NY, USA</td>
<td>5.37 ± 3.34</td>
<td>0.23 ± 0.13</td>
<td>0.14 ± 0.07</td>
</tr>
<tr>
<td><em>Terrapene carolina triunguis</em></td>
<td>3</td>
<td>1 US zoo</td>
<td>2.01 ± 0.95</td>
<td>n.d.</td>
<td>0.07 ± 0.04</td>
</tr>
<tr>
<td>Three-toed box turtle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aAll data from Wildlife Conservation Society Nutrition Laboratory, summarized through Jan 99.
bNone detected.
<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Sampling Location(s)</th>
<th>α-tocopherol μg/ml</th>
<th>γ-tocopherol μg/ml</th>
<th>retinol μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Geochelone carbonaria</em></td>
<td></td>
<td>US zoo</td>
<td>0.68</td>
<td>n.d.</td>
<td>0.32</td>
</tr>
<tr>
<td>Red-footed tortoise</td>
<td></td>
<td>US zoo</td>
<td>0.47</td>
<td>n.d.</td>
<td>0.47</td>
</tr>
<tr>
<td><em>Geochelone chilensis</em></td>
<td></td>
<td>US zoo</td>
<td>2.36</td>
<td>n.d.</td>
<td>0.08</td>
</tr>
<tr>
<td>Chilean tortoise</td>
<td></td>
<td>Guatemalan zoo</td>
<td>1.54 ± 0.68</td>
<td>0.08 ± 0.02</td>
<td>0.23 ± 0.06</td>
</tr>
<tr>
<td><em>Geochelone denticulata</em></td>
<td></td>
<td>US zoo</td>
<td>1.54 ± 0.68</td>
<td>0.08 ± 0.02</td>
<td>0.23 ± 0.06</td>
</tr>
<tr>
<td>Yellow-footed tortoise</td>
<td></td>
<td>US zoo</td>
<td>2.20 ± 2.09</td>
<td>0.27 ± 0.02</td>
<td>0.33 ± 0.06</td>
</tr>
<tr>
<td><em>Geochelone elephantopus</em></td>
<td></td>
<td>US zoo</td>
<td>6.58 ± 6.99</td>
<td>0.71 ± 0.44</td>
<td>0.56 ± 0.66</td>
</tr>
<tr>
<td>Galapagos tortoise</td>
<td></td>
<td>US zoo</td>
<td>2.60 ± 1.38</td>
<td>0.39 ± 0.19</td>
<td>0.25 ± 0.12</td>
</tr>
<tr>
<td><em>Geochelone gigantea</em></td>
<td></td>
<td>US zoo</td>
<td>1.56 ± 1.04</td>
<td>0.13 ± 0.07</td>
<td>0.17 ± 0.08</td>
</tr>
<tr>
<td>Aldabra tortoise</td>
<td></td>
<td>US zoo</td>
<td>0.52 ± 0.12</td>
<td>n.d.</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td><em>Geochelone pardalis</em></td>
<td></td>
<td>US zoo</td>
<td>0.52 ± 0.12</td>
<td>n.d.</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>Leopard tortoise</td>
<td></td>
<td>US zoo</td>
<td>4.66 ± 2.62</td>
<td>0.13 ± 0.07</td>
<td>0.61 ± 0.16</td>
</tr>
<tr>
<td><em>Geochelone radiata</em></td>
<td></td>
<td>US zoo</td>
<td>2.34 ± 1.17</td>
<td>0.21 ± 0.06</td>
<td>0.34 ± 0.12</td>
</tr>
<tr>
<td>Radiated tortoise</td>
<td></td>
<td>Tanzania - 2 sites</td>
<td>4.66 ± 2.62</td>
<td>0.13 ± 0.07</td>
<td>0.61 ± 0.16</td>
</tr>
<tr>
<td><em>Indotestudo elongata</em></td>
<td></td>
<td>US zoo</td>
<td>2.34 ± 1.17</td>
<td>0.21 ± 0.06</td>
<td>0.34 ± 0.12</td>
</tr>
<tr>
<td>Elongated tortoise</td>
<td></td>
<td>Tanzania - 2 sites</td>
<td>4.66 ± 2.62</td>
<td>0.13 ± 0.07</td>
<td>0.61 ± 0.16</td>
</tr>
<tr>
<td><em>Malacochersus tornieri</em></td>
<td></td>
<td>US zoo</td>
<td>2.34 ± 1.17</td>
<td>0.21 ± 0.06</td>
<td>0.34 ± 0.12</td>
</tr>
<tr>
<td>Pancake tortoise</td>
<td></td>
<td>Tanzania - 2 sites</td>
<td>4.66 ± 2.62</td>
<td>0.13 ± 0.07</td>
<td>0.61 ± 0.16</td>
</tr>
<tr>
<td><em>Testudo graeca nikolskii</em></td>
<td></td>
<td>Russia - 4 sites</td>
<td>4.66 ± 2.62</td>
<td>0.13 ± 0.07</td>
<td>0.61 ± 0.16</td>
</tr>
<tr>
<td>Russian spur-thighed tortoise</td>
<td></td>
<td>Russia - 4 sites</td>
<td>4.66 ± 2.62</td>
<td>0.13 ± 0.07</td>
<td>0.61 ± 0.16</td>
</tr>
<tr>
<td><em>Testudo hermanni</em></td>
<td></td>
<td>US zoo</td>
<td>2.34 ± 1.17</td>
<td>0.21 ± 0.06</td>
<td>0.34 ± 0.12</td>
</tr>
</tbody>
</table>

\(^{a}\)All data from Wildlife Conservation Society Nutrition Laboratory, summarized through Jan 99.
\(^{b}\)None detected.
CAUSES OF MORTALITY OF THE PUERTO RICAN PARROT

Laurie A. Baeten, DVM and F. Joshua Dein, VMD, MS

USGS-BRD National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711 USA

Abstract

The Puerto Rican parrot (*Amazona vittata*) was one of the first species listed under the U.S. Endangered Species Act and remains one of its most critical members. For over 30 yr, efforts have focused on the species life history, habitat conservation (Snyder et al., 1987; Wilson et al., 1994) and wild bird nest management. Starting in 1975, wild parrot eggs and chicks were brought in from the wild to develop a captive population. Currently there are approximately 100 parrots held in captive breeding facilities at Luquillo and Rio Abajo aviaries, Puerto Rico, under the stewardship of the U. S. Fish & Wildlife Service and the Puerto Rico Department of Natural and Environmental Resources. While significant progress has been made in habitat conservation, reproductive success has been low and/or variable among both the captive and wild populations.

As part of an ongoing effort to evaluate the captive flock health and management, an historic review of parrot mortality was undertaken. A total of 89 Puerto Rican parrot mortalities were documented in the medical records between February 1976 and March 1999. The majority of the carcasses were submitted to the National Wildlife Health Center or Southeastern Cooperative Wildlife Disease Study for post mortem evaluation. These consisted of 26 adult parrots held in captivity and 63 chicks that were wild caught or captive born.

Of the 26 adult (greater than 1 yr of age) mortalities, a definitive cause of death was determined in 58% (15) of the cases. Death secondary to trauma was the most frequent diagnosis (26%, 7). Visceral gout was diagnosed in three cases (11%); one being a parrot less than 2 yr of age with gout secondary to a nephroblastoma. Two other tumor types were noted in adult mortalities; a thyroid carcinoma was diagnosed in a 13-yr-old female, and a thymoma was an apparent incidental finding in a 5-yr-old male. Thyroid dysplasia was noted as an incidental finding in two other cases. Other miscellaneous causes of death included cardiovascular disease (2), hemorrhage (2) and peritonitis (1).

A group of 10 adult parrots have undetermined causes of death with similar histopathologic lesions within the liver or liver/kidney. This group, presented from 1988 to 1997, will be reevaluated with the knowledge that has emerged in avian medicine in the past 10 yr. Further testing of tissues and histopathologic review are planned.

Fifty-nine chick mortalities were noted from 1981 thru the 1998 breeding season. Seventy-one percent (42) of these chicks died before 30 days of age. Twenty-seven percent (16) died before 5 days of age. Twenty-two percent (13) of these cases did not have post mortem evaluations available for review. Cause of death could not be determined in 12% (7) of the cases due to the condition of the carcass.
Pneumonia was the most frequent cause of death in parrot chicks (29%, 17). Aspergillosis was the cause of mortality in 10% (6) of the 59 cases. Malnutrition/starvation/neonatal death was found in 7% (4) of the cases. Trauma was noted as the cause of death in 7% (4) of the cases. Myiasis was found to contribute to the cause of death in two chicks obtained from the wild. Scoliosis was noted in two chicks; one captive reared, the other wild born. Septicemia or omphalitis was noted in two cases.

Four fledgling (4-10 mo of age) mortalities were evaluated. The cause of death for these four cases included: visceral gout, pneumonia with pericarditis, zinc toxicosis, and a myocardial rupture.

The primary objective in this investigation was to determine if there was a pattern in mortalities that could be identified as contributing to the poor population growth. Adult parrot losses have been minimal, with an average of only four losses per year. Only two of the etiologies have exhibited five or more mortalities. As the data indicates, the only disease pattern that predominates is the high prevalence of pneumonia in the chick population. As the majority of the chick mortalities and those with pneumonia appear to be in the same age bracket (less than 30 days of age), further evaluation of the contributing factors will be pursued. In addition, continued efforts in flock management will revolve around reproductive and nutritional evaluations.

