SURGICAL REMOVAL OF AN ABDOMINAL MYOSARCOMA IN A KOI (Cyprinus carpio)

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Abstract

A 3-yr-old mature male showa koi (Cyprinus carpio) weighing 1792 g presented in October of 1996 to the Veterinary Teaching Hospital of the College of Veterinary Medicine, North Carolina State University (CVM-NCSU) with a 2-mo history of a distended abdomen. Water quality parameters were within normal limits and the fish had been fed a diet of commercially prepared koi pellets on the fish farm where it lived.

Multiple imaging procedures were performed to characterize the coelomic mass and to localize the organ of origin. Left to right lateral (RL) and right (RDL) and left (LLD) lateral decubital (horizontal beam) whole body radiographs, caudal coelomic cavity ultrasonography and computed tomography (CT) were performed.

The fish was anesthetized for an exploratory celiotomy. Anesthesia was induced by immersing the fish in a 200 mg tricaine methanesulfonate (Finquel®, Argent Chemical Labs, Redmond, WA 98052 USA) solution/L water for 5 min until the fish had only mild opercular movements (the opercula could be observed moving but the motion was not sufficient to generate water flow across the gills). The fish was placed on the surgery area and the delivery tubes of a recirculating anesthesia machine were positioned into its mouth. A stock solution of tricaine methanesulfonate was prepared by dissolving 10 g Finquel® in 1 L of deionized water and buffering it to pH 7.0 with sodium bicarbonate. Anesthesia was maintained with a constant 160 mg/L tricaine methanesulfonate at a flow rate of 3 L/min for 55 min. The concentration of tricaine methanesulfonate was gradually reduced to 38 mg/L over the next 40 min as the surgical procedure was being completed. Total duration of anesthesia was 100 min. The patient was also given a 10 mg/kg intraperitoneal dose of enrofloxacin (Baytril®, Bayer Corp., Shawnee Mission, KS 66201 USA) during surgery to reduce the possibility of a secondary bacterial infection. Butorphanol tartrate (Torbutrol®, Fort Dodge Laboratories, Inc., Fort Dodge, IA 50501 USA) at a dose of 0.1 mg/kg was given subcutaneously at the end of the surgery for pain management.

The scales were removed from the area of the planned incision site with forceps. A ventral midline abdominal incision was made beginning immediately caudal to the base of the pectoral fins and extended to within 1 cm of the cloaca. An osteotome was used to separate the pelvic girdle along its midline. Gelpi perineal retractors were used to maintain retraction of the coelomic incision. A large solid mass was present in the abdominal coelom extending caudally from the liver to the pelvic
inlet, and was compressing the internal organs dorsally and to the left. Sharp and blunt dissection was used to free the mass from its attachments to the liver, body wall, and kidneys. Larger vessels supplying the mass were divided and ligated using 4-0 polypropylene (Prolene®, Ethicon, Somerville, NJ 08876 USA) suture and bipolar cautery was used to electrocoagulate smaller vascular pedicles. The pelvic osteotomy was repaired using 2-0 stainless steel cerclage wires. The muscle wall was closed with 3-0 polyglyconate (Maxon®, Davis & Geck, Manati, Puerto Rico 00701 USA) in a simple continuous pattern and the skin was closed with 4-0 polypropylene in a continuous Ford interlocking pattern.

The fish recovered without complication from the anesthesia and surgical procedure. The air space in the coelomic cavity caused the fish to be positively buoyant, thus 110 ml of air was aspirated from the abdomen, correcting the problem. The skin sutures were removed 25 days post operatively.

Microscopically the mass was identified as a myosarcoma but could not be morphologically distinguished as a rhabdomyosarcoma or a leiomyosarcoma. Immunohistochemistry and transmission electron microscopy results are pending.

Myosarcomas are not common in fish but have been reported in an African lungfish (Protopterus dolloi) and a cutlass fish (Trichiurus lepturus).1,2 We found no reports of this tumor in koi or carp, but papillomas, squamous cell carcinomas, and a branchioblastoma have all been described in koi.4,5

Tricaine methanesulfonate is approved by the Food and Drug Administration (FDA) and is a widely used anesthetic for fishes.3 Most fish experience an excitatory stage of anesthesia followed by sedation, a loss of equilibrium and finally, a loss of any reactivity. It is desirable to maintain an anesthetic plane at this level. Mild opercular movements should be maintained and the gills must be bathed sufficiently with anesthetic water. Anesthetic effects of tricaine methanesulfonate are quickly reversed by diluting the concentration and placing the fish into a solution of fresh, clean water.

The surgical removal of this tumor relieved unnatural pressure on the internal organs and the body wall. The fish appeared clinically normal 6 mo after the procedure.

ACKNOWLEDGMENTS

The authors thank Drs. Michael Stoskopf and Craig Harms for their assistance with case management.

LITERATURE CITED


OVARIECTOMY OF A BROOK TROUT (Salvelinus fontinalis)

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Abstract

A 1.7 kg mature female brook trout, Salvelinus fontinalis, was diagnosed with egg retention. Anesthesia was induced with 150 mg/ml of tricaine (Finquel®, Argent Chemical Labs, Redmond, WA 98052 USA) and the fish maintained at 75-95 mg/ml with a flow rate of 2 L/min using a recirculating machine.1,2 The fish was placed in dorsal recumbency in a custom water permeable open-cell foam rubber trough and the incision site covered by a fenestrated transparent surgical drape. A midline incision was made approximately 1 cm cranial to the pelvic girdle. Ovariectomy was performed by retracting each ovary individually to expose their respective dorsal mesovarium which was carefully dissected from the caudal pole of the ovary. The ovarian artery and vein were clamped with two mosquito forceps approximately 2 mm apart prior to placement of a transfixiation ligature using 4-0 polyglycolic acid (Dexon, Davis and Geck Inc., Manati, PR 00701) around the ovarian artery and vein between the two clamps. The vasculature was incised between the ligature and distal forceps. The body wall and coelomic musculature were sutured with a two layer closure using 4-0 polyglycolic acid in a simple-continuous patterns. The skin was sutured using 4-0 polyglycolic acid in a simple interrupted pattern. The trout recovered from anesthesia within 20 min, but residual air in the coelomic cavity following closure created a transient positive buoyancy which resolved within an hour. No other complications were noted and the fish resumed eating within 2 days. Sutures were removed 21 days later. Ovariectomy in fish is a viable option for display aquarium female fish with ovarian disease.

LITERATURE CITED

REPLACEMENT OF A PROLAPSED STOMACH IN A BANDED WOBEGONG SHARK
(Orectolobus ornatus)

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1Veterinary and Quarantine Centre, Taronga Zoo, PO Box 20, Mosman, NSW, 2088, Australia; 2Veterinary Department, Western Plains Zoo, Obley Road, Dubbo, NSW, 2830, Australia

Abstract

A 48 kg, male, banded wobbegong shark (Orectolobus ornatus) presented with a history of having prolapsed its stomach through one of its gill slits 48 hr previously. The shark had been successfully kept in a 1.25 × 10^6 L mixed species oceanarium for several years prior to this.

On examination, 20-30 cm of brown-purple tissue was protruding from the shark’s right caudal external gill slit. The animal was resting on the floor of the aquarium and there appeared to have been minor bleeding from the everted tissue.

The wobbegong was anesthetized with 1,750 mg ketamine (36.5 mg/kg) (Ketamine HCl; 1 g powder Parke Davis, North Caringbah, New South Wales, Australia) combined with 500 mg xylazine (10.4 mg/kg) (Rompun Dry, 500 mg base, Bayer, Pymble, New South Wales, Australia) administered intramuscularly by a scuba diver using a pole syringe.

Loss of righting reflex occurred after 12 min. Surgical anesthesia occurred after approximately 20 min and lasted about 50 min. The shark was placed in an isolation tank in 15 cm of water, allowing the gills to be covered. An oxygen cylinder, regulator and aerator pump bubbled oxygenated water into the shark’s mouth. Spontaneous respiratory movements continued throughout the anesthetic period, at a similar rate to pre-induction (12-18 gill slit movements/min) although at a shallower amplitude.

The prolapse consisted of a mass of swollen tissue 15 × 25 cm thought to be the stomach protruding from the right caudal external gill slit. An area of more dense tissue, with an outlet tract inverting towards the oral cavity was presumed to be the pylorus. There were areas of abrasion and superficial necrosis of the gastric mucosa, but the tissue bled easily and appeared viable.

The prolapse was reduced with difficulty by traction from within the oral cavity by an arm inserted through a length of PVC piping to provide protection from the shark’s teeth. Concurrently, a second person repulsed the tissue externally whilst manually stretching the external gill slit. Once the stomach was retracted into the shark’s mouth, a single “swallowing” movement occurred and the organ rapidly disappeared. It was assumed to have been repositioned.

Dexamethasone, 20 mg (Dexason, Ilum Veterinary Products, Smithfield, New South Wales, Australia), 100 ml of 5% glucose (5% Glucose Intravenous infusion, Baxter Healthcare, Old
Toongabbie, New South Wales, Australia) and 500 ml of Hartmann’s solution (Compound Sodium Lactate Intravenous Infusion, Baxter Healthcare, Old Toongabbie, New South Wales, Australia) were administered i.v. into the ventral caudal vein in an attempt to decrease the effects of shock and metabolic acidosis. Six daily doses of 600 mg oxytetracycline i.m. (Oxytet-200 LA, Ilium Veterinary Products, Smithfield, New South Wales, Australia) were given prophylactically as the gastric mucosal barrier was compromised.

Respiratory amplitude and rate increased to 25-30 gill movements/min after Doxapram 100 mg i.v. (Dopram-V, Bomac Laboratories, Castle Hill, New South Wales, Australia). Yohimbine 20 mg i.v. (Reverzine, Parnell, Silverwater, New South Wales, Australia) was given to reverse xylazine induced anesthesia. Anesthetic recovery was prolonged but uneventful, and the shark was behaving and swimming normally 36 hr after chemical immobilization.

Blood was collected from the ventral caudal vein at the time of surgery and 2 days later (Table 1). The shark continued to behave normally over the following 2 wk although it refused food. Fourteen days after replacement of the prolapsed stomach, the animal was found dead.

At postmortem examination, the wobbegong weighed 47 kg. The stomach was correctly positioned and was empty. There was no discernable esophago-gastric sphincter and the mucosa was discolored red-brown in several places. Histologically, there was focal hemorrhagic necrosis of the right caudal gill lamellae, with surface necrosis and deeper congestion of the surrounding gill slit area. In the stomach, there was edema and focal, hemorrhagic, mucosal necrosis with extensive submucosal necrosis. The liver showed intense, foamy vacuolation of hepatocytes, probably due to fat accumulation.

There was no evidence of bacterial infection, and microbiological culture of brown tinged fluid from the coelomic cavity failed to grow any organisms. Heart blood was not cultured. There are no blood reference values published for the banded wobbegong shark, hence values were compared with those of other sharks.1

No references were found to gastric prolapse in sharks, although there are anecdotal reports from fishermen of temporary stomach eversion through the mouth on capture of several shark species. The anesthetic regime used for this procedure provided surgical anesthesia of sufficient duration without the need for supplemental doses, although this was at the expense of a prolonged recovery period.

The patches of gastric mucosal and submucosal necrosis may have contributed to the death of the wobbegong, but were not considered severe enough to have caused death in the apparent absence of bacterial infection. The cause of the gastric prolapse and the death of the shark 2 wk after replacement, remain unknown.
ACKNOWLEDGMENTS

The authors wish to thank the staff of Sydney Aquarium, Darling Harbour, Sydney, for referring this case, and for the care of the wobbegong.