LITERATURE CITED

IMMOBILIZATION OF SOUTHEAST ASIAN PRIMATES WITH MEDETOMIDINE, ZOLAZEPAM AND TILETAMINE, AND REVERSAL WITH ATIPAMEZOLE

Åsa Fahlman, DVM, Edwin J. Bosi, DVM, MPhil, and Görel Nyman, DVM, PhD

1Swedish University of Agricultural Sciences, Department of Large Animal Sciences, Box 7018, 750 07 Uppsala, Sweden; 2Sepilok Orangutan Rehabilitation Center, Wildlife Department Sabah, W.D.T. 200, 90009 Sandakan, Sabah, Malaysia

Abstract

The purpose of this study was to evaluate the combination of medetomidine (M), zolazepam and tiletamine (ZT) for immobilization of four species of Southeast Asian primates. Twenty-three immobilizations, including translocation of three free-ranging orangutans (Pongo pygmaeus pygmaeus), were performed at Sepilok Orangutan Rehabilitation Center and its surroundings in Sabah, Malaysia. Dosages of M 0.02-0.06 mg/kg and ZT 0.8-2.3 mg/kg i.m. produced a smooth induction without excitement and complete immobilization with good muscle relaxation within 1-7 min (x = 4 min) in all four species. Heart and respiratory rates, ear canal and rectal temperatures, and systolic blood pressure were recorded every 10 min. Pulse rate and percent oxygen saturation of hemoglobin (SpO2) trends were monitored continuously. Statistical analysis was performed for orangutans (n = 12). No significant change over time occurred in heart rate, respiratory rate or SpO2. Systolic blood pressure and body temperature decreased significantly during the immobilization. The only complication during all immobilizations occurred during translocation of one orangutan which developed hyperthermia and apnea, but it was successfully resuscitated. Atipamezole administered after 23-54 min (x = 37 min), at five times the dosage of M, reversed the immobilization in all species. First signs of recovery were observed 3-27 min (x = 10 min) after reversal. Medetomidine in combination with ZT and reversal with atipamezole was a safe and effective immobilization protocol for South-East Asian primates during clinical conditions at the dosages used in this study. As additional doses of ZT were required during all three translocations, further studies are needed to establish optimal dosage ranges of MZT for immobilization of free-ranging orangutans.

ACKNOWLEDGMENTS

We thank the personnel at Sepilok Orangutan Rehabilitation Center for their assistance during the immobilizations; and Dr. Bengt Röken for useful advice concerning medetomidine use in wild animals. We also thank the Swedish University of Agricultural Sciences; the Faculty of Veterinary Medicine; and Orion Animal Health for supporting this study.
**Dirofilaria immitis INFECTION IN A COLOBUS (Colobus guereza caudatus)**

*Kathy J. Topham, DVM, 1* Roberto F. Aguilar, DVM, 1 and Michael M. Garner, DVM, DACVP 2

1Audubon Zoo, 6500 Magazine Street, New Orleans, LA 70118 USA; 2Northwest Zoopath, 18210 Waverly Drive, Snohomish, WA 98296 USA

**Abstract**

In December, 1998 at Audubon Zoo, a 27-yr-old female colobus (*Colobus guereza caudatus*) was diagnosed with a spontaneous infection of *Dirofilaria immitis* at necropsy. The day of death it was found down in its exhibit, with profound dyspnea, and died while in transport to the hospital. Historically, it had no record of cardiopulmonary disease. Two months prior to its death, it had an episode of nasal discharge and rostral edema.

At necropsy, the animal had abundant subcutaneous edema in all tissues, particularly in dependent regions like perineum, limbs, hands, and feet. Abundant straw colored fluid was found in the abdomen, thorax and pericardium. The liver was rounded and heavy. The lungs were heavy and edematous as well. The heart was flaccid, thin walled and enlarged. Multiple, coiled nematodes were found within the right atrium and ventricle of the heart, the longest measuring 20 cm in length. Both male and female worms were mature. The female worm was gravid.

Identification as *Dirofilaria* was made based on the histologic features seen, as well as on the location they were found. The cuticle of the worms was thick, smooth, and bulged into the pseudocoelom in the region of the lateral chords forming lateral internal ridges. These features are consistent with that of a *Dirofilaria. D. immitis* is the only member of that species that has been found in the circulatory system of nonhuman primates (C. Gardiner, personal communication).

Naturally occurring infections with *D. immitis*, the canine heartworm, are a very rare occurrence in nonhuman primates, and in fact, its presence in humans is more common (C. Gardiner, personal communication). 5,6,8 Reports on *D. immitis* infection in orangutans, a rhesus monkey, and a gibbon have been published. 1,4 Microfilaremia has been found in pale-headed saki monkeys, which suggests a patent heartworm infection had occurred. 2 Experimental infections have been attempted in gibbons and macaques with some success. 4,7

Heartworm infections have occurred in humans in regions of the world where canine heartworm is endemic. 6,8 Infection with *D. immitis* most often results in an immature worm that does not survive in the heart, and embolizes to the lung. Once embedded in lung parenchyma, it creates a granuloma that can be seen radiographically. 3

The case seen at Audubon Zoo in a colobus indicates *D. immitis* can infect nonhuman primates and cause clinically relevant disease and death similar to that seen in its natural canine host.
LITERATURE CITED


TOXOPLASMOSIS IN Leontopithecus chrysomelas (KUHL, 1820) AND Saguinus imperator (GOELDI, 1907)

Sabrina Epiphanio, DVM, MSc,1* Marcelo A.B.V. Guimarães, DVM, PhD,2 Daniel L. Fedullo, DVM, MSc,2 Sandra H.R. Correa, DVM,2 and José L. Catão-Dias, DVM, PhD1*

1Departamento de Patologia. Faculdade de Medicina Veterinária e Zootecnia; Universidade de São Paulo. Av. Prof. Dr. Orlando Marques de Paiva, 87 – Cidade Universitária- CEP: 05508-000 – São Paulo – SP – Brazil; 2Fundação Parque Zoológico de São Paulo, Av. Miguel Stefano, São Paulo, SP, Brazil

Abstract

Four Leontopithecus chrysomelas (one male, three female golden-headed lion tamarins) and four Saguinus imperator (two male, two female emperor marmosets), belonging to the New World primate colony, Fundação Parque Zoológico de São Paulo, were studied. Table 1 lists the animals according to species, sex, age, origin and necropsy date. The monkeys died from 1991 to 1995. Six animals were found dead without previous clinical signs. A female L. chrysomelas showed extreme apathy, dyspnea and hemoptysis for 1 day prior to death. A male S. imperator presented moderate apathy and dyspnea for a few hours prior to death. All animals were necropsied and specimens from major organs were collected, fixed in 10% buffered formalin, embedded in paraffin, processed routinely for histology, sectioned at 4-6 µm, and stained with hematoxylin and eosin (H&E).

Immunohistochemistry assay for T. gondii was performed on all cases, using a polyclonal antibody to T. gondii (DAKO). The assays were achieved by the strepto-avidin-biotin-peroxidase method and the dilution used for the primary antibody was 1:10.000. All individuals exhibited good to regular nutritional conditions upon necropsy and no pathogenic bacteria or fungi were isolated from any case. All animals had pulmonary changes characterized by moderate to severe congestion, edema, hemorrhage and acute to subacute interstitial pneumonia. Other significant lesions reported were discrete to moderate acute necrotizing hepatitis, severe fibrin-hemorrhagic lymphadenitis affecting mainly the mesenteric lymph nodes, necrotizing splenitis and multifocal ulcerative enteritis. In all cases oval to crescent-shaped, 1-6 µm structures compatible with T. gondii were reported. The immunohistochemistry assays confirmed the agent to be T. gondii in all eight cases. The primates of the New World are highly susceptible to toxoplasmosis, rarely surviving after illness. The reason for such phenomenon is unknown. Of eight cases of toxoplasmosis observed in the present report, seven happened without previous clinical signs and the deaths occurred at the same day the animals were seen at hospital. Such a situation is in accordance with previous reports which refer to the fast course of this infection in neotropic primates. The means by which the monkeys were infected remains obscure. Due to age (adult) of seven individuals, the possibility of transplacental infection was rejected for those animals. On a similar basis, all meat supplied to the animals was previously frozen and cooked. Therefore, we believe that the infection was not acquired through ingestion of host tissue cysts. Yet another aspect requiring special attention is the control of access of feral cats to the zoo grounds. In spite of not having been reported, the presence of those animals during the outbreak is a possibility that cannot be completely rejected.
ACKNOWLEDGMENTS

This research was supported by FAPESP, grants Nos. 95/3621-6 and 97/13970-3.

LITERATURE CITED


Table 1. Distribution of the affected animals according to species, sex, age, origin, period on captivity and necropsy date. 1991 to 1994. São Paulo, SP. Brazil.