LITERATURE CITED


Table 1. Hematology and plasma biochemistry from an adult male wobbegong shark.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First sample</th>
<th>Second sample</th>
<th>Value for sharks</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell count (10⁹/L)</td>
<td>17.82</td>
<td>8.8</td>
<td>25.9-28.1</td>
</tr>
<tr>
<td>Red blood cell count (10⁹/L)</td>
<td>0.11</td>
<td>0.14</td>
<td>0.35-0.665</td>
</tr>
<tr>
<td>Hematocrit (L/L)</td>
<td>0.16</td>
<td>0.13</td>
<td>0.10-0.22</td>
</tr>
<tr>
<td>Heterophils (10⁹/L)(%)</td>
<td>2.67 (15)</td>
<td>4.58 (52)</td>
<td>56-58%</td>
</tr>
<tr>
<td>Lymphocytes (10⁹/L)(%)</td>
<td>11.23 (63)</td>
<td>3.87 (44)</td>
<td>30-40%</td>
</tr>
<tr>
<td>Monocytes (10⁹/L)(%)</td>
<td>1.25 (7)</td>
<td>0.18 (2)</td>
<td>1.0-1.4%</td>
</tr>
<tr>
<td>Eosinophils (10⁹/L)(%)</td>
<td>0.90 (5)</td>
<td></td>
<td>0.0-1.0%</td>
</tr>
<tr>
<td>Atypical lymphocytes (10⁹/L)(%)</td>
<td>1.78 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>325</td>
<td>321</td>
<td>302.4-404.8</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>0.02</td>
<td>0.01</td>
<td>0.04-0.09</td>
</tr>
<tr>
<td>Protein (g/L)</td>
<td>37</td>
<td>38</td>
<td>17-46</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>6</td>
<td>6</td>
<td>4-6</td>
</tr>
<tr>
<td>Globulin (g/L)</td>
<td>31</td>
<td>32</td>
<td>11-42</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>136</td>
<td>28</td>
<td>4-42</td>
</tr>
<tr>
<td>Creatine phosphokinase (U/L)</td>
<td>5233</td>
<td>1372</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>1.5</td>
<td>1.7</td>
<td>0.8-2.1</td>
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<tr>
<td>Magnesium (mmol/L)</td>
<td>1.8</td>
<td>1.3</td>
<td>1.7-1.9</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>4.7</td>
<td>5</td>
<td>3.3-4.3</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.3</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>330</td>
<td>286</td>
<td>262-287</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.1</td>
<td>5.1</td>
<td>3.1-4.3</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>330</td>
<td>254</td>
<td>218-257</td>
</tr>
</tbody>
</table>

a The first sample was taken 48 hr after the stomach prolapsed.

b The second sample was taken approximately 4 days after the prolapse.

c Range of values for serum from wild and captive animals (n<20, up to 8 different species)1 converted to SI units.2
FUNGAL KERATITIS IN A GROUP OF IMPORTED HELMETED WATER TOADS
(Caudiverbera caudiverbera)

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Abstract

A group of eight unidentified frogs were imported to a herpetologic distributor in December 1996. The frogs were apparently normal. No information was supplied by the importer as to the animal’s identification or care. The frogs were placed into a 29-gal glass aquarium (24” × 13” × 30”) filled with approximately 10 gal of chlorinated water. Approximately 15 pounds of river rock were added as substrate (0.5-in pebble). Two concrete bricks were placed in the aquarium to provide an area for basking/drying. The water was not aerated. No external heat or ultraviolet light sources were provided. The frogs were offered a diet of crickets and common shiners (Notropis cornutus).

After 1 wk, several frogs developed opaque lesions on the eyes. Five frogs were evaluated at Louisiana State University School of Veterinary Medicine. The frogs were identified as helmeted water toads (Caudiverbera caudiverbera). A water sample was collected and the following parameters were measured: temperature 65-68°F, total hardness 120 ppm, alkalinity 80 ppm, pH 7.6, nitrate 100 ppm, and nitrite 0.5 ppm. On physical examination the frogs were found to have varying degrees of ocular lesions. Lesions were mostly bilateral and included diffuse corneal edema, keratoconus, central corneal thinning and ulceration, aqueous flare and hyphema. Corneal edema preceded the formation of corneal ulcers and keratoconus in all five cases. A bacteriologic culture and cytologic examination of the corneas were performed. Standard aerobic cultures were evaluated at room temperature and at 37°C. Mixed populations of organisms were isolated including Alcaligenes spp., Alpha-Streptococcus spp., and Pseudomonas maltophilia. Corneal cytology revealed a mixed population of inflammatory cells, predominantly heterophils and macrophages, and bacteria. Blood samples were collected from the ventral abdominal vein. Packed cell volumes were performed on all five frogs and ranged from 12-29%. White blood cell estimates ranged from 9.8-22.6 × 10³/µl. Enemas were performed on the frogs to collect fecal material for parasite examination. Direct saline smears and fecal flotations were performed. Fecal evaluation revealed moderate numbers of ciliated and flagellated protozoa.

Treatment was started by correcting husbandry methods and instituting systemic and topical antimicrobials. Enrofloxacin was given at 7.5 mg/kg i.m. in the front limbs i.d. Topical triple antibiotic ointment was instilled in each eye i.d. to lubricate and protect the eyes from drying. The
frogs did not respond to treatment after 10 days resulting in the need for euthanasia.

Necropsies were performed on all five animals. Gross necropsy findings were unremarkable except for the ocular lesions. Globes and multiple tissues were collected for histopathologic examination. Ocular lesions were mostly bilateral with variable severity and included central corneal thinning, diffuse corneal edema with central corneal ulceration, and a mixed population of inflammatory cells in the corneal stroma. Blood intermixed with inflammatory cells and cellular debris was present in the anterior chamber and was adhered to the inner corneal stroma in areas devoid of corneal endothelial cells. Uniform septate fungal hyphae, approximately 1-2 microns in diameter, were present in some corneas. A mixed population of inflammatory cells were present within the anterior uvea and vitreous body. Sensory retinal detachment was present within some globes.

Cultures of the liver, kidney, spleen, and small intestines were performed. Small numbers of *Providencia* spp. were isolated from the liver and spleen of two of the frogs. Large numbers of *Enterobacter cloacae* were isolated from the intestines of three of the frogs and small numbers of *Escherichia coli* were isolated from the intestines of another frog.

Keratitis in anurans has been associated with bacterial infections and possible ultraviolet light exposure. Severe vascularizing keratitis associated with a syndrome of corneal melting is less common and often reveals no pathogens, although gram-negative bacteria have been isolated on occasion. The finding of fungal elements in the corneas of these anurans has not been previously described. Although the source of the fungi was not determined, the prey items (fish) and poor water conditions may have introduced and maintained the fungi within the environment. Stress associated with transport and the inadequate husbandry methods could have also played a role in pathogenesis by compromising the immune response. This case illustrates the importance of fungi as opportunistic pathogens in captive anurans.

LITERATURE CITED

BUOYANCY PROBLEMS IN SEA TURTLES: CAUSES AND DIAGNOSIS

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Abstract

Buoyancy problems in captive and free-ranging sea turtles are common and are seen as either difficulties in diving or floating at an angle to the surface. Buoyancy problems can result from infectious diseases and noninfectious diseases such as trauma and congenital defects in organ development. Viral, bacterial, fungal and parasitic infections may result in respiratory tract disease and may be manifested as buoyancy problems. Gas collecting in the gastrointestinal tract may result in the turtle floating with the affected side up. Trauma to the lung and/or penetrating wounds such as from boat injuries may result in pneumocoelom. Blunt trauma to the caudal carapace from boat injuries, often result in cord transection and flotation problems. Diagnostic techniques for determining causes of buoyancy problems in sea turtle include conventional radiographic imaging, magnetic resonance imaging and computerized axial tomography (CAT) scans, and endoscopy/bronchoscopy.
TO FORM THE MORE PERFECT STOOL: FEEDING TRIALS ON THE GALAPAGOS TORTOISE (*Geochelone nigra*) AND ALDABRA TORTOISE (*Geochelone gigantea*) POPULATION AT THE PHILADELPHIA ZOOLOGICAL GARDEN

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Abstract

Poor growth, infertile eggs, shell problems, and loose stools were problems consistently noted in the giant tortoises at the Philadelphia Zoo, and are problems that appear to be associated with captive populations at many other zoos in the United States. Many of these problems are suspected to have an underlying nutritional etiology, so in March 1992, the Philadelphia Zoo began dietary trials on 1.2 Galapagos tortoises (*Geochelone nigra*) and 5.6 Aldabra tortoises (*Geochelone gigantea*) to develop a more nutritionally sound feeding program. The giant tortoises used in this study were given a health exam that included documenting weights, curved carapace lengths, and hematological parameters (complete blood count, plasma chemistry profile including vitamin A and E levels) prior to the start of the trial, and were sampled periodically throughout the trials. Feed intake and stool quality were assessed by the keepers on a regular basis.

The original diet that the tortoises were fed prior to 14 June 1992 is described in Table 1. The initial trial used an alfalfa hay and biscuit diet (Table 2) similar to one that had been used in a 3-mo trial at another institution. The biscuit was the Mazuri ADF 16 pellet. This diet was changed incrementally until the new alfalfa hay and biscuit diet was the sole diet fed in June 1992. This alfalfa hay and biscuit diet was offered daily and a small amount of produce was offered 3 times/wk. Based on estimated feed intake, the diet appeared successful while the animals were maintained in an outdoor enclosure. A better assessment of palatability and overall consumption was obtained when the animals were moved to their indoor enclosures. Palatability of the alfalfa hay and biscuits were poor. Despite various treatments to increase palatability of the alfalfa hay (e.g., soaking hay in water for 4 hr, chopping hay into 2.5-in lengths, soaking the chopped hay for 2 and 4 hr, soaking chopped hay in orange juice), acceptance continued to be poor. In September 1992, the biscuits were dyed orange in an effort to increase palatability using iron oxide, a dye that is not absorbed by other animals. The efficacy of this treatment was questionable. A side effect of this diet was that the overall hue of the tortoises turned orange, most likely the result of the dye used to make the biscuits orange. In January 1993, since the tortoises did not seem interested in the food on a daily basis, the alfalfa hay and biscuit diet was offered only 5 times/wk and the produce maintained at the same level (Table 3) until the end of March 1993 when the tortoises were returned to the original diet. The orange hue gradually faded when the tortoises returned to the original diet. The keepers perceived a decrease in general activity level and breeding behavior throughout the duration of this dietary trial.
No significant changes in tortoise morphometrics were noted during this trial, nor were perceptible growth lines noted on the carapace scutes. Tortoise stools ranged from loose to soft-formed, but never achieved the firm-formed texture noted in wild tortoises.

Based on this trial, the following conclusions were made: 1) giant tortoises seem to self adjust their nutrient intake consuming the same proportion of major nutrients on the original diet and the biscuit based diet; 2) giant tortoises become depressed when fed a high concentrate-low produce diet as indicated by a keeper-perceived decrease in breeding behavior and activity level; 3) giant tortoises seem to metabolize iron oxide differently than mammals; 4) giant tortoises will not consume large amounts of alfalfa hay; 5) collection of feed intake information is not practical when tortoises are maintained outside; and, 6) the consumed diet did not appear to meet the needs of the tortoises due to the lack of significant growth and weight changes in any of the tortoises, and the poor stool quality noted.

The Walkabout Mix diet (Nutrition Support Services, Walkabout Farm, Pembroke, VA) was used for the next dietary trial, and consisted of a produce-based diet to which a premix is added to balance various nutrients. This is a nutritionally complete diet based on dietary analysis of the components, and is similar in analysis to the diet known to be consumed by free-ranging giant tortoises. (Tables 4 and 5). Produce rather than hay was chosen as the bulk of the diet to avoid palatability problems. The Walkabout Mix diet was offered starting in March 1994. There were no problems with acceptance of this diet. Significant weight changes occurred in young female Aldabra tortoises while being fed this diet, and growth lines on the carapace scutes were noted even in mature and aged individuals. Stool consistency was firm and similar to that noted in free-ranging tortoises. Although an ethogram for this population has not been produced, keepers believe that more reproductive activity occurs and the tortoises are more active than in previous years.

Additional items consumed that are not part of either the alfalfa hay and biscuit diet, the original diet, or the Walkabout Mix included grass and browse. The grass of the outdoor pen was 3-Way Fescue (40% Falcon, 40% Thunderbird, 20% Bonanza) in 1994, but in fall of 1995 the mix was changed to Liberty Mix (44% perennial rye [Lorolium perenne], 34% red fescue [Festuca rubra], and 18% Kentucky blue [Poa pratensis]). Browse is added to the diet in season (e.g., mulberry Morus sp., forsythia Forsythia sp., poplar Populus sp.) on a daily to weekly basis.

Based on this dietary trial, the following conclusions were made: 1) giant tortoises prefer a high produce diet over a hay-based diet and keepers perceive increased breeding behavior and activity level when the nutrients for the produce diet are balanced; 2) the Walkabout Mix is a nutritionally complete diet since the consumed diet did appear to meet the needs of the tortoises based on the significant growth and weight changes in the tortoises, and the good stool quality noted.

ACKNOWLEDGMENTS

We thank the staff of the Department of Animal Health for their assistance with this project: Donna Ialeggio, DVM for her role in obtaining blood samples and Sandy Skeba, AHT for performing routine hematological analyses. We thank
Ellen Dierenfield of the Wildlife Conservation Society for performing vitamin A and E analyses of the plasma samples.