<table>
<thead>
<tr>
<th>Case</th>
<th>Species</th>
<th>Age</th>
<th>Sex</th>
<th>Origin</th>
<th>Captivity</th>
<th>Necropsy date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>L. chrysomelas</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Unknown</td>
<td>Female</td>
<td>Confiscated</td>
<td>1 yr 2 mo</td>
<td>15/7/91</td>
</tr>
<tr>
<td>2</td>
<td><em>L. chrysomelas</em></td>
<td>Adult</td>
<td>Female</td>
<td>Born at the zoo</td>
<td>1 yr 11 mo</td>
<td>6/12/91</td>
</tr>
<tr>
<td>3</td>
<td><em>L. chrysomelas</em></td>
<td>Adult</td>
<td>Male</td>
<td>Donation</td>
<td>6 mo</td>
<td>29/3/95</td>
</tr>
<tr>
<td>4</td>
<td><em>L. chrysomelas</em></td>
<td>Adult</td>
<td>Female</td>
<td>Donation</td>
<td>6 mo</td>
<td>30/3/95</td>
</tr>
<tr>
<td>5</td>
<td><em>S. imperator</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Adult</td>
<td>Female</td>
<td>Born at the zoo</td>
<td>1 yr 5 mo</td>
<td>16/3/94</td>
</tr>
<tr>
<td>6</td>
<td><em>S. imperator</em></td>
<td>Adult</td>
<td>Male</td>
<td>Born at the zoo</td>
<td>4 yr 2 mo</td>
<td>16/3/94</td>
</tr>
<tr>
<td>7</td>
<td><em>S. imperator</em></td>
<td>Adult</td>
<td>Male</td>
<td>Exchanges</td>
<td>9 yr 1 mo</td>
<td>23/3/94</td>
</tr>
<tr>
<td>8</td>
<td><em>S. imperator</em></td>
<td>Adult</td>
<td>Female</td>
<td>Exchanges</td>
<td>11 yr 9 mo</td>
<td>23/4/94</td>
</tr>
</tbody>
</table>

<sup>a</sup>Golden-headed lion tamarin
<sup>b</sup>Emperor marmoset
ORAL FOCAL EPITHELIAL HYPERPLASIA IN A HOWLER MONKEY (Alouatta fusca)

Lílian R. M. Sá, DVM,1* Celso DiLoreto, DM,2 Mário C. Leite, DVM,1 A Wakamatsu,2 R. T. M. Santos,2 and José L. Catão-Dias, DVM, PhD1

1Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo. Av. Prof. Orlando Marques de Paiva, 87 – Cid. Universitária. CEP: 05508-000. São Paulo, SP, Brazil; 2Divisão de Patologia, Instituto Adolfo Lutz; Av. Dr. Arnaldo. São Paulo, SP, Brazil

Abstract

An adult male howler monkey was kept in captivity for approximately 6 mo for rehabilitation purposes. Multiple physical exam and laboratory tests were performed during this period, and no signs compatible with focal epithelial hyperplasia (FEH) were observed. About 10 days after the reintroduction, the animal was found on the ground, in agony. The gross exam revealed dog bites in multiple sites, associated with extensive hemorrhages. In the oral cavity, the lower lip mucosa and the antero-lateral-medial aspects of the tongue, showed multiple, 2-5 mm in diameter, soft, whitish, circumscribed mucosal raised plaques, either isolated or coalescent. Other findings were pulmonary edema, presence of Enterobius sp. and focal ulcerative colitis. Samples of the lower lip and all organs were fixed in 10% buffered formalin and embedded in paraffin, sectioned at 4-6 μm and stained with hematoxylin and eosin (H&E). Immunohistochemistry was performed for the detection of the viral capsid antigen (code no. B580, diluted 1:8,000, Dako) and for the detection of HPV antigens (clone 4C4/F10/H7/83, code NCL-HPV-4C4, diluted 1:40, Novocastra). In situ hybridization with a wide spectrum biotinylated probe of HPV types 6, 11, 16, 18, 30, 31, 33, 35, 45, 51 and 52 (code K0012, Dako) was performed on the formalin-fixed, paraffin-embedded tissue section. Major histopathologic findings of the lower lip included moderate acanthosis, with elongation and fusion of the epithelial cones, and koilocytosis. The immunohistochemistry test of the generic papillomavirus antigen (PV) showed strong nuclear positivity in the koilocytes. On the other hand, the specific reactions for detection of HPV 6, HPV 11 and HPV 18 were negative. There was no cross-hybridization with the HPV probe. The gross and microscopic characteristics found in this howler monkey are compatible to those previously described in humans and chimpanzees with FEH. It was shown by immunohistochemistry that the agent involved in this case is a PV with positive reactions to the viral capsid antigens (L1) only in the cells with koilocytosis on the upper stratum spinosum, which indicates a productive infection by PV. The negative result to in situ hybridization for the HPV wide spectrum DNA probe is very interesting and eliminates the possibility of the PV of this howler monkey being related to any of these HPV types. It is emphasized that this PV is probably not related either to HPV 13 or 32, as they have a partial homology with types 6, 11, 16 and 18. These data suggest that the PV identified in this case is a mucosotropic virus specific of the howler monkey oral cavity. Further studies should be carried out so as to provide a better understanding and to characterize the epidemiology of FEH cases in neotropic primates.

ACKNOWLEDGMENT

This research was supported by FAPESP, grants Nos. 95/3621-6 and 97/4815-4.

1999 PROCEEDINGS AMERICAN ASSOCIATION OF ZOO VETERINARIANS
LITERATURE CITED

ENTEROPATHY WITH EPITHELIAL INTRACYTOPLASMIC MICROVILLUS INCLUSIONS IN A GOELDI’S MONKEY (Callimico goeldii)

Laura S. Zwick, DVM,1* Michael T. Dutcher, DVM,1 Sherri L. Yong, MD,2 Robert D. Murnane, DVM, PhD,1 and Michael J. Kinsel, DVM1

1Zoological Pathology Program, University of Illinois College of Veterinary Medicine, Loyola University Medical Center, Suite 400, Building 120, 2160 South First Avenue, Maywood, IL 60153 USA; 2Department of Pathology, Loyola University Medical Center, Building 103, 2160 South First Avenue, Maywood, IL 60153 USA

Abstract

A captive, 701-g, 9-yr-old, spayed female, Goeldi’s monkey (Callimico goeldii) with a history of intermittent diarrhea beginning at 1 yr of age, and a 3-mo history of progressive weight loss, uremia, panleukopenia, anemia, and declining clinical condition was euthanatized and submitted for necropsy in good postmortem condition and fair nutritional status. Gross examination revealed bilateral chronic nephritis, multiple hepatic masses, and moderate diffuse glossitis.

Histologic examination revealed marked, multifocal villus atrophy with frequent crypt abscesses and crypt loss within the small intestine. Crypts within affected areas were often moderately to markedly ectatic with hyperplastic or dysplastic epithelium. The lamina propria had a moderate diffuse increase in the number of lymphocytes and plasma cells. Small numbers of epithelial cells within the apical mucosa, and rarely crypts, contained single, round, 10-30μm diameter, intracytoplasmic inclusions which centrally contained basophilic material. Periodic acid-Schiff stains highlighted the intracytoplasmic inclusions, which exhibited a targetoid appearance. The colon also had rare intraepithelial vacuolated inclusions, as well as marked, multifocal crypt abscesses and crypt loss. Small and large intestinal mucosa also contained few variably-sized erosions with superficial accumulations of yeast organisms and pseudohyphae consistent with Candida sp.

Additional histologic lesions in this individual included marked, diffuse, membranoproliferative glomerulonephritis with moderate, multifocal chronic interstitial nephritis, marked, diffuse myeloid dysplasia and erythroid hypoplasia, multiple hepatic myelolipomas, moderate, diffuse glossal candidiasis, mild, multifocal myocardial fibrosis, and moderate, multifocal, thyroid cystic hyperplasia.

An area of the affected jejunum was evaluated by transmission electron microscopy, and surface epithelial cells occasionally exhibited shortened and disorganized microvilli. In other areas the integrity of the brush border was maintained. The most striking ultrastructural feature was the presence of large, intraepithelial, membrane bound, vacuolated inclusions lined by either complete microvillus brush borders or intermittent disorganized tufts of microvilli.

Progressive weight loss and the progressive declining clinical condition in this animal were attributed to severe renal disease with likely some contribution from malabsorption due to enteric lesions. Chronic
intermittent diarrhea may have been secondary to malabsorption, although this individual and other members of the colony also had several documented episodes of intestinal campylobacteriosis. Enteric and glossal candidiasis were consequences of debilitation subsequent to multiple disease processes.

Enteric changes observed in this monkey were similar to changes described in a severe, generalized enteropathy in human infants known as microvillus inclusion disease (MID), which causes intractable diarrhea, usually culminating in death. Familial cases occur, with a pattern consistent with autosomal recessive inheritance. The underlying etiology of MID has been localized to the cytoskeleton of the enterocyte. The constellation of biochemical and ultrastructural findings have led some to propose a defect of binding between actin and myosin in the terminal web as the cause. Others have postulated heterotopic formation of brush border proteins within the cell cytoplasm, rather than at the cell surface.

Histopathologic hallmarks of MID are diffuse hypoplastic to normoplastic villus atrophy with normal to decreased numbers of lamina propria inflammatory cells. Enterocytes lack the normal linear brush border staining pattern with PAS, and often aggregates of PAS positive material are noted in the apical cytoplasm. Ultrastructurally, epithelial cells often lack brush borders, or possess markedly shortened and disorganized microvilli. The apical cytoplasm frequently contains large membrane bound vacuoles lined by microvilli, and smaller vesicular bodies containing small membrane fragments and sparsely lined by microvilli. Abundant secretory granules are also typically observed in the apical cytoplasm. Similar ultrastructural abnormalities are variably observed in mucosal epithelial cells of the colon, rectum, pyloric antrum, renal tubules, and gallbladder in patients with MID. Similar microvillus inclusions have been observed in animals or cell cultures treated with microtubule or microfilament inhibitors such as colchicine, vinblastine, and cytochalasin, and have been described in a human metastatic intestinal adenocarcinoma, and are sometimes observed in cultured Caco-2 cells, a human colonic carcinoma cell line.

The histologic and ultrastructural appearance of enteric epithelial inclusions noted in this monkey were similar to microvillus inclusions and vesicular bodies of MID. Marked enteric villus atrophy was also similar, though in this case crypts were hyperplastic, not hypoplastic as in MID. Additionally, the moderate increase in lamina propria inflammatory cells was unlike MID, where normal or decreased inflammation is typical. Possibly lymphoplasmacytic inflammation in this case was a sequela of prior campylobacteriosis. The early age of onset and intractable diarrhea seen in MID were not observed, although intermittent diarrhea was noted clinically. The enteropathy in this monkey may have developed at a later age, or possibly was less severe. This monkey was not treated with any microtubule or microfilament inhibiting drugs, and the underlying etiology in this case remains unknown, but may be similar to MID. This is the first reported case of an enteropathy with epithelial microvillus inclusions in a nonhuman primate.