**Table 1.** Giant tortoise diet used at the Philadelphia Zoo prior to 14 June 1992.

<table>
<thead>
<tr>
<th>Tuesday</th>
<th>Friday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 lbs kale</td>
<td>22 lbs kale</td>
<td>22 lbs kale</td>
</tr>
<tr>
<td>-----</td>
<td>22 lbs spinach</td>
<td>-----</td>
</tr>
<tr>
<td>3 lbs beets</td>
<td>3 lbs beet pulp</td>
<td>-----</td>
</tr>
<tr>
<td>75 lbs carrots</td>
<td>75 lbs carrots</td>
<td>22 lbs carrots</td>
</tr>
<tr>
<td>11 lbs Zoo Cake</td>
<td>11 lbs Zoo Cake</td>
<td>-----</td>
</tr>
<tr>
<td>4.5 lbs bananas</td>
<td>4.5 lbs bananas</td>
<td>-----</td>
</tr>
<tr>
<td>4.5 lbs apples</td>
<td>3 lbs apples</td>
<td>-----</td>
</tr>
<tr>
<td>1 lb oranges</td>
<td>3 lbs oranges</td>
<td>-----</td>
</tr>
<tr>
<td>0.5 lb escarole</td>
<td>2.25 lbs escarole</td>
<td>22 lbs escarole</td>
</tr>
<tr>
<td>12 hard boiled eggs</td>
<td>12 hard boiled eggs</td>
<td>-----</td>
</tr>
<tr>
<td>0.5 lbs grapes</td>
<td>0.25 lbs grapes</td>
<td>-----</td>
</tr>
<tr>
<td>12 lbs potatoes</td>
<td>-----</td>
<td>12 lbs potatoes</td>
</tr>
<tr>
<td>3 tbsp Vionate</td>
<td>3 tbsp Vionate</td>
<td>-----</td>
</tr>
<tr>
<td>-----</td>
<td>0.33 lbs mineral mix</td>
<td>-----</td>
</tr>
<tr>
<td>-----</td>
<td>1 lap salt marsh hay</td>
<td>-----</td>
</tr>
</tbody>
</table>

*aZoo Cake analysis can be provided on request.

**Table 2.** Tortoise biscuit diet trial, offered 14 June 1992 through 1 January 1993.

The base of the diet was 15 lbs ADF 16 pellet (Mazuri) and alfalfa hay *ad libitum* offered daily. In addition to this staple diet, items were offered on the following schedule.

<table>
<thead>
<tr>
<th>Tuesday</th>
<th>Friday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 lbs escarole</td>
<td>-----</td>
<td>4 lbs escarole</td>
</tr>
<tr>
<td>-----</td>
<td>10 lbs kale</td>
<td>6 lbs kale</td>
</tr>
<tr>
<td>3 lbs apples</td>
<td>3 lbs apples</td>
<td>-----</td>
</tr>
<tr>
<td>10 lbs carrots</td>
<td>10 lbs carrots</td>
<td>10 lbs carrots</td>
</tr>
<tr>
<td>-----</td>
<td>10 lbs mustard greens</td>
<td>-----</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>3 lbs potatoes</td>
</tr>
</tbody>
</table>
Table 3. Tortoise biscuit diet trial, offered 2 January 1993 through 29 March 1993.

The base of the diet was 10 lbs tortoise biscuit (Mazuri) and 5 lbs of alfalfa hay soaked for 2 hrs in orange juice offered Tuesday, Wednesday, Friday, Saturday, Sunday. In addition to this staple diet, items were offered on the following schedule.

<table>
<thead>
<tr>
<th>Tuesday</th>
<th>Friday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 lbs escarole</td>
<td>-----</td>
<td>4 lbs escarole</td>
</tr>
<tr>
<td>-----</td>
<td>15 lbs kale</td>
<td>10 lbs kale</td>
</tr>
<tr>
<td>6 lbs apples</td>
<td>6 lbs apples</td>
<td>-----</td>
</tr>
<tr>
<td>15 lbs carrots</td>
<td>15 lbs carrots</td>
<td>15 lbs carrots</td>
</tr>
<tr>
<td>-----</td>
<td>15 lbs mustard greens</td>
<td>-----</td>
</tr>
<tr>
<td>60 g Vionate</td>
<td>60 g Vionate</td>
<td>60 g Vionate</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>6 lbs potatoes</td>
</tr>
</tbody>
</table>

Table 4. Walkabout Mix Diet Trial, begun 24 March 1994 and offered currently.

<table>
<thead>
<tr>
<th>Tuesday</th>
<th>Friday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 lbs greens (endive, romaine, escarole, mustard greens, kale)</td>
<td>35 lbs greens (endive, romaine, escarole, mustard greens, kale)</td>
<td>35 lbs greens (endive, romaine, escarole, mustard greens, kale)</td>
</tr>
<tr>
<td>20 lbs bananas</td>
<td>20 lbs bananas</td>
<td>20 lbs bananas</td>
</tr>
<tr>
<td>15 lbs oranges</td>
<td>15 lbs oranges</td>
<td>15 lbs oranges</td>
</tr>
<tr>
<td>5 lbs chopped timothy hay</td>
<td>5 lbs chopped timothy hay</td>
<td>5 lbs chopped timothy hay</td>
</tr>
<tr>
<td>3 lbs beet pulp</td>
<td>3 lbs beet pulp</td>
<td>3 lbs beet pulp</td>
</tr>
<tr>
<td>5 lbs Walkabout Diet premixa</td>
<td>5 lbs Walkabout Diet premixa</td>
<td>5 lbs Walkabout Diet premixa</td>
</tr>
</tbody>
</table>

*Premix includes certified organically grown clover, certified organically grown dandelion, dry whole egg (culture Salmonella-negative), dried strawberry, dried yellow squash, dried carrot, soybean meal, fresh vitamins, USP-grade calcium carbonate, trace minerals.
Table 5. Nutritional analysis of the Walkabout Mix Diet (dry matter basis).\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>3.0 kcal/g</td>
</tr>
<tr>
<td>Protein</td>
<td>16%</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>13%</td>
</tr>
<tr>
<td>Fat</td>
<td>4%</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>12.5 IU/g</td>
</tr>
<tr>
<td>Vitamin D\textsubscript{3}</td>
<td>3.8 IU/g</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>254 ppm</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.3%</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.6%</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.6%</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.5%</td>
</tr>
<tr>
<td>Iron</td>
<td>400 ppm</td>
</tr>
<tr>
<td>Zinc</td>
<td>45 ppm</td>
</tr>
<tr>
<td>Copper</td>
<td>11 ppm</td>
</tr>
<tr>
<td>Manganese</td>
<td>48 ppm</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.3 ppm</td>
</tr>
<tr>
<td>Iodine</td>
<td>0.7 ppm</td>
</tr>
</tbody>
</table>

\textsuperscript{a}In addition, B vitamins and amino acids were analyzed, but are not included in this table.
\textsuperscript{b}Moisture of the “as fed” diet is 73%.
SINGLE DOSE PHARMACOKINETICS OF CEFTAZIDIME IN LOGGERHEAD SEA TURTLES (Caretta caretta)

M. Andrew Stamper,1,2* Mark Papich,3 Greg Lewbart,1,2 Delta Plummer,3 Stuart May,4 and Michael Stoskopf1,2

1Environmental Medicine Consortium, North Carolina State University, 4700 Hillsborough Street Raleigh, NC 27606 USA; 2Department of Companion Animal and Special Species Medicine, North Carolina State University, 4700 Hillsborough Street Raleigh, NC 27606 USA; 3Department of Anatomy, Physiology, and Radiology College of Veterinary Medicine, 4700 Hillsborough Street Raleigh, NC 27606 USA; 4North Carolina Aquarium, Pine Knoll Shores, Atlantic Beach, NC 28512 USA

Abstract

Antibacterial dosage regimens are poorly established in many reptiles and especially for sea turtles. Ceftazidime is a broad spectrum antimicrobial which is particularly active against gram-negative bacteria such as Vibrio spp., Aeromonas spp. and Pseudomonas spp. often associated with morbidity and mortality of sea turtles.1-3 This study was performed to determine the pharmacokinetics of a single injection of ceftazidime in yearling loggerhead sea turtles (Caretta caretta). Eight juvenile loggerhead sea turtles weighing 1.25 (± 0.18) kg were divided into two groups. Four animals received 20 mg/kg of ceftazidime (Tazidime, Eli Lilly and Company, Indianapolis, IN 46284 USA) intravenously and four received the same dose intramuscularly. Intravenous doses were given in the left cervical sinus. Intramuscular doses were injected into the left deltoid muscle. Repeated blood sampling was performed at times: 0, 0.5, 1.5, 3, 6, 12, 24, 48, 72, 96 and 120 hr post introduction of the drug. Blood collection sites were alternated between right and left cervical sinuses. Blank (untreated) sea turtle plasma was spiked with known concentrations of ceftazidime and analyzed to determine quality control values. Ceftazidime plasma concentrations were detected at all time points for all turtles and were above the MIC for Pseudomonas as long as 72 hr after the i.m. and i.v. injection.

LITERATURE CITED

STUDY ON HERPESVIRUS INFECTIONS IN LAND TORTOISES IN SWITZERLAND

Horst Posthaus, med. vet.,¹* Rachel E. Marschang, med. vet.,¹² Marcus Gravendyck, Dr. med. vet.,¹² and Luca N. Bacciarini, Dr. med. vet.¹

¹Institute of Animal Pathology, University of Berne, Laenggassstr. 122, 3012 Berne, Switzerland; ²Institute for Avian and Reptile Medicine, University of Giessen, Frankfurterstr. 87, 35392 Giessen, Germany

Abstract

Diseases caused by herpesviruses have been reported in different species of turtles. Many of these reports are based on observations of single or isolated cases,²⁻⁶,⁹,¹² but several epizootics caused by herpesviruses were also reported.⁷,⁸,¹⁰,¹¹,¹³ In Europe a herpesvirus infection which causes typical pathological lesions in land tortoises seems to be responsible for significant morbidity and mortality among these animals in captivity.⁵,⁹,¹⁰,¹¹ A similar disease associated with herpesvirus particles was also described in the United States.⁴,⁷,¹² However, current data about the prevalence of herpesvirus infections in land tortoises are lacking.

In a retrospective study we investigated the occurrence of herpesvirus infections in land tortoises which had been necropsied since 1988 at the Department of Zoo Animal Pathology, Institute of Animal Pathology, Berne. Moreover, systematical examinations on the distribution of herpesvirus induced lesions have been performed since 1995.

From 1988 to 1996 a total of 914 postmortem examinations of land tortoises were carried out. In 142 cases (15.5%) a herpesvirus infection was diagnosed based on the presence of typical eosinophilic intranuclear inclusion bodies in histological sections. Selected and ambiguous cases were examined by electron microscopy to confirm the histological diagnosis. Among the 142 affected animals were 99 spur tailed tortoises (Testudo hermanni, 69.7 %), 29 spur thighed tortoises (T. graeca, 20.4 %), 8 leopard tortoises (T. pardalis, 5.6 %), 4 marginated tortoises (T. marginate, 2.8 %), 1 four-toed tortoise (T. horsfieldii, 0.7 %), and 1 yellow-footed tortoise (T. denticulata, 0.7 %). Many of the animals had been captive bred in Switzerland but some were also imported.

Pathological lesions were mainly characterized by lesions in the upper digestive tract. An ulcerative to diphtheroid-necrotizing stomatitis and glossitis was seen in most of the cases. Histologically, epithelial necrosis in the oral cavity, the pharynx, and the esophagus were found. Eosinophilic intranuclear inclusion bodies were present in and around degenerating epithelium. The second most frequently affected organ system was the respiratory tract. Intranuclear inclusion bodies were found in areas of epithelial necrosis in the trachea and lung. Bacterial superinfections frequently caused a severe inflammatory reaction. Moreover intranuclear inclusion bodies in neurones and glial cells of the brain were detected in about 25 % of our cases. Other organs that were inconsistently found to be affected are the stomach, small and large intestine, cloaca, liver, and spinal cord. In all of these organs intranuclear inclusion bodies were detected. Electron microscopy revealed intranuclear and
intracytoplasmic herpesvirus-like particles.

In our study no sex or age predisposition was evident. However an increase in the frequency of the disease was noted in spring, when tortoises wake up from hibernation.

Our data demonstrate that herpesvirus infections frequently occur in captive land tortoises in Switzerland and suggest a wide distribution of this disease. A similar situation can be assumed for other European countries. Like in mammals and birds, reptilian herpesviruses possibly induce latent and persistent infections of their hosts. Virulent herpesviruses could be easily distributed and be introduced into immunological naive populations resulting in a high mortality. Therefore the disease is of concern for owners, breeders, the pet trade, zoos, and conservation programs.