ACKNOWLEDGMENTS
The authors acknowledge the excellent technical assistance of Jane Chladny and the Histopathology Laboratory, Laboratories of Veterinary Diagnostic Medicine, and the Ultrastructural Imaging Laboratory, Department of Veterinary Biosciences of the University of Illinois College of Veterinary Medicine.

LITERATURE CITED

ANIOCENTRIC CEREBRAL SARCOMA IN A COMMON MARMOSET (Callithrix jacchus)

Carles Juan-Sallés, DVM,1* Daniel Borràs, DVM,2 Xavier Valls, DVM,1 Michael M. Garner, DVM, Dipl ACVP,3 Alberto Marco, DVM, PhD, Dipl ECVP,2 Javier Vergés, DVM,1 and José A. Ramos-Vara, DVM, PhD, Dipl ECVP4

1Clínica Exòtics, c/Balmes 454, E-08022 Barcelona, Spain; 2U.D. Histologia i Anatomia Patològica, Facultat de Veterinària (UAB), E-08193 Bellaterra, Barcelona, Spain; 3Northwest ZooPath, 18210 Waverly Drive, Snohomish, WA 98296-4815 USA; 4Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia, College of Veterinary Medicine, PO Box 6023, Columbia, MO 65205 USA

Abstract

The animal of this report was a 466-g, 6.5-yr-old female pet common marmoset (Callithrix jacchus) with a diet consisting of a commercial cereal mix, fruits, calcium supplements, and, occasionally, meat and fish. This marmoset was presented at the Clínica Exòtics (Barcelona, Spain) with a 4-day history of weakness, anorexia, occasional vomiting, ptyalism and sialorrhea, and a previous unremarkable clinical history.

Upon clinical examination, some of the very striking findings were the unexpected, marked docility of this animal, that did not react to any of the clinical procedures (e.g., auscultation, positioning for radiographs, or taking of the rectal temperature), abdominal distension, enlarged kidneys and venous stasis, especially visible in both hindlimbs. Rectal temperature (37ºC) was normal. Dorsoventral and lateral radiographs demonstrated the abdominal distension associated with a diffuse moderate radiodensity that did not allow to identify abdominal organs. There was cranial displacement of the diaphragm, at least of two intercostal spaces; the lung fields and cranial abdominal structures overlapped markedly as a result of such displacement. Besides, the diaphragm was more rounded than normal. These radiographic findings suggested hepatomegaly and/or ascites. The animal died a few hours after presentation with convulsions, seizures and generalized tremors.

At necropsy, fat stores were almost completely depleted. The liver, spleen and, to a lesser extent, the kidneys and adrenal glands were markedly enlarged and pale. Most abdominal and hindlimb veins were prominently engorged, and there was generalized lymphadenopathy, especially of mesenteric lymph nodes. The brain had a reddish mass occupying most of the caudal half of the left cerebral hemisphere. The heart was apparently enlarged, with a moderately enlarged right atrium and thickened left ventricular wall. There were no gastric contents. A complete set of tissues was fixed in 10% buffered formalin and routinely processed for histopathology and stained with hematoxylin and eosin. Special stains on selected tissues included periodic acid Schiff (PAS), Ziehl-Neelsen (ZN), Masson’s trichrome, Congo red, and chloroacetate esterase. An immunohistochemical panel including keratin, vimentin, lymphocyte common antigen (LCA), MAC 387 (histiocytes), HMB-45 (melanocytes), glial fibrillar acid protein (GFAP), and CD31 (endothelial cells) was done on the brain tumor.
Microscopic examination of the brain mass revealed an angiocentric proliferation of a relatively homogeneous cell population characterized by large nuclei and moderately abundant, slightly basophilic cytoplasm; intravascular invasion or growth within the tumor was a prominent finding, occasionally associated with thrombosis, necrosis and hemorrhage. The tumor was poorly delimited and, although it mostly grew in the white matter, there were focal perivascular proliferations in the grey matter as well. The results of the immunohistochemical panel were as follows: keratin negative, vimentin positive, and MAC 387-, LCA-, HMB45-, CD31- and GFAP-negative. The tumor did not have myeloperoxidase activity. Histopathology also revealed important lesions in multiple organs, especially the kidneys and liver. Some of the renal lesions (chronic diffuse mesangiproliferative glomerulonephritis with interstitial fibrosis) resembled those reported in mesangial nephropathy of callitrichids, but were associated with abundant hyaline and cellular casts; multinucleate giant cells within tubules and phagocytosis of hyaline casts were prominent. A striking finding in the kidneys and liver (and less prominent in adrenal glands, adipose tissue and gastrointestinal tract) was the presence of marked infiltrates predominantly of mature myeloid cells (relatively homogeneous in morphology, with indented or polylobulated nuclei, and eosinophilic cytoplasm). The liver also had diffuse hydropic degeneration, and perivascular fibrosis (especially of sublobular veins). There was a myelolipoma in one of the adrenal glands. Other findings were: neuronal lipofuscinosis of sympathetic ganglia, mild granulomatous pansteatitis with ceroid, decreased erythroid:myeloid ratio in the bone marrow (that was hypercellular), hyperplasia of splenic red pulp, excessive erythrophagocytosis, and generalized lymphoid hyperplasia.

The results of the immunohistochemistry panel and stain for myeloperoxidase activity on the brain tumor are consistent with a diagnosis of undifferentiated angiocentric cerebral sarcoma. The absence of neoplastic growths in other tissues despite extensive histologic studies suggests that this tumor is a primary cerebral neoplasia. Neoplasia of the central nervous system has not been previously reported in New World primates and occurs sporadically in Old World species.²⁴⁸

The disseminated myeloid infiltrates were considered to correspond to extramedullary hematopoiesis (EMH). EMH with predominating myeloid cells and leukemoid reaction occur chiefly as a result of severe, localized pyogenic infections (e.g., pyometra in the bitch); despite extensive histologic studies no evidence of such infections was found in this animal. Prominent EMH is a common microscopic finding in callitrichids in captivity (Dr. B. Rideout, personal communication). Adrenal and hepatic myelolipomas are not uncommon lesions in callitrichids and Goeldi’s monkeys.³⁵⁷ Most renal lesions were considered to fit well in the overall picture of mesangial nephropathy commonly found in callitrichids,¹ except for the extensive hyaline and cellular cast formation. Pansteatitis with ceroid was an incipient lesion in this animal that may have been the result of an inadequate diet (deficient in vitamin E as for its contents in polyunsaturated fatty acids) and/or increased production of peroxides in severely damaged tissues such as the liver and kidneys.

ACKNOWLEDGMENTS

The authors thank Hospital Sant Jaume de Calella for assistance with immunohistochemistry, and Blanca Pérez and Pere Losada for histotechnology.
LITERATURE CITED


TOXOPLASMOSIS AND NEONATAL MORTALITY IN PALLAS’ CATS: A SURVEY OF NORTH AMERICAN ZOOLOGICAL INSTITUTIONS

William F. Swanson, DVM, PhD

Center for Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, Cincinnati, OH 45220 USA

Abstract

During 1995 and 1996, a total of 16 (eight male and eight female) wild-caught Pallas’ cats (Otocolobus manul) were imported into the U.S. and Canada from Russia, providing a rare opportunity to re-establish a small felid population using known founder animals. Captive propagation of these wild-caught cats remains a high priority. However, anecdotal reports from several zoological institutions suggested the occurrence of excessive neonatal deaths in the offspring of these paired Pallas’ cats, presumably caused by infection with Toxoplasma gondii. In February 1999, a detailed questionnaire was sent to the five North American institutions (Birmingham Zoo, Cincinnati Zoo & Botanical Garden, Denver Zoological Gardens, Mountain View Farms Conservation & Breeding Center, Oklahoma City Zoological Park) that currently held breeding pairs of Pallas’ cats to investigate these reports. All five institutions responded within 2 mo of survey distribution and the survey findings were compiled.

Between 1996 and 1998, these five institutions reported the birth of nine Pallas’ cat litters, comprising a total of 32 kittens (Table 1). Of these kittens, 23 (72%) were either stillborn or died within the first 16 wk after birth. Of the stillbirths or neonatal deaths, 16 (70%) kittens were subjected to necropsy, six (26%) were eaten by the dam and one (4%) was not evaluated (Table 2). For the 16 necropsied kittens, 13 (81%) died due to toxoplasmosis, 1 (6%) due to sepsis, 1 (6%) due to disseminated intravascular coagulation and 1 (6%) to unknown causes. Kitten deaths attributed to confirmed toxoplasmosis were biphasic, with many deaths (38%) occurring at or near birth (one stillbirth, four neonatal deaths within 23 days of birth) and the remaining deaths (62%) occurring after 60 days (eight neonatal deaths between 62 and 112 days of birth).

Antibody titers against T. gondii were evaluated in 14 of the original wild-caught Pallas’ cats shortly after their importation into North America (Table 3). Of these cats, 11 (79%) tested positive for antibodies against T. gondii within 6 wk of arrival from Russia. For some cats (pairings 2, 3, 6 and 7), an IgG-specific ELISA was used for testing, demonstrating high IgG titers (≥ 1:512 dilution) in seven of eight cats within 3 wk of their arrival from Russia. Four of five institutions also reported the presence of anti-T. gondii antibodies in other cat species in their collection. However, none have observed any cases of confirmed toxoplasmosis as causes of stillbirths or neonatal death in any of these other species. At all five institutions, Pallas’ cats have access to dietary items potentially containing T. gondii tissue cysts. These potential dietary sources include whole prey items, such as baby/adult mice, chicks, and quail (five institutions), commercially-available raw horse meat (four institutions), wild rodents and/or birds in outdoor exhibits (four institutions) and raw organ meat from chickens or cattle (one institution).
At four institutions, newborn kittens were treated with clindamycin (25-50 mg/kg BW, s.i.d. or b.i.d., p.o.) for suspected toxoplasmosis (Table 4). However, clindamycin treatment appeared to be ineffective in kittens showing any symptoms of toxoplasmosis before the initiation of therapy. All surviving kittens were generally asymptomatic for toxoplasmosis and were treated with clindamycin based on deaths or symptoms observed in siblings. It is possible that these surviving kittens never had active *T. gondii* infections.