LITERATURE CITED

CLINICAL SIGNIFICANCE OF Cryptosporidia IN CAPTIVE AND FREE-RANGING CHELONIANS

Bonnie L. Raphael, DVM, Dipl ACZM,* Paul P. Calle, VMD, Dipl ACZM, Nicole Gottdenker, DVM, Stephanie James, DVM, William R. Karesh, DVM, Michael J. Linn, DVM, Tracey McNamara, DVM, AVCP, and Robert A. Cook, VMD

Wildlife Health Sciences, Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, NY 10460 USA

Abstract

Cryptosporidium spp. are protozoa known to infect a wide range of vertebrates. Cryptosporidium parvum and C. muris are mammalian species, C. baileyi and C. meleagridis infect avians, and C. nasorum are found in fish. A single species, C. serpentis, has been documented in reptiles, although there is evidence to suggest that there may be multiple species or subspecies. C. serpentis in snakes may be clinically inapparent or cause signs ranging from mid-body swelling, regurgitation, and loss of condition, to death. In other reptiles, clinical signs are not as dramatic if they occur at all, and have seldom been reported. It has been shown that C. parvum is not infective for snakes, fish or amphibians, and that C. serpentis is not transmissible to laboratory mice. Transmission of C. serpentis among different groups of reptiles has not been documented.

Diagnosis of Cryptosporidium in feces is best achieved by performing combined battery of tests consisting of a modified acid fast stain (MAFS), enzyme immunoassay (EIA), and immunofluorescence antibody test (IFA). As many as 66-100% false-negative results may occur using only MAFS testing, and positive tests may, in fact, reflect the presence of nonpathogenic Cryptosporidia. When all three tests are run, it is possible to differentiate C. serpentis from C. parvum.

Fecal samples from captive bred radiated tortoises (Geochelone radiata) located at three different sites, recently imported Indian star tortoises (Geochelone elegans) and travancore-like tortoises (Indotestudo sp.), and free-ranging gopher tortoises (Gopherus polyphemus) and Russian tortoises (Testudo graeca nikolskii) were collected and stored fresh, in formalin, or in PVA (Polyvinyl alcohol, Transcaddy, Baxter Health Care Corp, West Sacramento, California 95691 USA) until processed. Samples were examined by MAFS, EIA, and IFA.

Nineteen of 38 radiated tortoises, 8/10 Indian star tortoises, 3/4 Indotestudo, 8/20 gopher tortoises and 4/29 Russian tortoises were positive for Cryptosporidium sp. by MAFS and IFA, and negative by EIA. None of the tortoises had clinical signs indicative of enteric protozoa infections. Of animals that have been examined post mortem, one had numerous Cryptosporidia-like organisms associated with microvillar borders, without any significant pathology noted, and another had severe necrotizing transmural gastroenteritis with massive numbers of bacterial colonies, sloughed epithelial cells, karyorrhectic cellular debris and clusters of Cryptosporidia-like organisms admixed within the luminal debris. Prolonged contact between positive and negative animals has not been associated...
with the onset of clinical signs, or with conversion of all animals to a positive test status. However, since it is not known if the organism found in chelonia is infective for snakes, it is prudent to maintain test positive turtles and tortoises separate from Squamata.

ACKNOWLEDGMENTS

The authors thank Dr. Thaddeus Graczyk at the Johns Hopkins University School of Hygiene and Public Health, Department of Molecular Microbiology and Immunology, 615 N Wolfe Street Baltimore MD, 21205 USA, (410)614-4984 for performing the laboratory procedures, to Dr. Bill Zovickian for his participation and financial support, to Jeff Spratt for collection of samples, and to John Behler for facilitation of surveys.

LITERATURE CITED

THE EFFECTS OF HEMOLYSIS ON PLASMA ELECTROLYTE AND CHEMISTRY VALUES IN THE COMMON GREEN IGUANA (*Iguana iguana*)

Keith G. Benson, DVM,1* Joanne Paul-Murphy, DVM, Dipl ACZM,2 and Peter MacWilliams DVM, Dipl ACVP3

1Department of Medical Sciences, 2Department of Surgical Sciences, 3Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI  53706 USA

Abstract

Hemolysis of serum and plasma samples is a common problem in veterinary diagnostic laboratories. Hemolysis occurs when red blood cells contact foreign surfaces, when small gauge needles are used to obtain samples or when samples are handled inappropriately. Samples taken during field investigation of free-ranging wildlife, or by inexperienced phlebotomists frequently result in erythrocyte disruption. Significant changes in plasma and serum electrolyte and chemistry values have been described in domestic species3, zoological species1 and human beings.2-4 The species specific effects of hemolysis depend upon variations in the intracellular concentrations of the analytes. Methodology and instrumentation will also have a profound effect of the degree to which hemolysis will effect clinical chemistry. Previous studies have indicated that many of these alterations are related to the severity of hemolysis.

Ten common green iguanas (*Iguana iguana*) were individually housed at the University of Wisconsin School of Veterinary Medicine animal housing facility and fed a commercially available formulated diet. Animals were anesthetized using 30 mg/kg of ketamine i.m. in the triceps muscle. Three to 5 cc of non-hemolyzed whole blood was collected from the right atrium using a 1.5-in 22-ga needle and a 5 cc syringe. Samples were immediately placed in lithium heparin tubes. One aliquot of blood was centrifuged and the non-hemolyzed plasma harvested. A second aliquot was severely hemolyzed by rapid freezing and the plasma harvested. Consistent levels of hemolysis were achieved by adding known quantities of hemolyzed plasma to the original non-hemolyzed samples. The degree of hemolysis in the plasma was determined using a Kodak Interference Guide color chart. Two levels of hemolysis, moderate and severe were developed, and the hemolyzed and non-hemolyzed samples were frozen at -70 °C for 8 mo. Nine serum analytes were measured using a Kodak Echtachem 500 Chemistry analyzer. Mean values and SD for non-hemolyzed, moderately hemolyzed and severely hemolyzed plasma were compared (Table 1).

The presence and degree of hemolysis must be considered when interpreting clinical chemistries in the common green iguana (*Iguana iguana*).

As an adjunct to this study we developed a technique to draw a relatively large sample of blood from the smaller (0.5 kg) iguana. Anesthetized animals were placed in dorsal recumbency and held at the edge of an examination table. A 22-ga 1.5-in needle on a 5 cc syringe was inserted into the thoracic inlet and advanced towards the opposite pelvic limb. Sampling by this method was quick, effective
and without complication in all animals sampled. To document the site of cardiopuncture, digital subtraction angiograms were performed and contrast media (iohexal) was found to be present in the right atria before proceeding to throughout the circulation.

ACKNOWLEDGMENTS

We would like to acknowledge Dr. Chet Thomas DVM, PhD for his help with the statistical analysis.

LITERATURE CITED


Table 1. Effect of hemolysis on plasma electrolytes and chemistries; values are means (n = ---)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>No Hemolysis</th>
<th>Mild Hemolysis</th>
<th>Marked</th>
<th>Statistical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (Na)</td>
<td>166</td>
<td>164</td>
<td>163</td>
<td>NS</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>4.1 a</td>
<td>4.6 a,b</td>
<td>5.3 b</td>
<td>P&lt; 0.02</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>12.0</td>
<td>11.7</td>
<td>11.4</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>6.8 a</td>
<td>8.7 b</td>
<td>10.7 c</td>
<td>P&lt; 0.01</td>
</tr>
<tr>
<td>Uric acid</td>
<td>1.5</td>
<td>1.4</td>
<td>1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Total protein</td>
<td>5.6 a</td>
<td>6.8 a,b</td>
<td>7.0 b</td>
<td>P&lt; 0.05</td>
</tr>
<tr>
<td>Albumin</td>
<td>2.9 a</td>
<td>3.0 b</td>
<td>3.1 b</td>
<td>P&lt; 0.07</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST)</td>
<td>32 a</td>
<td>50 a,b</td>
<td>144 b</td>
<td>P&lt; 0.04</td>
</tr>
<tr>
<td>Creatine kinase (CK)</td>
<td>280</td>
<td>308</td>
<td>477</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: Means with different superscript letters in each row are significantly different at the P level indicated.
TERATOMA IN DESERT GRASSLAND WHIPTAIL (*Cnemidophorus uniparens*)

Maryanne E. Tocidlowski, DVM, Michael R. Loomis, DVM, MA, Christine L. Merrill, DVM, and James F. Wright, DVM, PhD

1Department of Companion Animal and Special Species Medicine and 3Department of Microbiology, Pathology, and Parasitology, North Carolina State University, College of Veterinary Medicine, Raleigh, NC 27606 USA; 2North Carolina Zoological Park, 4401 Zoo Parkway, Asheboro, NC 27203 USA

Abstract

This report outlines the findings of teratoma in two desert grassland whiptails (*Cnemidophorus uniparens*), a southwestern lizard species of the Family Teiidae. Case one was a wild born female *C. uniparens* from New Mexico, received into quarantine at the North Carolina Zoological Park at approximately 2-yr-old. It had no history of medical problems while in captivity. One year after capture, it became lethargic, anorexic and appeared distended. One month previously, it had successfully laid eggs. On physical examination, it became slightly dyspneic. No eggs were palpated. Dorsoventral radiographs showed fluid density in the caudal coelomic cavity, lungs slightly compressed cranially, and no evidence of calcified eggs. The lizard became progressively debilitated after a bout of normal feeding behavior and defecation and was later found dead in its cage. At gross necropsy, the primary abnormality was a large (1.5 × 2.0 cm) dark colored cystic structure in the coelomic cavity. Histologic examination showed the walls of the cyst structure contained fronds of pseudostratified to columnar epithelium with occasional subadjacent follicular structures. The majority of the section contained irregular masses composed of well differentiated keratinizing stratified squamous epithelium overlaying haphazardly arranged blood filled spaces, cartilage, clumps of glandular epithelium, adipose tissue, and neuroglial cells.

Case two was a captive born female lizard with no direct relationship to the first case. At 2-yr-old, it developed coelomic distention and appeared gravid for a long period of time. Activity and feeding remained normal. Physical examination revealed palpable round masses in the caudal coelomic cavity which were presumed to be eggs. The animal was placed back in its enclosure for monitoring and was found dead 3 days later. At gross necropsy, a 2-cm-diameter mass was identified and the colon was impacted with feces. Histopathologic examination of the mass showed a disorganized arrangement of well differentiated tissues representing skin, digestive tract, fat, bone, respiratory tract, cartilage, and smooth muscle surrounded by a thin fibrous capsule.

*Cnemidophorus uniparens* is a parthenogenic species. Most parthenogenic species are polyploid (2-4N), are of female gender, and often have both male and female sexual behaviors. Individuals are considered to be clones of each other although family lines may be far removed. Although the two animals in this report were not directly related, they technically have the same genetic make-up. Parthenogenic reproduction for *C. uniparens* involves a premeiotic endoreduplication followed by two meiotic divisions preserving the triploid chromosomal number.
Teratoma is a rare neoplasm of domestic animals. Ovarian teratoma has been reported in the green iguana (*Iguana iguana*) and ovarian teratoadenocarcinoma also in the green iguana. It is a tumor of the germ cells, is comprised of one or more of the primary germ layers (ectoderm, mesoderm, endoderm), occurs in both males and females, and can be gonadal or extragonadal. Teratomas are believed to be parthenogenic tumors that have developed from a single germ cell after the first meiotic division. In humans they are characterized as benign or cystic (dermoid cyst), immature or malignant, or specialized. Usually teratomas in animals are well differentiated and benign and are composed of a combination of recognizable tissues of germ cell origin.

No chromosomal analysis was conducted on the teratoma tissues; therefore, it is unknown at what stage of division the neoplasia occurred or the chromosomal number present in the tumors of these lizards. It is assumed that the formation of teratoma in this species would be similar to formation in mammals, but it is unknown if a parthenogenic neoplasia in a parthenogenic lizard species is different than that found in mammals. An etiology of teratogenesis was not identified.