The findings from this survey indicate that Pallas’ cats are experiencing very high losses of newborn kittens due to toxoplasmosis, with two peaks in deaths: at or near birth (i.e., within 24 days) and near weaning (i.e., between 60 and 120 days of age). The early deaths are most likely due to in utero infection of fetuses with *T. gondii*. Most wild-caught Pallas’ cats have persistent antibody titers against *T. gondii*, reflecting previous exposure either in the wild or in captivity in Russia, and presumably indicating encysted *T. gondii* within their tissues. In contrast to domestic cats and all other nondomestic cats, maternal immune responses apparently are not protective during pregnancy in Pallas’ cats, allowing cyst reactivation and in utero infection to occur. Chronic captive stress in these wild-caught cats also may be a factor.

The later deaths (near weaning) may reflect delayed proliferation of *T. gondii* acquired in utero from the dam or newly-acquired infection from tissue cysts ingested in the diet, both potentiated by decline of anti-*T. gondii* colostral antibodies and reduced access to any milk-derived antibodies. It is difficult to eliminate all potential exposure to *T. gondii*, especially if cats are maintained outdoors on relatively natural diets (raw meat, whole prey). Treatment of Pallas’ cat kittens with clindamycin after observation of clinical signs has a very poor prognosis.

At this time, options are limited for addressing in utero transmission and early deaths caused by toxoplasmosis. Hand-raising healthy kittens (either from shortly after birth or beginning at approximately 6-8 wk of age) in a tightly controlled environment (no access to wild prey, *T. gondii*-free whole prey, cooked meat and/or processed diet) and/or prophylactic treatment with clindamycin beginning approximately 8-12 wk of age are possible alternatives for reducing *T. gondii*-related mortality at later time points. However, diarrhea can be a problem with clindamycin treatment and hydration must be monitored closely. Maturation of the kitten’s immune system after 4 mo of age hopefully will provide improved immunologic responsiveness to subsequent *T. gondii* exposure (as in domestic cat kittens). Studies are being initiated to investigate immune responses of Pallas’ cats against *T. gondii* to gain a better understanding of the unusual etiology of toxoplasmosis in this species.

**ACKNOWLEDGMENTS**

The assistance of the following individuals and institutions with this survey are very much appreciated: Dr. Mel Shaw, Birmingham Zoo; Dr. Mark Campbell, Cincinnati Zoo & Botanical Garden; Dr. David Kenny, Denver Zoological Gardens; Mr. Gordon Blankstein, Mountain View Farms Conservation & Breeding Center; and Dr. Michael Barrie, Oklahoma City Zoological Park.

<table>
<thead>
<tr>
<th>Year</th>
<th>Births (litters)</th>
<th>Deaths (SB/NNa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>3 (1)</td>
<td>2 (1/1) in 1 litter</td>
</tr>
<tr>
<td>1997</td>
<td>4 (1)</td>
<td>3 (0/3) in 1 litter</td>
</tr>
<tr>
<td>1998</td>
<td>25 (7)</td>
<td>18 (4/14) in 7 litters</td>
</tr>
</tbody>
</table>

by litter:
- 3 (1) 2 (2/0)
- 2 (1) 1 (0/1)
- 5 (1) 3 (0/3)
- 3 (1) 3 (0/3)
- 4 (1) 4 (2/2)
- 4 (1) 4 (0/4)
- 4 (1) 1 (0/1)

Total 32 (9) 23 (5/18) in 9 litters

aSB = stillbirth; NN = neonatal (birth to 16 wk of age)


<table>
<thead>
<tr>
<th>Year</th>
<th>Deaths (SB/NNa)</th>
<th>Toxoplasmosis</th>
<th>Other</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>2 (1/1)</td>
<td>1 (NN)</td>
<td>0</td>
<td>1 (SB)</td>
</tr>
<tr>
<td>1997</td>
<td>3 (0/3)</td>
<td>2 (NN)</td>
<td>0</td>
<td>1 (NN)</td>
</tr>
<tr>
<td>1998</td>
<td>18 (4/14)</td>
<td>10 (1 SB/9 NN)</td>
<td>2 (NN)</td>
<td>6 (3 SB/3 NN)</td>
</tr>
</tbody>
</table>

by litter:
- 2 (2/0) 0 0 2 (SB)
- 1 (0/1) 0 0 1 (NN)
- 3 (0/3) 0 1 (NN) 2 (NN)
- 3 (0/3) 3 (NN) 0 0
- 4 (2/2) 3 (1 SB/2 NN) 0 1 (SB)
- 4 (0/4) 4 (NN) 0 0
- 1 (0/1) 0 1 (NN) 0

Total 23 (5/18) 13 (1/12) 2 (0/2) 8 (4/4)

aSB = stillbirth; NN = neonatal (birth to 16 wk of age)
Table 3. Anti-\textit{Toxoplasma} antibody status of wild-caught Pallas’ cats.

<table>
<thead>
<tr>
<th>Male/Female Pair</th>
<th>Duration (days) in NA Zoo(^a)</th>
<th>Antibody Status (+/-)</th>
<th>Proven Pair</th>
<th>Confirmed Deaths d/t Toxoplasmosis (y/n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>M+/F+</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>M+/F+</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>M+/F+</td>
<td>Yes</td>
<td>No (but some eaten)</td>
</tr>
<tr>
<td>4</td>
<td>N/A</td>
<td>Not tested</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>M-/F+</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>M+/F+</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>7</td>
<td>36381</td>
<td>M-/F+</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>36381</td>
<td>M+/F-</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

\(^a\) Period of time between arrival of Pallas’ cats at the North American institution (from Russia) and collection of blood samples for anti-\textit{T. gondii} antibody testing.

Table 4. Treatment of \textit{T. gondii}-infected kittens.

<table>
<thead>
<tr>
<th>Litter No.</th>
<th>Initial Observation</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 stillborn kitten (toxoplasmosis suspected initially)</td>
<td>1 kitten treated with clindamycin (50 mg/kg) s.i.d., p.o. for 21 days beginning the day after birth</td>
<td>survived</td>
</tr>
<tr>
<td>2</td>
<td>1 kitten died ~80 days d/t toxoplasmosis</td>
<td>1 kitten treated with clindamycin (25 mg/kg) b.i.d., p.o. for 14 days beginning at 80 days of age</td>
<td>survived</td>
</tr>
<tr>
<td>3</td>
<td>toxoplasmosis in previous litter (#2 above)</td>
<td>3 kittens treated prophylactically with clindamycin (25 mg/kg) b.i.d., p.o. for 14 days beginning at 5-6 wk of age</td>
<td>all died at ~12 wk of age d/t \textit{T. gondii}</td>
</tr>
<tr>
<td>4</td>
<td>1 kitten died ~62 days d/t toxoplasmosis</td>
<td>2 kittens (1 w/ symptoms) treated with clindamycin (25 mg/ml) b.i.d., p.o. beginning at ~62 days of age</td>
<td>1 kitten lived, 1 kitten (w/ symptoms) died at ~63 days</td>
</tr>
<tr>
<td>5</td>
<td>2 kittens died ~6-19 d/t toxoplasmosis, 1 kitten died ~108 days d/t toxoplasmosis</td>
<td>1 kitten (w/symptoms) treated with clindamycin (25-37.5 mg/kg) s.i.d., p.o. beginning at 108 days of age</td>
<td>died 4 days after starting treatment</td>
</tr>
</tbody>
</table>
PRELIMINARY EXPERIMENTAL CANARYPOX VECTORED RECOMBINANT CANINE DISTEMPER VACCINE EVALUATION IN THE SIBERIAN POLECAT (*Mustela eversmanni*)

Jeffrey Wimsatt, DVM, PhD, 1* Dean Biggins, MS, 2 Bobbi Taylor, 1 Kim Innes, PhD, MSPH, 3 and Della Garell, DVM 4

1Department of Clinical Sciences, College of Veterinary and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523 USA; 2Biological Resources Division, Midcontinent Ecological Science Center, USGS, Ft. Collins, CO 80525 USA; 3Department of Preventative Medicine and Biometrics, University of Colorado Health Sciences Center, Denver, CO 80262 USA; 4Cheyenne Mountain Zoo, Colorado Springs, CO 80906 USA

Abstract

Mustelids and (primarily) other members of the Carnivora are highly susceptible to canine distemper virus (CDV) infection, a ubiquitous disease affecting susceptible captive and wild populations throughout the world. The endangered black-footed ferret (BFF, *Mustela nigripes*) is exquisitely sensitive to canine distemper, and a safe and effective vaccination strategy would be central to BFF reintroduction, and to protect other species at risk of contracting CDV-induced disease. An experimental canarypox recombinant subunit vaccine for eventual application in BFF and other susceptible species was evaluated in a captive-raised group of Siberian polecats (SP; *M. eversmanni*), believed to be the closest living relative to the BFF.

Study Goals

The purpose of the present study was to test the efficacy of an experimental canarypox vectored recombinant CDV subunit vaccine (ReCDV) for oral and parenteral administration and to determine its safety and efficacy via these routes.

Methods

SP with defined pedigrees were studied; their progenitors were collected from Inner Mongolia. ReCDV was administered at three (104.5, 105.0, 105.5) dose levels subcutaneously over the back, or by spraying (105.5, 108) into the oropharynx (orally). In all cases, two doses of reconstituted (1 ml) vaccine were delivered 4 wk apart, followed by live virus challenge 3 wk after the second vaccination. Based on preliminary data, the challenge consisted of Synder Hill strain CDV NVSL (#18-90) injected intraperitoneally at a dose four times the concentration shown to induce 100% mortality in SP previously. Clinical signs were observed daily and weights were obtained weekly, then biweekly during the challenge period.
Results

ReCDV at the highest oral dose induced a 83.3 % (5/6) survival rate following live challenge, as compared to 0% (0/6) in the controls.

Conclusions

This study indicates the potential efficacy of oral vaccine delivery. Results presented here suggest that further studies are needed to evaluate the safety and efficacy of vectored recombinant vaccines in highly susceptible species, and especially in those species where CDV modified-live vaccination has precipitated disease. The potential to use such vaccines in the field in appropriate baits must be further explored.
SUSCEPTIBILITY OF THE SIBERIAN POLECAT (M. eversmanni dauricus) TO SUBCUTANEOUS AND ORAL PLAGUE (Yersinia pestis) EXPOSURE

Kevin T. Castle, BS, MS1* Dean Biggins, MS,1 Kim Innes, PhD, MSPH,2 Leon G. Carter,3 May Chu, PhD,3 and Jeffrey Wimsatt, DVM, PhD4

1USGS, Midcontinental Ecological Science Center, 4512 McMurray Blvd., Ft. Collins, CO 80525 USA; 2Department of Biometrics and Preventive Medicine, University of Colorado Health Sciences Center, E. 9th Ave. Denver, CO 80262 USA; 3Plague Branch, Centers for Disease Control and Prevention, Ft. Collins CO 80521 USA; 4Department of Clinical Sciences, Colorado State University, Fort Collins, CO 80523 USA

Abstract

Plague (Yersinia pestis) causes periodic and dramatic die-offs of prairie dogs. Plague may thus adversely affect black-footed ferret (Mustela nigripes) reintroduction efforts both by reducing ferret prey populations and by killing ferrets, a species also susceptible to plague. The development of a standard model for plaque pathogenesis using a well-characterized virulent Y. pestis strain is essential for vaccine testing. The Siberian polecat (M. eversmanni) is the closest living relative of the black-footed ferret and is indigenous to the old world, where plague originated and was endemic. To determine if this species would offer a suitable model for plaque pathogenesis and prevention in the black-footed ferret, we assessed plague susceptibility and pathogenesis in pure defined-strain Siberian polecats (M. eversmanni dauricus). We exposed 33 individually housed polecats to $10^3$ ($n = 7$), $10^7$ ($n = 7$), or $10^{10}$ ($n = 7$) Y. pestis organisms by s.c. injection, or by feeding an intact, freshly plague-killed mouse ($n = 12$). An additional group of seven unexposed animals given otherwise identical care and housing served as controls. Plague exposure led to an 88% mortality overall in polecats (71% mortality in the $10^3$ group, 100% mortality in the $10^7$ and $10^{10}$ groups, and 83% mortality in the mouse-fed group). None of the controls died or showed signs of illness during the 21-day trial period. Within the challenged group, mean survival time post-challenge ranged from 3.6-12.6 days. Animals that received the lowest parenteral dose survived significantly longer than those receiving higher parenteral doses. The survival time of polecats ingesting plague-killed mice was intermediate between that of low and that of high parenteral dose groups. Age, gender, pedigree, and baseline weight were not significantly related to survival status. Within the challenged group, mean survival time was lower in animals presenting with significant weight loss by day 3, lethargy, and low fecal output; onset of lethargy and other signs was also inversely related to risk of dying and/or plague dose. The results of this study confirm that the Siberian polecat is susceptible to plague and suggest that this species will offer an appropriate surrogate for black-footed ferrets in future plaque studies and related vaccine trials.
REPRODUCTIVE FUNCTION TESTING IN MALE SIBERIAN POLECATS (Mustela eversmanni)

Heather Branvold, BS,1* James K. Graham, PhD,2,3 Torrance M. Nett, PhD,2,3 and Jeffrey Wimsatt, DVM, PhD1,3

1Department of Clinical Sciences, 2Department of Physiology, and 3Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO 80523 USA

Abstract

The Siberian polecat (Mustela eversmanni) is the closest living relative to the endangered black-footed ferret (M. nigripes), and is widely used as an investigational model. In polecats, ferrets and other mammals, gonadotropin releasing hormone (GnRH) stimulates the release of luteinizing hormone (LH) from the anterior pituitary. LH in turn elicits the release of testosterone, promoting sperm production and reproductive competence. By challenging polecats with GnRH and LH while in peak breeding condition, and again during the nonbreeding (refractory) period, hormonal responses can be characterized to determine if normal hypophyseal-gonadal function has been reached in preparation for breeding.

Methods

A reproductive group of genetically defined Siberian polecats was studied. Blood sampling was performed via brief isoflurane/O2 immobilization. After baseline serum sampling, seven male Siberian polecats in peak breeding condition (peak testicular size and firmness) received 200 ng of a GnRH analog (Cystorelin®) intramuscularly. Serum was collected 15, 60, and 90 min after challenge and LH was measured by specific radioimmunoassay. One week later, these polecats were similarly challenged with 1 mg of ovine LH (NICHD, NIH, Bethesda, MD), and again blood samples were collected and assayed for LH. Identical GnRH and LH challenges were performed during the middle of the nonbreeding season (testicles soft, retracted, and greatly reduced). Wilcoxon-sign rank tests were used for statistical analysis at times 0 and 60 min after challenge to compare values from the same polecats at breeding and nonbreeding periods.

Results

At peak breeding condition, baseline LH (x ± SE in pg/ml) was 702 ± 63, prior to GnRH challenge. LH increased to 2183 ± 166 at 15 min, then decreased to 2027 ± 217 at 60 min and to 762 ± 165 at 90 min after Cystorelin® administration. Baseline LH was 636 ± 174 before LH challenge. LH increased to 2484 ± 302 by 15 min, and then dropped to 1965 ± 313 by 60 min and to 647 ± 92 at 90 min after LH challenge. During the nonbreeding season (refractory period), baseline LH was 672 ± 97 before GnRH challenge, increased to 1310 ± 296 by 15 min, then dropped to 762 ± 165 at 60 min and 603 ± 108 at 90 min. Baseline LH averaged 941 ± 131 before LH challenge, decreased to 710 ± 116 by 15
min, to 643 ± 68 by 60 min, and to 592 ± 107 by 90 min. Baseline LH values during breeding and nonbreeding prior to GnRH ($P = 0.8125$) and LH ($P = 0.3125$) challenge were not significantly different. Siberian polecats responded to GnRH by increasing circulating LH levels during both the breeding and nonbreeding period challenges; however, after 60 min during the nonbreeding period, LH was significantly ($P = 0.0078$) lower than values from the same animals just prior to breeding. Likewise, 60 min following LH challenge, nonbreeding LH concentrations were significantly ($P = 0.0312$) lower than just prior to breeding.

**Conclusions**

Although further evaluation of this technique is required, these data suggest that GnRH or LH response testing may provide a quick and simple assessment of reproductive readiness for potential breeders, based on a single LH measurement 60 min after i.m. agonist administration. For example, this noninvasive technique could be of value in species where few outward indications of reproductive fitness are observed, and as a valuable adjunct to other techniques such as semen evaluation. In addition, combined response-testing as evaluated here could help to pinpoint the level of reproductive dysfunction in nonreproductive males.
EVALUATION OF MODIFIED LIVE CANINE DISTEMPER VACCINE BOOSTERING AND CHALLENGE IN BLACK-FOOTED FERRETS (Mustela nigripes) PREVIOUSLY VACCINATED WITH A KILLED VACCINE

Sharon Harthorn,1* Jeffrey Wimsatt, DVM, PhD,1 Dean E. Biggins, MS,2 Jerry L. Godbey, MS,2 and H. Branvold, BS1

1Department of Clinical Sciences, College of Veterinary and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523 USA; 2Biological Resources Division, USGS, Midcontinent Ecological Science Center, 4512 McMurry Ave, Ft. Collins, CO 80525 USA

Abstract

Effective vaccination regimes are of critical importance to protect mustelids and other highly susceptible species against lethal canine distemper virus (CDV) infection. An experimental killed vaccine (KV), when available, has been used to protect endangered black-footed ferrets (BFF) and other high-risk species, yet live virus challenge studies have never been done for this vaccine to prove its value in species where it has been used. In addition, a readily available modified live vaccine (Galaxy D, MLV) should potentially provide a more robust and longer lasting immunity if safe boostering of KV vaccinates is possible. The goals of this study were to determine (1) if boostering with a commercial modified live virus (MLV) vaccine (Galaxy D®) was safe and effective in ferrets having received the killed vaccine previously, and (2) if previous KV use protects against virulent CDV challenge.

Fourteen non-releasable non-reproductive black-footed ferrets were identified and designated by the BFF SSP for these studies. The 14 KV protected (seven male, seven female) black-footed ferrets (Mustela nigripes), 4-6 yr of age, were stratified by CDV SN serotiters into two groups before study. The first group was vaccinated with the Galaxy D vaccine, and the second group went unvaccinated. The results of this first part of the study indicated that the survival rate was 85.7% (6/7) following MLV vaccination of previous KV black-footed ferret recipients. The animal that developed typical CDV signs had the lowest pre-vaccination SN titer of the group (pre-study titer 1:8). One animal from the unvaccinated group in a separate bank of cages in the same room developed CDV specific signs 30 days after MLV vaccination of the first group (pre-study titer 1:64).