**LITERATURE CITED**

PRELIMINARY SINGLE-DOSE PHARMACOKINETICS OF ENROFLOXACIN AFTER ORAL AND INTRAMUSCULAR ADMINISTRATION IN GREEN IGUANAS (Iguana iguana)

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Abstract

Enrofloxacin (Baytril, Bayer Corp., Shawnee, KS 66201 USA) is a broad-spectrum fluoroquinolone antibiotic with good activity against many gram-negative bacteria and some gram-positive bacteria. Its use in green iguanas has previously been empirical as no enrofloxacin pharmacokinetic studies have been published in any lizard. In this study, six green iguanas, ranging in size from 374-1740 g, were given injectable enrofloxacin p.o. at 5 mg/kg. Three iguanas (672-916 g) were given enrofloxacin at 5 mg/kg i.m. Blood samples were taken at 0, 0.5, 1, 3, 6, 12, 24, 48, 72, and 96 hr post-administration. The plasma concentrations of both ciprol ofloxacin and enrofloxacin were measured using high-performance liquid chromatography (HPLC). After oral and i.m. administration, the plasma concentration-time curve followed a two-compartment model. The elimination half-life of enrofloxacin was 22.3 ± 11.3 hr (x ± SD) after p.o. administration and 26.0 ± 10.2 hr after i.m. administration. Enrofloxacin was absorbed fairly rapidly with a mean time to reach peak plasma concentration of 2.7 ± 2.6 hr following oral administration and 1.0 ± 0 hr following i.m. administration. The mean maximal plasma concentration (Cmax) achieved was 1.16 ± 0.54 µg/ml after oral administration and 2.03 ± 0.52 µg/ml after i.m. administration. While therapeutic enrofloxacin plasma concentrations (> 0.2 µg/ml) were obtained in all iguanas administered enrofloxacin, there was considerable variability in the time that enrofloxacin was maintained at or above therapeutic levels following oral dosage. Therapeutic enrofloxacin plasma concentrations were maintained for 31.7 ± 32.1 hr after oral administration and 16 ± 6.9 hr following i.m. administration. As plasma ciprofloxacin levels were always below the limit of quantitation of the assay and usually below the limit of detection, enrofloxacin does not appear to be metabolized to ciprofloxacin in significant amounts in green iguanas. Although therapeutic plasma enrofloxacin concentrations were achieved following oral administration, the marked variability of the pharmacokinetic parameters of enrofloxacin after oral administration may make the parenteral route more suitable for the treatment of critical infections in green iguanas.
METRONIDAZOLE PHARMACOKINETICS IN YELLOW RAT SNAKES (Elaphe obsoleta quadrivitatta)

Christine M. Kolmstetter, MS, DVM,* Donita Frazier, DVM, PhD, Sherry Cox, MS, and Edward C. Ramsay, DVM

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Abstract

Anaerobic bacterial infections are a common problem in reptiles, and although they are frequently treated with metronidazole, there are no reported pharmacokinetic studies on metronidazole in any reptile. This study examined metronidazole pharmacokinetics in wild-caught, yellow rat snakes, Elaphe obsoleta quadrivitatta. A preliminary dosage trial evaluated 20 mg/kg and 100 mg/kg p.o., each dose given to two rat snakes. These snakes were bled by cardiocentesis at 0, 4, 8, 11.5, and 23 hr. Plasma metronidazole levels were analyzed by high performance liquid chromatography and indicated that the 20 mg/kg dose produced peak metronidazole levels above reported minimum inhibitory concentrations (MIC) for most anaerobic bacterial pathogens (MIC 2-4 µg/ml). Steady state pharmacokinetics were then evaluated in five rat snakes. The snakes were dosed with metronidazole at 20 mg/kg p.o. every 48 hr for six doses. Snakes were bled by cardiocentesis at 0, 4, 8, 12, 24, and 48 hr prior to and following the initial and the final treatment, and plasma metronidazole levels were obtained. Based on these data, maximum plasma metronidazole concentrations following the initial and final treatment were 14 µg/ml and 13 µg/ml, respectively. Time of maximum concentration following the initial and final treatment were 1.4 and 4.0 hr, respectively. Mean metronidazole levels remained above 4.0 µg/ml for 24 hr following initial treatment and 48 hr following the last treatment. No adverse effects were observed in any snake. These data indicate that a metronidazole dosage of 20 mg/kg p.o. every 48 hr should be adequate for the treatment of most anaerobic infections in yellow rat snakes.
PRELIMINARY KINETICS OF SINGLE-DOSE INTRAVENOUSLY ADMINISTERED ENROFLOXACIN AND OXYTETRACYCLINE IN THE AMERICAN ALLIGATOR (Alligator mississippiensis)

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Abstract

In September 1995, an epizootic characterized by progressive lethargy, anorexia, ocular discharge, edema, weakness, and generalized paraparesis resulted in the death or euthanasia of 74 captive American alligators (Alligator mississippiensis) from a male herd of 89 conspecifics located in St. John’s County, Florida. Pathologic findings included a fibrinous tracheitis, pneumonia, polyarthritis, and fibrinous coelomitis. In addition to a variety of gram-negative bacteria isolated from multiple tissues, a new Mycoplasma, tentatively named Mycoplasma lacerti, was cultured from the lung, trachea, joints, and cerebrospinal fluid of symptomatic alligators.1

Nine Mycoplasma isolates were obtained from six symptomatic alligators and the minimum inhibitory concentration (MIC) for nine antibacterial agents were determined by serial dilution in broth and plate culture of each mycoplasma isolate. The MIC obtained for doxycycline, enrofloxacin, sarafloxacin, oxytetracycline, tilmicosin, and tylosin (<1 µg/ml) were lower than that of clindamycin (1-8 µg/ml), chloramphenicol (8-16 µg/ml) and erythromycin (32-128 µg/ml). Based upon these results, enrofloxacin and oxytetracycline were chosen for pharmacokinetic evaluation.

The seven alligators used in this study were captive-reared, of unknown gender, clinically healthy, and ranged in weight from 2.85-4.70 kg. Alligators were acclimated at 27°C for 5 days prior to the study and maintained at this temperature during the period of sample collection. Prior to drug administration, a time zero blood sample was obtained for baseline analysis and generation of a standard curve. Five alligators received enrofloxacin (Baytril, 22.7 mg/ml, Bayer Co., Shawnee Mission, KS 66201 USA) at 5 mg/kg and two alligators received oxytetracycline (Liquamycin LA-200, 200 mg/ml, Pfizer, New York, NY 10017 USA) at 10 mg/kg as a single intravenous bolus in the supravertebral vein. All alligators were manually restrained and 2.0 ml of blood collected from the supravertebral vein into lithium heparinized tubes at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 84, and 96 hr following drug injection. Plasma was separated by centrifugation, aliquotted into 1 ml plastic cryotubes and frozen at -10°C.

Sample analysis for both drugs were performed using high-performance liquid chromatography (HPLC) (North Carolina State University, Raleigh, NC 27606 USA). The limit of quantitation
(LOQ) for enrofloxacin was 0.05 µg/ml and the limit of detection (LOD) 0.02 µg/ml. For oxytetracycline, the LOQ was 0.25 µg/ml and the LOD approximated 0.20 µg/ml. For each animal, a plasma drug concentration-versus-time curve was generated. Compartmental modeling was performed using computer software for polyexponential curve stripping, fitting and least squares parameter estimation of the data (RSTRIP II version 1.0, MicroMath, Salt Lake City, UT 84110 USA).

Both enrofloxacin and oxytetracycline were best described by a two-compartment model. At the doses administered, both enrofloxacin and oxytetracycline exceeded the target MIC for *Mycoplasma lacerti* of 1.0 µg/ml. Enrofloxacin achieved a mean peak plasma concentration of 6.03 µg/ml and oxytetracycline a mean peak plasma concentration of 98 µg/ml at time zero. Plasma enrofloxacin concentrations were maintained above 1.0 µg/ml for an average of 31 hr, while the mean plasma oxytetracycline level at 96 hr was 6.52 µg/ml. In order to maintain plasma concentrations above the target MIC for two-thirds of the dosing interval, enrofloxacin at 5 mg/kg should be administered every 36 hr for *Mycoplasma lacerti* infections. Further sampling beyond 96 hr is necessary before an accurate dosing interval can be proposed for oxytetracycline.

**LITERATURE CITED**

PULMONARY NOCARDIOSIS IN A GROUP OF BLACK CRAKES (*Limnocorax flavirostra*)
AT THE BASLE ZOO, SWITZERLAND

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Abstract

Natural nocardial infection has been reported in many different species including mammals and fish.5,7 Reports in birds remain uncommon.1–6

From September 1996 to February 1997, eight juvenile black crakes (*Limnocorax flavirostra*) from a group of 12 died unexpectedly at the Basle Zoo. The birds had been housed in an aviary together with a pair of red-billed hornbills (*Tokus erythrorhynchus*). The exhibit consisted of an outdoor cage with a natural sand ground, and an indoor part with a substrate of chopped bark. The volume of the aviary was approximately 110 m³.

Necropsy revealed that five of the submitted crakes were in poor body condition. Disseminated white, raised, firm, well circumscribed nodules, 1–3 mm in diameter, were noted throughout the lung parenchyma of all birds. Furthermore, a severe splenomegaly was observed. Histologically, the lungs had multiple, often confluent, granulomas with moderate to severe central necrosis. The necrotic center was surrounded by a margin composed of heterophils, macrophages, lymphocytes, and some multinucleated giant cells. Various numbers of delicate, gram-positive, 0.5–1.0 μm wide, branching, occasionally beaded, filamentous organisms were visible in the necrotic centers. These organisms were acid-fast if stained with Fite-Faraco, but not with the Ziehl-Neelsen acid-fast stain. No histologic lesions were seen in the other organs.

A microbiological examination was carried out in three cases and *Nocardia asteroides* nova was isolated from samples of the liver, spleen, kidney and lung. A diagnosis of severe granulomatous and necrotizing nocardial pneumonia with agonal septicemia was made. This suggests an aerogenous infection.

Fecal samples were collected at necropsy and from crakes in the aviary. No parasites were detected with flotation, sedimentation, and direct mount examination.

To resolve the problem in the exhibit, the aviary was cleaned, disinfected, and the chopped bark was changed. To our knowledge, epizootic outbreaks of nocardiosis in birds are not reported in the literature. Whether nocardiosis is a truly rare disease in birds, or is simply not recognized, is unknown. In the past, nocardiosis has often been confused with mycobacterium, actinomyces, and...
streptomyces infections in both animals and man.

LITERATURE CITED

SERIAL Aspergillus ANTIBODY LEVELS AND SERUM PROTEIN ELECTROPHORESIS AS A DIAGNOSTIC AND TREATMENT MONITORING TECHNIQUE IN HUMBOLDT PENGUINS (Spheniscus humboldti)

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Abstract

An outbreak of aspergillosis occurred in the Woodland Park Zoo’s flock of Humboldt penguins. The outbreak was thought to be related to changes in keepers, cleaning procedures, and fish quality. After several deaths, a program of intensive treatment and monitoring was begun. Penguins were treated with itraconazole, multiple antibiotic regimes, fluids, and tube feedings. Monitoring was accomplished with complete blood counts, clinical chemistries, serum protein electrophoresis, and Aspergillus levels. White blood cell counts, Aspergillus levels, and electrophoretic albumin levels were helpful in diagnosis and in monitoring treatment success. Sick birds tended to have high white blood cell counts, high Aspergillus antibody levels, and/or low albumin values. As birds improved, their white blood cell counts and Aspergillus antibody levels decreased and their albumin levels increased. Very low albumin levels did not indicate a poor prognosis in this study.

Introduction

Penguins are extremely susceptible to Aspergillus infections and mortality from these infections is often high.1,2,4,5 Antemortem diagnosis of aspergillosis can be difficult but several authors have reported on the usefulness of Aspergillus antibody levels and serum protein electrophoresis (SPE) in identifying affected birds.3,5 In addition, albumin and globulin levels determined by electrophoresis have shown some promise as prognostic indicators.5

An outbreak of aspergillosis and suspected secondary bacterial infections occurred in the Woodland Park Zoo’s flock of Humboldt penguins (Spheniscus humboldti). Only one bird died of confirmed aspergillosis between 1987 and 1995. However, six birds died of aspergillosis between March 1995 and September 1996. In September 1996, one bird died and five others were noted to be clinically ill and a program of intensive monitoring and treatment was begun with the remaining birds in the flock. The goals of this program were to use complete blood counts, clinical chemistries, Aspergillus levels and SPE in an attempt to (1) rapidly detect clinical cases, (2) effectively monitor treatment in birds who became ill, and (3) to further evaluate the efficacy of SPE and Aspergillus antibody levels as diagnostic and prognostic tools.

Methods

At the beginning of the study period, the 13 birds in the flock were bled for complete blood counts, clinical chemistries, Aspergillus antibody levels, and SPE. Serum protein electrophoresis was
performed by Phoenix Central Laboratory in Everett, WA in a process that has been described by other authors. Aspergillus antibody levels were determined by The Raptor Center at the University of Minnesota (1920 Fitch Ave, University of Minnesota, St Paul, MN 55108 USA) using an ELISA test. Reported numbers are optical density readings with 0.12 being the minimal threshold for a positive result. Because serial dilutions are not performed, these values are not considered titers (Patrick T. Redig, personal communication).

All birds were vaccinated with an Aspergillus vaccine (Willamette laboratories, 9108 NE Sandy Blvd., Portland, OR 92270 USA) and placed on oral itraconazole (Sporanox™, Janssen Pharmaceutica, Inc., Titusville, NJ 08560 USA) at a dosage of 10 mg/kg s.i.d. Sick birds were placed on broad spectrum injectable antibiotics and other supportive treatments (s.c. fluids, tube feeding, etc.) as needed. Serial white blood cell counts, Aspergillus antibody levels, SPE, and clinical impressions were used to evaluate treatment protocols. In addition, a computerized axial tomography (CAT) scan was performed under isoflurane anesthesia on the sickest bird (bird 1) to determine the extent of pulmonary and air sac involvement. A postmortem CAT scan on a confirmed Aspergillus negative bird (bird 9) was used for comparison.

Blood work was repeated on all birds after 6 mo to reevaluate the health of the flock. Trends in white blood cell counts, protein electrophoretic data, and antibody levels were evaluated using correlation coefficients and scattergrams. These coefficients and scattergrams were generated with STATVIEW 4.02 on an Apple Macintosh computer.

Results

Initial evaluation of the 13 birds in the flock revealed that five birds had positive Aspergillus antibody levels (birds 1,3,6,7,8) ranging in value from 0.135 to 0.248. Five birds (birds 1-5), only two of which had positive levels (birds 1,3) on initial evaluation, were clinically ill at that time. Birds 2 and 5 developed positive antibody tests within the next month. Early clinical signs of aspergillosis in our penguins included lethargy, decreased swimming time, and dehydration. Later signs included a decrease in appetite, regurgitation, gaping, sitting back on the tail instead of upright, and in very late stages standing stooped over. The sick birds had an average white blood cell count of 27,400 cells/ml and an average albumin of 1.61 g/dL. The eight healthier birds, three of which had positive Aspergillus levels, had an average white blood cell count of 15,800 cells/ml and an average albumin of 2.33 g/dL.

Three (birds 3,4,5) of the five sick birds were treated intensively for 3 mo, then taken off antibiotics and continued to do well. The other two birds (birds 1 and 2) remained on intensive treatment for an additional 2 mo.

During this time, one bird who was not ill (bird 9), fought with another bird, became septic and died from severe gout. No Aspergillus lesions were seen on postmortem examination and cultures were negative. In addition, one of the previously healthy birds (bird 6), who had initially had a positive Aspergillus test became clinically ill with aspergillosis, just as birds 1 and 2 were improving.
When blood analyses were repeated at 6 mo, bird 6 was the only sick bird. Two birds, 1 and 6, had positive *Aspergillus* antibody levels of 0.181 and 0.176 respectively. The flock had an average white blood cell count of 20,277 cells/ml$, an average albumin of 2.77 g/dL, and an average *Aspergillus* level of 0.115.

Serial measurements in the five original sick birds revealed a general decrease in *Aspergillus* antibody level and total white blood cell counts with a general increase in albumin level as the birds improved clinically (Fig. 1). Although albumin levels tended to increase and total white blood cell counts tended to decrease as *Aspergillus* antibody levels decreased, the correlations between these variables were not statistically significant. Albumin levels were lowest in birds who were doing poorly and tended to increase as birds became healthier.

A CAT scan done on bird 1 revealed lesions in the cranial thoracic air sacs that suggested fungal infiltration. These lesions were visualized as multi-septated planes of soft-tissue within the air sacs. No lesions were seen in the lungs.

**Discussion**

Changes in keeper coverage and cleaning procedures in the unit are thought to have contributed to this outbreak. In particular, the excessive use of bleach in cleaning the penguin pool is thought to have been an important stressor. The use of new keepers in the area who were less familiar with penguin behavior may have delayed rapid identification of sick birds and increased the initial mortality rate. Finally, poor fish quality was also a contributing factor. The severity of this outbreak and the expense of treatment reiterates the importance of careful evaluation of changes in routine when dealing with penguins. In addition, having keepers who can recognize early clinical signs of illness and who are willing to aggressively pursue fluid and nutritional therapy is crucial in bringing an outbreak under control.

Since the program of intensive monitoring and treatment began, only one bird has died and this death was not attributable to aspergillosis. In addition, the health of the whole flock has improved as evidenced by a decrease in the number of sick birds (five to one), a decrease in the average total white blood cell count, an increase in the average albumin level, and a decrease in the number of birds with positive levels (five to two).

Serum protein electrophoresis did give important diagnostic information. Three of the original five sick birds (birds 2, 4, and 5) strongly suspected to have aspergillosis based on clinical signs and high white blood cell counts had negative *Aspergillus* levels. However, these birds did have low albumin levels. These birds are suspected to have had negative levels and low albumin levels due to immunosuppression. This is consistent with Reidarson’s report of birds confirmed with aspergillosis on postmortem examination that had negative levels. Two of our birds (2 and 5) did develop positive *Aspergillus* levels later in the disease course, possibly when they were improving enough to mount an immune response.
Single SPE data points did not appear to give reliable prognostic information. In Reidarson’s report,\textsuperscript{5} 27 of 29 birds with albumin values less than 1.8 g/dL died.\textsuperscript{5} In contrast, all six of our sick birds had albumin values less than or equal to 1.8 g/dL at some point in the disease. These albumin values then increased as the birds were treated. One explanation for this difference in results may be the intensive treatment that our very sick birds received.

Although total white blood cell count, albumin level, and antibody levels all give important clinical information, a significant correlation is not present among these variables. Thus, none of these tests can be used to predict values in the other tests (e.g., animals with low albumin values may not show high white blood cell counts). Therefore, diagnosis is likely to be most accurate when using all three tests. In our flock, for example, it was important to treat birds who had some clinical signs of aspergillosis, elevated white blood cell counts, and decreased albumin levels even when they had negative \textit{Aspergillus} antibody tests.

Because immunosuppression is a feature of aspergillosis, birds are susceptible to secondary bacterial infections. Some of the elevations in white blood cell count seen in our birds may have been due to these infections as white blood cell counts were somewhat responsive to antibiotics. Deep tracheal cultures were not performed in our birds due to the stressful nature of this procedure so it is difficult to confirm the presumptive joint contributions of fungal and bacterial pathogens.

CAT scans, although not widely accessible, can give useful information about the extent of fungal infiltration of the air sacs. The lesions seen in bird 1’s scan would not have been apparent on radiographs. Location of lesions can be accurately determined which is important if considering surgical debulking of fungal plaques. Thus, this may be a useful tool when available. Difficulties with this procedure include the need for anesthesia and the need for the bird to be recumbent during the scan. In addition, since lesions can be subtle, scans need to be interpreted by someone familiar both with CAT scans and with bird anatomy.

ACKNOWLEDGMENTS

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LITERATURE CITED


**Figure 1.** Changes in white blood cells, *Aspergillus* titer, and albumen levels over time.
A TECHNIQUE FOR SEX IDENTIFICATION OF IN OVO AVIAN EMBRYOS

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Abstract

A technique for identifying the sex of a bird embryo in the egg was requested by managers of endangered species conservation programs, to choose the sex of offspring to meet demographic and genetic goals, and to place single sex eggs in wild bird nests as a tool for reintroduction programs. A study was initiated at the International Crane Foundation (ICF) to determine if small blood samples could be safely collected from developing crane eggs and then used to determine the embryo’s sex using a DNA probe/restriction fragment length polymorphism technique.

Thirty-five fertile eggs from seven crane species (brolga [Grus rubicundus], demoiselle [Anthropoides virgo], hooded [Grus monachus], red-crowned [Grus japonensis], sandhill [Grus canadensis], siberian [Grus leucogeranus], white-naped [Grus vipio]) that were identified as surplus to the Species Survival Plan’s (SSP) breeding and reintroduction program goals were used in the study. The 12 control and 23 “sexed” eggs were artificially incubated using standard ICF protocols. Embryonic development was monitored by weighing and candling. Between the 16th and 25th day of incubation, a 1-cm circular area of the shell above the equator of the egg was removed using a belt sander. A drop of sterile mineral oil was applied to the exposed shell membrane, and with candler transillumination, underlying chorio-allantoic vessels were visualized. A vessel was cannulated using a 27-30-ga needle and 1 cc syringe, and 0.01-0.05 cc of blood was collected and then transferred to a tube containing 2 cc of 70% ethanol. The egg shell hole was covered with a piece of sterile adhesive semi-occlusive wound/IV dressing (Tegaderm, 3M Animal Care Products, St. Paul, MN) that extended 2-4 mm beyond the hole. The eggs were then incubated until they hatched or until the normal incubation period was exceeded. Chicks were euthanatized on the day of hatch. All the chicks and unhatched eggs were necropsied, and gonadal sex was determined grossly and histopathologically. Fixed blood samples from four species were submitted to AviGene Services, Inc., Madison, WI where the DNA was isolated and subjected to RFLP analysis using a commercial turkey DNA probe that marks both Z and W chromosomes.

There were no differences in the egg weight loss rates between control and “sexed” eggs. Nine of 12 (75%) of control eggs successfully hatched; 15 of 23 (65%) of “sexed” eggs hatched. However, hatching success of “sexed” eggs improved markedly during the second half of the breeding/research season (83% versus 45% for the first half), as the blood collection technique was refined. Of the eight “sexed” embryos that died, six died within a few days of the blood collection procedure, and the remaining two died close to the time of hatch. Infection was a possible factor in only one of these eggs; a coagulase negative Staphylococcus sp. was cultured. Blood was successfully collected from 20 of the 23 eggs (87%). There was 100% (11/11) concurrence between the DNA probe and gonadal sex determination results.
The sex of avian embryos can be determined in the egg by collecting blood for DNA probe analysis, with a good chance for continued normal development and successful hatching of the egg. Factors that seem to increase the success of this technique include using it on species whose eggs are thin-shelled and lightly pigmented so chorio-allantoic vessels can be identified by candling, collecting the blood during the second half of the incubation period, and careful management of the eggs after the shell membrane has been exposed, especially during hatching. This technique for in ovo avian sex determination has the potential to be a useful tool for the management of commercial, zoo, and endangered species breeding flocks.

LITERATURE CITED

BALI MYNAH (Leucopsar rothschildi) CAPTIVE MEDICAL MANAGEMENT AND REINTRODUCTION PROGRAM

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Abstract

The Bali mynah (Leucopsar rothschildi) is currently a critically endangered species. The 1996 pre-breeding population census ranged from 17 to 22 birds (R. Seibels, personal communication). The American Zoo and Aquarium Association (AZA), along with several other organizations, have been involved in an intensive captive propagation and reintroduction program. Unfortunately, these efforts have been stagnant for the last 3 yr due to political problems. Poaching the Bali mynah for the Indonesian pet trade and habitat destruction, are the primary reasons for its decline. Atoxoplasmosis and hemochromatosis are the primary medical problems in captive Bali mynahs. Atoxoplasma oocysts have been found in the feces of wild Bali mynahs; however, it is unknown whether this disease is contributing to its current decline in the wild. Two drugs, sulfachlorpyrazine (Esb3) and toltrazuril (Baycox), are currently being evaluated for their efficacy against this organism. Sulfachlorpyrazine is not absorbed systemically, thus is only useful in reducing oocyst load in the gastrointestinal tract. Toltrazuril has significantly reduced the mortality of canaries in Europe with systemic atoxoplasmosis. Neither of these drugs are available in the United States, but can be obtained through the Bali mynah Species Survival Plan (SSP) veterinary advisor. In a recent study, the Bali mynah Atoxoplasma was reclassified to Isospora rothschildi.

LITERATURE CITED

DEVELOPING A ZOOLOGICAL AVIAN NUTRITION PROGRAM

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Abstract

In the absence of a staff nutritionist, the task of overseeing a zoological nutrition program often falls to the veterinarian. Exotic avian nutritional requirements have yet to be determined across all taxa. Given the diversity of an avian collection, the most up-to-date veterinarian can spend an inordinate amount of time trying to develop a nutrition program. Time may be better spent devoted to veterinary medicine.

Diet at the Houston Zoo had evolved over time with good intentions but with little basis on science. In order to provide the best nutritional program, it is necessary to systematically collect data on existing diets and seek professional help in the analysis of diets fed in the past and proposed improved diets. We discovered that waste and overfeeding was more significant than initially expected and that time saving protocols could work without affecting the health of the animals.

No single member of the staff had all the answers and, a team approach, utilizing the expertise of curator, keeper, veterinarian, and consultants resulted in a successful program with mutual understanding and compliance.

Introduction

The Houston Zoo bird collection consists of approximately 900 specimens of 225 species. The collection specializes in four main family groups: turacos, corvids, curassows, pigeons and doves. These four groups have been maintained and bred consistently over the past 20 yr. Sixteen forms of turaco and 10 species of corvid have been successfully raised. It would not be unusual to have 70-80 specimens of turaco and 25-30 corvids housed at the zoo at the same time.