In the second part of the study, all black-footed ferrets from both groups were challenged with a virulent defined CDV strain intranasally/intraorally using Synder Hill strain CDV (NVSL #90-18) at a dose four times the previously established 100% lethal dose (LD) in Siberian polecats. CDV was verified by typical clinical sign development or at necropsy. In this part of the study, black-footed ferrets vaccinated with MLV vaccine had a survival rate of 66.7% (4/6). Black-footed ferrets previously vaccinated only with KV and challenged with the virulent strain of CDV had a 50% (3/6) survival. Although, one death appeared to result from sepsis with E. faecalis, and may have been indirectly related to CDV infection-induced immunosuppression.
Based on the survival data, we concluded that serologic evidence of having received KV previously was at least partially protective against MLV vaccination and during CDV challenge. Whereas, boostering with MLV vaccine appeared of marginal value, and could in fact be detrimental. A partial explanation may have been that previous KV titers blocked the protective titer increases expected from MLV vaccination. Therefore, MLV vaccine boostering as reported here, added minimal protection to black-footed ferrets already vaccinated repeatedly with KV against CDV, and Galaxy D by itself might induce clinical distemper disease in susceptible animals, including black-footed ferrets. Our results suggest that the potential benefits of boostering susceptible animals with a MLV CDV product needs to be carefully weighed against likely risks.
USE OF THERMOGRAPHY AS A DIAGNOSTIC AND PROGNOSTIC TOOL IN SELECTED CETACEAN CONDITIONS

Michael T. Walsh, DVM* and John Thompson2

1SeaWorld Adventure Park Orlando, 7007 SeaWorld Dr., Orlando, FL 328212 USA; 2Emerge Interactive, 10315 102nd Terrace, Sebastian, FL 32958 USA

Abstract

The measurement of change in core body temperature, and its relation to infection or inflammation, is one of the oldest and most widely recognized diagnostic tools in medicine. The use of a thermometer is considered a basic part of the initial physical exam in most species and is often followed by other more sophisticated techniques to try to isolate the source of illness. With the development of affordable heat sensitive cameras the clinician can now detect general or specific areas of abnormal tissue temperatures. Detectable changes in temperature may be related to superficial tissue involvement or a reflection of heat production at a deeper level. These manifestations may include isolated or general areas involving such conditions as abscess, trauma, cellulitis, dermatitis, tendonitis, myositis, and pyothorax.

A thermographic camera was used in clinical cases in cetaceans to refine previous findings that indicated it’s potential applications in diagnosis and prognosis. Individuals which showed clinical signs compatible with trauma, dental disease, and dermal conditions were examined with an EVS DTIS - 500 camera (Emerge Interactive, 10315 102nd Terrace, Sebastian, Fl 32958 USA) and therapy monitored with periodic thermal scans.

Dental disease including trauma to oral tissues, periodontal abscess, and mandibular infections could be readily located, temperature measurements taken, and the size of area of involvement noted. Post therapy follow-up illustrated the ability to gauge the effect of therapy as evidenced by temperature decrease and a decrease in the size of the area involved. The clinician can also better determine the length of drug use based on the response. In one individual case it showed the infection from an abscessed tooth spreading down the lingual side of the mandible.

External trauma to the skin can be monitored for extent, complications and speed of resolution. Rake marks received from other dolphins have shown an inflammatory response present much longer than expected. A loss of normal temperature can also be used as a clue to the presence of material that may require debridement.

Dermatitis is currently being investigated for possible application of this technology. A Tursiops truncatus female with an extensive visual roughening of the skin showed substantial heat in the affected areas of the skin with thermography but no signs of inflammation on bloodwork. The skin inflammation was readily monitored by thermography until total resolution.
CAPTURE OF NARWHALS (Monodon monoceros) IN THE CANADIAN ARCTIC FOR INSTALLATION OF SATELLITE-TRACKED TRANSMITTERS

Mads Peter Heide-Jorgensen, PhD,1 Rune Dietz, MSc,2 and Stéphane Lair, DMV, DVSc3†*

1Greenland Institute of Natural Resources, Tagensvej 135, DK-2200 Copenhagen N, Denmark; 2Danish Department of Arctic Environment, Department of Arctic Environment, Tagensvej 135, DK-2200 Copenhagen N, Denmark; 3Toronto Zoo, 361A Old Finch Avenue, Scarborough, Ontario M1B 5K7 Canada, †Present address: University of British Columbia, 6199 South Campus Road, Vancouver, B.C. V6T 1W5 Canada

Abstract

The study of secretive marine mammals, like narwhals (Monodon monoceros), has been greatly facilitated by the development of autonomous satellite-tracked transmitters. In order to attach these transmitters, the animal to study has to be captured and restrained, which is not an easy task when dealing with large mammals like narwhals. We describe as follows the technique used to capture individuals of this species for the installation of such transmitters. These whales were caught in Tremblay Sound, a narrow inlet in Baffin Island, Northwest Territories. This 45 km long fjord with maximum depths of up 275 m is known to be explored by large numbers of narwhals at the end of the summer. Three 5 × 50 m stationary nets, made of 4 mm in diameter green nylon twines forming meshes of 20 × 20 cm, were mounted in a row close to the surface. The net was set perpendicularly to the shore and was constantly monitored. Between 10 and 23 August 1997 four males, and one female, measuring from 2.4-4.4 m, were caught. When the narwhals were captured, they vigorously struggled and became rapidly entangled into the net. Afterward they were restrained at the surface of the water using three industrial straps secured on two inflatable boats located on each side of the whale. These straps were installed cranially to the pectoral fins, under the abdomen, and around the caudal peduncle. Radio-transmitters weighing from 950-1450 g in air (150-225 g in water) were installed on the tusk of the adult males using steal collars, and on the dorsal ridge of a female and a juvenile male using transcutaneous plastic rods. Skin biopsies were also taken for genetic study. The total procedure lasted 45-60 min. The initial response to the capture was strong, but of short duration. All five animals remained relatively calm when removed from the net and supported by the straps. Their breathing remained stable throughout the procedure, and all whales dived for a long period of time when released. Lesions caused by the net were limited to superficial cutaneous lacerations of most likely little significance for animals of this size. Based on these observations we believe that these animals experienced a significant, but acceptable, level of stress during a limited length of time. Fatal captures of non-target species were limited to one ringed seal, and two arctic chars. Several Greenland sharks were also caught in the net but could be released. The method of capture used in this study was therefore proven to be safe for narwhals, and did not cause any significant harm to the local fauna.
DESIGN OF A COMPUTER DATABASE TO TRACK ANIMAL ADMISSIONS AND PRIMARY DIAGNOSES AT A WILDLIFE REHABILITATION CENTER

Catherine M. Brown, DVM, MSc

Willowbrook Wildlife Center, Forest Preserve District of DuPage County, 525 S. Park Blvd., Glen Ellyn, IL 60137 USA

Abstract

The use of microcomputers to aid in record keeping in wildlife facilities has lagged behind their use in zoological facilities primarily due to financial and personnel time limitations. However, the need for a standardized system of data management is not less, and may actually be greater due to the steep learning curve that currently exists in the field of wildlife medicine. So many wildlife rehabilitators are single individuals working out of their home or backyard that implementation of computerized record keeping is not likely in their foreseeable future. Larger wildlife facilities need to take the lead in developing a system that enables them to collate and disseminate information easily. Establishing an internal database is usually the first step towards adopting a more comprehensive shared system.

Since 1997, Willowbrook Wildlife Center had used a database written in FileMaker Pro that tracked animal admissions only. With a recent computer system upgrade at the Center, this program became obsolete, and we had the opportunity to develop a new system. Some of the problems with the original program were: 1) lack of standard set of diagnoses, 2) maintenance of separate animal admission, bird-banding, and release databases, 3) lack of standard admission information from police and animal control agencies, and 4) inconsistent style of data entry, particularly in species names. We chose Microsoft Access 97 for our new database program by default. This program was already in use throughout the Forest Preserve District for other record-keeping purposes, and we had an on-site staff member who was superficially familiar with the program and able to learn enough to write and maintain the database.

The current system provides several advantages.
1. It tracks admissions from local agencies by providing a drop-down menu of police departments and animal control agencies. Admissions from individuals are tracked by recording last +/- first names;
2. Animal admissions are recorded and sorted by species. A separate category is used for species not accepted for admission based on our policies, but which we wish to track as they represent a significant time commitment for our staff.
3. It has a standardized drop-down list of diagnoses, which allows us to track up to three primary diagnoses per animal. With this we can evaluate trends in cases and track success rates by injury or disease in order to allocate our resources most productively.
4. Records of US Fish and Wildlife bird band numbers and Forest Preserve District release locations are incorporated into the admissions program. This enables us to locate the records of birds with
returned USFWS bands and to track numbers of species released at different sites to prevent too
great a stocking density.
5. Recording the town each animal was found in enables us to watch for localized outbreaks of disease,
such as botulism.
6. Reports built into the database provide information on birds at the center more than 90 days that
need to be reported according to our permit; annual summary information formatted for submission
to the USFWS for permit maintenance; and annual summary information required by the Forest
Preserve District to justify our activities and budget.

The biggest problem with the system continues to be human-related data entry errors although the use
of drop-down menus has eliminated a significant number of problems. We have also found that
educating data entry personnel about the functions this data collection serves and the impact that data
entry errors can have, has reduced the incidence of errors. As staff members and volunteers become
used to the system and recognize the usefulness of timesaving report functions over manual collation
of data, the percentage of human errors should continue to decrease.
COST EFFECTIVE METHOD TO TRANSPORT MANUALLY COLLECTED GORILLA SEMEN FOR LONG TERM STORAGE

Jennifer M. Finnegan, BS,* Naida M. Loskutoff, PhD, and Corrine S. Brown, DVM

Center for Conservation and Research, Henry Doorly Zoo, 3701 S. 10th Street, Omaha, NE 68107 USA

Abstract

Since 1994, semen samples have been collected from two western lowland gorillas (Gorilla gorilla gorilla) housed at the Henry Doorly Zoo (HDZ) using a positive reinforcement training program.1 Semen quality collected from gorillas by manual palpation is superior to electroejaculation for obtaining ejaculates with higher percent (up to 80%) motile sperm.2 A protocol for the cryopreservation of gorilla sperm was developed (Loskutoff, unpublished data) and to date, over 500 samples have been stored at the HDZ genome resource bank. The storage of gorilla semen is important not only for use in assisted reproductive techniques, but to maintain genetic diversity in the captive population. Using cryopreserved gorilla sperm collected from a trained animal, embryos have been produced4 and a gorilla baby was born in 1995 by in vitro fertilization.5 Furthermore, gorilla embryos have been produced by intracytoplasmic sperm injection from oocytes collected post mortem.3 Currently, few institutions have the capability to house cryopreserved samples due to the expense and labor involved in maintaining liquid nitrogen (LN2) storage tanks. With an increasing interest by zoos to develop their own training programs for semen collections from gorillas, the goals of this study were to develop a cost-effective method for transporting processed gorilla semen to cryogenic storage facilities and to develop a short term storage alternative for LN2.