Few changes had occurred in diets over the years. Though successful in many ways, dietary inadequacies had resulted in medical problems as well as problems in preparation time, presentation and waste. The goal of the diet review was to improve nutrition, simplify ingredients and eliminate costly waste. But more important was the need to determine the nutritional value of successful diets, fed over many years, and developed from trial and error not necessarily from sound science.

This paper emphasizes the need for a combination of expertise and collaboration in the art and science of avian husbandry.
Methods

Mazuri/Purina Feeds assisted the Houston Zoo in developing diets for their collection. An avian nutritionist from Purina and Houston’s staff veterinarian oversaw the process. The following steps are meant to be a guideline, each facility will undoubtedly vary.

Steps to Follow

Data Collection

*Have staff at all levels “buy-in” and get involved with the process!*  
Designate a keeper liaison to help collect and disseminate information.  
Let keepers design the form to be used for data collection, it will save you many re-writes if their input is sought from the beginning.

*Insure that staff at all levels understand basic avian nutrition!*  
This may require extra efforts and several presentations but will pay off in the long run if everyone is on the same track and understands the vocabulary.

*Determine what is being fed,* not what is written on the diet cards but exactly what each keeper is putting on the plates (live foods, treats, vitamin supplementation and the basic diet). It will be necessary to provide several digital scales and several sets of the same spoons, cups and other measuring devices. Be sure accurate measurements are being taken and everyone is using the same conversion methods.

*Calculate the waste.* Carefully weigh food left at the end of the day and identify preferred items. Ask about rodent, insect and other feral pests and determine amounts of food consumed by them.

Ingredient Database

Once the data collection (diet ingredients and amounts) were provided by the zoo personnel, it was found that the nutrient data set available was not very complete with respect to key ingredients (anoles, mealworms, crickets, specific commercial diets, mouse pups, etc.) used at the zoo. An extensive analysis of certain ingredients was done for moisture, protein, fat, fiber, macro- and micro-minerals, and fat and water soluble vitamins. These values were added to the data set of the formulation programs being used (The Animal Nutritionist, N-Squared Computing & Durango Software, 1985, Silverton, OR 97381 USA).

Diet Nutrient Requirements and New Diet Recommendations

The diet ingredients (items) and amounts supplied by the zoo were inputted into the formulation program (The Animal Nutritionist) and a nutrient content profile was obtained for the diet being used (original diet). Diet nutrient content was compared to a developed standard (recommended nutrient
levels), potential problem areas were identified (deficiencies, excesses, and imbalances), and a new diet recommendation was provided to the zoo. The diet recommendations given were designed to use items available at the zoo with known palatability to the birds, to simplify the diet, to minimize the possibility of error, and to meet the developed nutritional recommendations. The diets were fine-tuned and reevaluated 1.5 yr after the initial recommendations were given. Reevaluation was based on a review of palatability, breeding performance, health problems, overall physical condition of the birds and chick survivability (this included parent reared birds as well as the utilization of the basic diet as a hand rearing diet). The reevaluation processes should be considered ongoing. A true test of the new diet will not be complete until the birds experience the seasonal challenges of breeding, laying eggs, raising chicks and also growing older.

Development of the Recommended Nutrient Levels

Limited information is available on nutrient requirements for avian species not under intensive commercial production. Given that no nutrient recommendations exist for the avian species being reviewed, recommended nutrient levels had to be developed to use as a reference point when reviewing diets.

In developing the nutrient recommendations several factors were considered:
• feeding habits in the wild;
• behavioral differences such as corvid species that wash their food before ingestion;
• known nutritional idiosyncrasies (e.g., the magenta wing colors in turacos result from a red pigment, turacin, which is copper based);
• where available, nutrient content of diets that have been successfully used;
• the baseline used was the National Research Council’s *Nutrient Requirements of Poultry*; and
• where available, general nutrition information available in the scientific literature, mainly in relation to pheasants, quail, pigeons, parrots, finches, flamingos and cranes.

Breeding and maintenance recommendations were developed and named. Diets were assigned on the basis of feed preferences (with some exceptions) as follows:
• nectar-eating birds;
• fruit-eating birds;
• fruit, vegetable, and seed-eating birds;
• seed and vegetable-eating birds;
• insect, fruit, and vegetable-eating birds;
• meat, insect, and fruit-eating birds;
• meat and insect-eating birds; and
• psittacines.

Results

The original diets reviewed contained approximately 80 different items. In most cases the ingredients used were included because they had traditionally been used, but the reason for inclusion
in the diet was not evident. Several vitamin and mineral supplements were used in amounts described as “a pinch”. “A pinch” was weighed in the different feed preparation areas and within the same feed preparation area over a period of 3 days. “A pinch” was found to weigh between 0.1 and 1.9 g, with differences existing within the same feed preparation area and between feed preparation areas. This lead to large nutrient variations leading in some cases to excesses and in others to deficiencies. Given the large number of items used, the high degree of variation between areas and with areas, the high probability of error given the system being used, the high level of wastage (estimated at 40 to 50%), it was important to develop diet recommendations that would address these problems. The number of items used was reduced by approximately 60%, all vitamin and mineral supplement were eliminated from the diets and wastage was reduced to less than 10%.

Concerns with acceptability of the diets that were initially recommended lead to some changes in the ingredients used and in the preparation of the diets. Ingredient mixing was implemented to increase consumption of the extruded diets and to discourage poaching by feral birds (mainly sparrows). For example Bird of Prey Diet (Animal Spectrum Inc., North Platte, NE) and Parrot Breeder 56A9 (Mazuri, St Louis, MO) were rolled together in a ball and offered as a mix, in other cases the chopped fruit mix and Parrot Breeder 56A9 (soaked or dry) were also mixed together.

For the purpose of this paper, and due to space limitations two species will be discussed, Beechey’s jays (*Cissilopha beecheii*) and red-crested turacos (*Tauraco erythrolophus*).

The original Beechey’s jay diet was made up of 17 items and varied considerably between exhibits and keepers. When the nutrient content of one of these sample diets was reviewed (on a dry matter basis) the following issues were of concern: very high vitamin A (42.6 IU/g), and vitamin D₃ (7.8 IU/g); low choline (786 ppm), manganese (33 ppm), selenium (0.09 ppm). Also, a vitamin supplement was being sprinkled in a “pinch” amount and this amount varied considerably.

The jays were very preferential in their consumption, choosing mostly the meat and insects. Thus much of the diet went to waste. Though breeding well with good egg production, chick mortality was high and cataracts were occasionally seen in young adult jays.

The initial diet recommendation for the Beechey’s jays was a diet based on 5 items (Bird of Prey Diet, mouse pups, fruit mix (25% apple, 25% papaya, 25% cooked sweet potato, 25% grapes and soaked raisins) a chopped greens mix (equal parts of endive, kale,), and Mazuri Parrot Breeder 56A9. This recommendation was modified over time to comprise rotational items such as crickets, mealworms, and an in season rotational fruit or vegetable. This last modification resulted in a 7 item diet that closely matched the recommended nutrient levels for meat, insect, and fruit-eating birds and that the birds ate well. By rolling small meatballs of Bird of Prey Diet in crushed Parrot pellets the birds were forced to consume some of the pelleted food. No further cases of cataracts have been observed and chick survivability has increased.

The original red-crested turaco diet contained 13 items and also had a vitamin supplement in powder form that was being sprinkled over the feed. Large variations in powder supplement levels were
observed and this lead to nutrient content variations. From a nutrient content perspective (on a dry matter basis) the diet was low in vitamin E (30.9 IU/kg), choline (790 ppm), biotin (0.1 ppm), zinc (51 ppm), copper (8.6 ppm), manganese (34 ppm), methionine (0.26%), and total sulfur amino acids (0.44%).

Turacos are primarily fruit eaters and will clean all fruit from an offered diet shortly after presentation. Keepers often overfeed the fruit component of a diet in their efforts to make sure the birds do not “go hungry” and sometimes overlook the protein portion of the diet contained in the commercial pellet. Though birds were breeding well often chicks would hatch with apparent calcium deficiencies (fractured and/or soft bones). Other nutrient deficiencies were manifested in “washed-out” coloration in juvenile plumages.

The breeding diet was modified to a four-item diet (mealworms, greens and fruit mix, and Mazuri Parrot Breeder 56A9) with no added supplements. To prevent poaching in certain exhibits where the enclosure wire allows sparrows access to the feed, the parrot breeder was ground and mixed with the fruit mix. The recommended diet nutrient levels were similar to those in the nutrient recommendations developed for vegetable, fruit, and seed-eating birds and contained 23.3 ppm copper.

Since incorporating the new diets, together with increased keeper awareness of avian nutrition, we have seen a marked decrease in the two observable deficiencies previously mentioned.

**ACKNOWLEDGMENTS**

Appreciation is given to the entire Houston Zoo Keeper staff for their diligence and patience in the continuance of this program.

**LITERATURE CITED**

FOOD ITEM SELECTION AND NUTRITIONAL MANAGEMENT OF THE PALM COCKATOO (Probosciger aterrimus) IN CAPTIVITY

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Abstract

Poor reproductive performance and suspected nutritional deficiencies initiated a 2-yr study into food item selection and nutrition of a group of 10 palm cockatoos (Probosciger aterrimus) at the White Oak Conservation Center, Yulee, FL. Daily food intake was measured by individually weighing food items before feeding and subtracting the remaining waste food after feeding. The actual weight of consumed food was then corrected to adjust for any change in water content that occurred over night. This data was collected for 10 days and entered into Animal Nutritionist computer software for analysis. Year 1 results suggest that the cockatoos were selectively eating only the larger nuts and a minimal amount of fruit and vegetables. The analysis determined that there was a dietary deficiency in most minerals. Fat consumption was seven times higher than the recommended level as determined by the Animal Nutritionist computer program data base. The diet was then modified to include a commercially-prepared pelleted parrot ration to establish a more balanced diet. The pelleted ration, fruits, and vegetables were fed daily in the AM with the large nuts being fed 4 times/wk in the PM. One year later (Year 2) this modified diet was re-analyzed and compared to Year 1 results to see if any improvements in nutritional standards had been made.

Year 2 results indicated that on average each parrot was eating 15 g of the pelleted diet a day. Intake of all minerals was determined to be acceptable, however calcium levels were still well below normal. Fat intake had decreased but was still considered high at three times normal. The results of this study are encouraging with the improvement in the nutritional status of the palm cockatoos. Approximately 1 yr after the improvement in diet the first palm cockatoo chick was raised and fledged by its parents at White Oak Conservation Center. The results of this study demonstrate the importance of food item selection, especially in species that are fed a variety of food items, such as birds and primates. Food selection studies are easily repeated over time and should be considered in the development of dietary protocols.

ACKNOWLEDGMENTS

We would like to thank Mike Taylor, Bird Supervisor, and John Lukas, Director of White Oak Conservation Center for their assistance and support of this project.
AN ADENOVIRUS OUTBREAK IN CAPTIVE APLOMADO FALCONS (*Falco femoralis septentrionalis*) CAUSING HIGH MORBIDITY AND MORTALITY

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Abstract

The Northern aplomado falcon (*Falco femoralis septentrionalis*) was extirpated from the United States in the 1950's, with reduced populations surviving only in Mexico. In 1986 a recovery program was begun to reestablish the U.S. population of this endangered species through captive propagation and release of hatch-year juveniles. More than 100 falcons have now been hatched and released in Southern Texas. The first nesting of this species in the United States since 1952 occurred in 1995. In 1996 the captive propagation effort yielded an unprecedented 110 hatchlings (85% hatchability). However, the 22nd chick to hatch died on 6 June 1996, at 9 days of age, beginning a viral outbreak which ultimately resulted in 57 mortalities. Affected birds ranged in age from 9-35 days and were either found dead without premonitory signs, or exhibited various combinations of lethargy, anorexia, diarrhea, and green mutes (feces). All 57 aplomado falcons received complete necropsies. The most consistent gross lesions were moderate to marked hepatomegaly and/or splenomegaly. Five birds also exhibited either intraluminal hemorrhage or fibrinonecrotic cast formation in the small or large intestine. The primary histopathologic findings included multifocal necrotizing hepatitis and splenitis with variably rare to abundant intranuclear viral inclusions. In cases with rare inclusions, the biliary epithelium was more prominently affected than hepatocytes. Most inclusions were large and basophilic, but smaller eosinophilic herpesvirus-like inclusions were also seen. Intranuclear inclusions were also seen in intestinal epithelial cells of the birds with enteritis. Polymerase chain reaction studies utilizing a nested set of consensus primers for herpesviruses were negative on samples of frozen liver, spleen, kidney, lung, and large intestine from four affected aplomado falcons (not all organs were tested from each falcon). Transmission electron microscopy on liver from three cases revealed 58-70 nm nonenveloped, hexagonal virions in the nucleus of hepatocytes, forming paracrystalline arrays in one case. These findings are compatible with an adenovirus infection. Virus isolation attempts using pooled falcon liver and spleen homogenates inoculated onto chicken embryo hepatocytes and embryonated eggs were negative, but limited cytopathic effect and viral replication were observed on a primary culture of quail fibroblasts. Scanning electron microscopy of sediment from the tissue culture supernatant revealed virions
m morphologically compatible with an adenovirus. Attempts to transmit the virus to day-old and adult coturnix quail using falcon liver and spleen homogenates were also negative. The inoculated quail remained clinically normal and seronegative for group I or II adenoviruses by agar gel precipitation. Epidemiologic investigations revealed a point source epidemic with two clusters of cases resulting from horizontal transmission. The two clusters followed the index case by 8 and 14 days, respectively. The median incubation period was 10 days, assuming 2 days of viral shedding prior to death. Supportive therapy was ineffective, serving only to prolong the period of viral shedding by 0.5 day. Management of the outbreak included strict isolation of birds showing clinical signs, segregation of caretakers to certain areas, utilization of foot baths, immediate disinfection of contaminated areas, and a change in diet from fresh ground coturnix quail (Coturnix coturnix; reared on site) to chickens and rodents. The last mortality occurred 5 July 1996. Chicks hatching on 21 June 1996 or later were not affected. Adult aplomado falcons were not affected during the outbreak, but 6 peregrine falcons (Falco peregrinus) ranging in age from 2 wk to 5 yr died during the period of the outbreak. All had gross and microscopic lesions indistinguishable from the affected aplomado falcons. The epidemiologic pattern suggests that the peregrines were secondarily infected through fomites. Serologic testing of 16 remaining aplomado and 6 peregrine falcons for type I and type II adenoviruses by agar gel precipitation was negative. Negative stain electron microscopic examination of feces from 10 surviving aplomado falcons was also negative for viral particles.