Three ejaculates collected by manual palpation were processed using the standard protocol: 1) dilution (1:1) with TEST – egg yolk buffer + 17.5% egg yolk at room temperature; 2) refrigeration (4-7°C) for 2 hr before dilution with cooled cryodiluent (same as above but with added glycerol for final concentration of 4%), loaded into 0.25 ml straws, and equilibrated for another hour. Straws were frozen using two methods: 1) placed on a rack in an ultralow upright freezer; 2) placed between two 10-lb blocks of dry ice in a Styrofoam container. As a control, some straws were frozen using the standard procedure of placing on a block of dry ice for 10 min and then plunged into LN2. Pre-freeze motilities for individual ejaculates were 74%, 68%, and 81% respectively. Post-thaw motilities, expressed as means ± SEM, were determined as percent motile over time from pre-freeze motility estimates (Table 1). Straws were thawed (37°C water bath for 10 sec) at approximate times corresponding to standard express mail delivery courier services: 16 hr, 28 hr, 45 hr, and 75 hr. Straws remaining at the end of 75 hr were plunged into LN2 (Table 2).

As shown in Table 1, the post-thaw motility of gorilla sperm held in the ultra-low freezer stayed fairly constant over time, while a decrease in motility occurred when straws were stored between dry ice. Overall post-thaw motility rates after plunging into LN2 after 3 days storage were very good with the ultralow being slightly higher than dry ice, although not statistically significant. This study shows that
SERUM CONCENTRATIONS AND BEHAVIORAL EFFECTS OF ORAL HALOPERIDOL IN BONGO ANTELOPE (Tragelaphus eurycerus)

Susan K. Mikota, DVM,1* Steven G. Kamerling, PhD,2 and Steven A. Barker, PhD3

1Audubon Center for Research of Endangered Species, 14001 River Road, New Orleans, LA 70131 USA; 2Pharmacia and Upjohn Animal Health 7923-190-41,7000 Portage Road, Kalamazoo, MI 49001-0199 USA; 3Louisiana State University, School of Veterinary Medicine, South Stadium Drive, Baton Rouge, LA 70803 USA

Abstract

Neuroleptic drugs have been used to reduce anxiety, excitement, and motor activity in ungulates. These drugs facilitate the handling and transportation of both captive and free-ranging animals. Haloperidol, an antipsychotic, tranquilizing agent, is used in humans to reduce psychomotor agitation and aggression. Parentally administered haloperidol has also been shown to produce desirable psychomotor effects and tractability in a number of ungulate species. The pharmacologic effects of haloperidol in bongo antelope have not been reported, and further, such effects have not been correlated with plasma concentrations. There is little information on the oral effectiveness of haloperidol in any nondomestic species. The ability to administer an oral tranquilizing agent could obviate the need for repeated administration by remote injection to maintain a desired level of tranquilization.

A dose of 1 mg/kg has been suggested as an oral haloperidol dose. Recent clinical observations (S. Mikota, unpublished data) have shown that a 1 mg/kg dose of haloperidol, administered once daily in the food for 5 days, produced a level of tranquilization which permitted restraint and blood sampling without incident in captive bongo conditioned to enter a custom designed chute. These observations suggested that haloperidol is palatable orally, and is absorbed and distributed to the central nervous system. The purpose of this study was to systematically evaluate the behavioral effects of orally administered haloperidol in bongo antelope and to correlate these effects with plasma concentrations.

As part of an ongoing project to evaluate steroid hormones and anti-tuberculosis drug levels, each of four adult female bongo received haloperidol at 4:00 PM for 28 days at an approximate dose of 1 mg/kg/day (1.04-1.62 mg/kg). Haloperidol is supplied as 20 mg tablets and oral dosing was accomplished by inserting 10 tablets into bananas which were hand fed to individual animals by a keeper who had established the animals’ trust.

A push wall directed bongo to enter a chute and squeeze box. Bongo were blind-folded upon entering the chute and two straps placed dorsally across the back to discourage jumping. Venipuncture sites were shaved with a disposable razor and a 4 % tetracaine ointment applied. (Application of tetracaine alone did not result in analgesia sufficient for blood sampling.; S. Mikota, unpublished data). Blood samples to measure plasma progesterone and estradiol were collected daily for 29 days.
On day 20, blood was drawn at 6:30 AM (time 0), haloperidol was administered orally as described, and anti-tuberculosis drugs were administered orally and/or parentally. Bongo were bled at 1, 2, 3, 5, 8, 10 and 12 hr post-administration to measure serum concentrations of anti-tuberculosis drugs and haloperidol over time.

Bongo were observed under four sets of conditions as follows:

- Condition 1: Confined to pen; on haloperidol
- Condition 2: Manipulation through chute; venipuncture; on haloperidol
- Condition 3: Manipulation through chute; venipuncture; not on haloperidol
- Condition 4: Manipulation through chute; venipuncture; on haloperidol (4-day study)

Score cards, devised to subjectively grade the degree of tranquilization observed under Conditions 1-4, were completed by all personnel involved in the procedure. Parameters included: behavior in pen, behavior in chute, appetite, and response to treats. Pen and chute behaviors were scored using a 7-point subjective scale that described the animal’s behavior in each setting. The behavioral scores ranged from unresponsive to violent. Overall Behavior was scored using a 4-point scale that described general impressions of the performance of the tranquilizer. These scores ranged from unacceptable to excellent. Temperature, pulse, and respiration were measured at each time point and animals were observed for possible side effects (repetitive muscle jerks (dystonia), drooling, worm-like movements of the tongue (lingular vermiculation), spontaneous rolling back of the eyes (oculogyric crisis) or slow, repetitive, purposeless movements (tardive dyskinesia). Behavioral data from all observers was averaged and a single score generated for each animal and variable.

Condition 4 behavioral data was collected for 4 consecutive days following 13 days during which bongo did not receive haloperidol.

Serum haloperidol was measured using a commercially available enzyme linked immunosorbent assay (ELISA) in a 96-well microtiter plate format. The assay was validated for bongo serum using samples from untreated animals and fortification of the samples with haloperidol reference standard. Samples were assayed in singlicate against blanks and blank fortified reference standard controls.

At time 0, 14.5 hr since the last haloperidol dosing, mean serum concentration of haloperidol was 12.6 ng/ml and Overall Behavior was scored as fair to good (1.7). Over the next 1-3 hr, the steepest increases in haloperidol concentration and Overall Behavior Score were observed. At 2 hr post-dose, Overall Behavior was scored as good to excellent (2.7) and serum haloperidol concentrations had risen to 16.2 ng/ml. While serum concentrations continued to rise for 10 hr post dose, increases became more gradual from 3-10 hr, reaching a peak at 10 hr (19.5 ng/ml). Overall behavioral scores remained fairly constant, from good to excellent, from 3-10 hr post. During this time the animals could be easily approached and did not attempt to escape the chute. They readily tolerated venipuncture, blindfolding, restraint with dorsally placed straps, stethoscopic auscultation, and the insertion of a digital thermometer. The bongo also complied with physical coaxing towards the chute and ambulated normally upon leaving the chute. Little or no change in chute or overall behavior was reported during
that time. Although behavior was not scored, serum haloperidol concentration began to decline (17.3 ng/ml) by 12 hr post dose.

General appetite, as measured by treat consumption, increased from partial consumption at time 0 to complete consumption by 3 hr post dose. Consumption remained complete for the remaining observation period. Respiratory rate, body temperature, and ambient temperature rose gradually during the 10-hr observation period. Pulse rate was variable.

The data obtained from Conditions 1-3 indicated that adequate plasma concentrations of haloperidol could be achieved in bongos via the oral route of administration. Furthermore, the data indicated that serum concentrations could be correlated with changes in behavior. The most dramatic improvements in tranquilization were seen during the most rapid increases in serum concentrations (i.e., 1-3 hr post dose). The data also demonstrated that plasma haloperidol concentrations appear to peak at 10 hr post dose and that good-to-excellent tranquilization could be achieved at 15-19 ng/ml.

Residual haloperidol concentrations of 8.38-8.5 ng/ml were measured at 24 hr post dose, suggesting that haloperidol is absorbed gradually and reliably from the gastrointestinal tract, even in the presence of food. Plasma concentrations of 5-15 ng/ml have been associated with positive therapeutic responses in humans. Similar plasma concentrations were achieved in our study, suggesting that the therapeutic range for humans and bongos is similar. In conclusion, a once daily oral dose (200 mg) of haloperidol produced a desirable level of tranquilization in bongos that permitted manipulation and blood sampling.

ACKNOWLEDGMENTS

This project was funded in part by grants from the Institute of Museum and Library Services, the Conservation Endowment Fund of the American Zoo and Aquarium Association and Ortho-McNeil. A special thank-you is extended to the hoofstock staff at the Audubon Park Zoo for their dedicated effort to make this project possible.

LITERATURE CITED