Conclusions

An unidentified adenovirus is capable of causing explosive outbreaks with high mortality in young aplomado falcons, and can cause fatal infections in peregrine falcons. Adenoviral infections in raptors are rare, with only two other reports in the literature. The source of this virus is unknown, but epidemiologic and experimental evidence suggests that the feeder coturnix quail were not the source. Because the median incubation period is 10 days, rapid postmortem diagnostics on early cases can provide a window of time for intervention to prevent propagation of an outbreak. The morphology of the viral inclusions by light microscopy is variable, therefore, additional diagnostics may be required to distinguish this adenovirus from falcon herpesvirus, which can cause similar lesions. Strict hygiene and quarantine of breeding facilities may be the best preventive strategy, and when combined with isolation and segregation procedures may be able to arrest an outbreak in progress. Because treatment is ineffective, and mortality approaches 100%, euthanasia of any birds showing clinical signs during an outbreak may be warranted.

LITERATURE CITED

HUSBANDRY AND MORTALITY DATA ON MALAGASY LEAF-TAILED GECKOS (Uroplatus sp.)

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Abstract

Leaf-tailed geckos of the genus Uroplatus are found only on the islands of Madagascar. There are 10 recognized species of Uroplatus: U. fimbriatus, U. sikorae, U. lineatus, U. ebenaui, U. allaudi, U. guentheri, U. phantasticus, U. malaheta, U. malama, and U. henkeli. In the wild, U. fimbriatus, U. sikorae, and U. phantasticus are relatively abundant while the other species have localized distributions and are rare. According to the International Species Information System (ISIS) Reptile Abstract (December 1995), the captive North American population of Uroplatus consisted of 245 individuals representing 6 of the 10 species, with U. henkeli, U. fimbriatus and U. sikorae the predominant captive species. Uroplatus are nocturnal active feeders (versus ambush feeders). They rest in a vertical position with head facing downward and tail flattened. Stress responses include color changes, high-pitched vocalization, and dropping of the tail. Males can be distinguished from females by thick hemipenal bulges.

Husbandry surveys and requests for medical records and necropsy reports were sent to 28 public institutions in North America that currently maintain or that have maintained Uroplatus sp. in the past. A 52% response rate was achieved and a total of 85 necropsy reports were reviewed. Health problems identified by keepers/curators included dehydration, reluctance to feed, anorexia, injuries to the rostrum due to aggressive feeding behavior, and dysecdysis with retained skin on digits. Keepers noted a high mortality rate in recently imported species and in hatchlings. Common medical problems identified by veterinarians included anorexia of unknown etiology, weakness, ocular disease, general unthriftiness, paresis, problems related to oviposition secondary to calcium and phosphorous imbalances, Salmonella sp. infections, and early neonatal death. The majority (80%) of respondents felt that medical problems were related to husbandry and nutrition. Diagnostic procedures included cloacal cultures, radiology, fecal examinations, and blood work.

Of the 32 necropsy reports which contained a final diagnosis, 19 (59%) of these diagnoses were infectious in nature and included septicemia, hepatitis, pneumonia, colitis, enteritis, inflammation of the hemipenes, egg yolk coelomitis, cloacitis, oviductitis, nephritis, salpingitis, and ophthalmitis (Tables 1, 2 and 3). A majority (49%) of infections involved the gastrointestinal and/or reproductive system. Noninfectious diseases included renal and articular gout, nutritional osteodystrophy, cystic ovaries, hepatic fibrosis, impaction, and trauma. Mortality in hatchlings was attributed to premature utilization of yolk sac prior to pipping possibly related to incubation humidity and temperature, congenital defects including contracted limbs, scoliosis, and shistothorax, impaction from vermiculite ingestion, and ocular infections. Gastrointestinal parasites were identified as pathological
agents in several cases and protozoal flagellates and unidentified amoeba were implicated. Although coccidia were a frequent finding on fecal exams, no pathology was attributed to their presence. Little is known about the distribution, natural history and diseases of *Uroplatus* sp. in the wild. Captive husbandry and medical management of these animals could be enhanced by applied research in this area.

ACKNOWLEDGMENTS

Recognition and gratitude goes to the veterinarians, keepers, curators, and pathologists who contributed this information. They represent the following institutions to date: Birmingham Zoo, Center for Endangered Reptiles, Central Florida Zoological Park, Chaffee Zoological Gardens of Fresno, Cincinnati Zoo and Botanical Garden, Dallas Zoo, Detroit Zoological Institute, Forth Worth Zoological Park, Louisville Zoological Garden, Milwaukee County Zoological Garden, Riverbanks Zoological Park and Botanical Garden, St. Augustine Alligator Farm, and Virginia Zoological Park, Wildlife Conservation Society, and The Zoological Society of San Diego.

LITERATURE CITED

Table 1. Most common findings in 41 necropsy reports from adult leaf-tailed geckos.

<table>
<thead>
<tr>
<th>Category</th>
<th>Finding</th>
<th>Percent (Occurrence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious</td>
<td>colitis/enteritis</td>
<td>29.3 (12/41)</td>
</tr>
<tr>
<td></td>
<td>egg yolk coelomitis/salpingitis/oophritis</td>
<td>21.4 (6/28) of all females</td>
</tr>
<tr>
<td></td>
<td>balantitis/hemipenal prolapse and infection</td>
<td>15.3 (2/13) of all males</td>
</tr>
<tr>
<td></td>
<td>hepatitis</td>
<td>17 (7/41)</td>
</tr>
<tr>
<td></td>
<td>bacterial pneumonia</td>
<td>14.6 (6/41)</td>
</tr>
<tr>
<td></td>
<td>septicemia</td>
<td>7.3 (3/41)</td>
</tr>
<tr>
<td>Metabolic Nutritional</td>
<td>hepatic lipidosis</td>
<td>14.6 (6/41)</td>
</tr>
<tr>
<td></td>
<td>articular and/or visceral gout</td>
<td>14.6 (6/41)</td>
</tr>
<tr>
<td></td>
<td>metabolic bone disease</td>
<td>4.8 (2/41)</td>
</tr>
<tr>
<td></td>
<td>inanition</td>
<td>7.3 (3/41)</td>
</tr>
<tr>
<td>Other</td>
<td>normal tissues</td>
<td>14.6 (6/41)</td>
</tr>
<tr>
<td></td>
<td>traumatic</td>
<td>4.8 (2/41)</td>
</tr>
<tr>
<td></td>
<td>mechanical: impaction</td>
<td>7.3 (3/41)</td>
</tr>
<tr>
<td></td>
<td>degenerative: hepatic fibrosis, cirrhosis</td>
<td>4.8 (2/41)</td>
</tr>
<tr>
<td></td>
<td>neoplastic</td>
<td>2.4 (1/41)</td>
</tr>
</tbody>
</table>

* Percentages do not equal 100% because more than one lesion could be reported per animal.

Table 2. Histopathological diagnosis in 32 adult leaf-tailed geckos.

<table>
<thead>
<tr>
<th>Category</th>
<th>Percent (Occurrence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious</td>
<td>59 (19/32)</td>
</tr>
<tr>
<td>Metabolic/Nutritional</td>
<td>15.6 (5/32)</td>
</tr>
<tr>
<td>Trauma/Impaction</td>
<td>9.4 (3/32)</td>
</tr>
<tr>
<td>Degenerative</td>
<td>6.2 (2/32)</td>
</tr>
<tr>
<td>Neoplastic</td>
<td>3.1 (1/32)</td>
</tr>
<tr>
<td>Normal Tissue</td>
<td>3.1 (1/32)</td>
</tr>
</tbody>
</table>
**Table 3.** Specific problems found in leaf-tailed geckos at necropsy.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>hepatitis, lipidosis, granulomas, melanomacrophagic melanosis, bilary trematodiasis, microfilariae, hydropic degeneration, hepatocellular degeneration, cirrhosis, fibrosis, lipofuscinosis</td>
</tr>
<tr>
<td>Adipose</td>
<td>steatitis, atrophy, cellulitis</td>
</tr>
<tr>
<td>Eye</td>
<td>cataract, progressive corneal opacities assoc. with keratitis and subspectacular abscessation, ophthalmitis, iridocyclitis and ulcerative keratitis</td>
</tr>
<tr>
<td>GI</td>
<td>enteritis, colitis, peritonitis, foreign body impaction, colibacillosis, mycobacteriosis</td>
</tr>
<tr>
<td>Kidney</td>
<td>visceral gout, nephritis, ureteritis, acute tubular necrosis, lipofuscinosis</td>
</tr>
<tr>
<td>Integument</td>
<td>cutaneous gout</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>articular gout, myositis, osteomyelitis, osteopenia, osteodystrophy, fracture</td>
</tr>
<tr>
<td>Reproductive</td>
<td>balantitis, egg yolk coelomitis, oviductitis, oophoritis, salpingitis, abscessed musk glands, cystic ovaries, xanthogranulomas</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>epicarditis, visceral gout, xanthoma, myocarditis</td>
</tr>
<tr>
<td>Spleen</td>
<td>splenitis, splenic necrosis, hemorrhagic splenopathy</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>bacterial pneumonia, parasitic pneumonia, thromboembolic pneumonia, tracheobronchitis, xanthogranulomas</td>
</tr>
<tr>
<td>Brain</td>
<td>xanthogranulomas</td>
</tr>
<tr>
<td>Other</td>
<td>septicemia, inanition, intestinal obstruction</td>
</tr>
</tbody>
</table>
SURGICAL CORRECTION OF URETHRAL OBSTRUCTION IN AN ELK (Cervus elaphus) BY PERINEAL URETHROSTOMY

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Abstract

A 5-yr-old castrated male elk (Cervus elaphus) was examined because of a 5-hr history of depression, anorexia, abdominal straining, and bruxism. The elk had been maintained in a 3-ha lot with a diet of cubed alfalfa hay, native grass hay, and a pelleted supplement. Water and a trace mineral block were available ad libitum. The elk was restrained in a 0.75 × 2 m chute for examination. Stranguria was noted with small amounts of red-brown urine released from the urethral orifice during straining. Frank blood was apparent in a free-catch urine sample. Blood was drawn for hematologic and serum biochemistry analyses. All serum biochemical and hematologic values were within normal reference ranges for adult elk.1 For further examination, anesthesia was induced by hand-injection of carfentanil citrate (Wildnil, Wildlife Pharmaceuticals Inc., Fort Collins, CO; 20 µg/kg i.m.) and the elk was placed in lateral recumbency. Attempts to fully extend the distal tip of the penis for visual inspection and palpation were unsuccessful due to the elk’s muscle tone. Transrectal abdominal palpation revealed a large distended urinary bladder.

Based on the history and physical examination findings, urethral obstruction was diagnosed and the elk was treated by perineal urethrostomy. The sigmoid flexure of the penis was palpated caudal to the scrotal remnant and an 8-cm skin incision was made along the ventral midline directly over the penis at this location. The sigmoid flexure was identified, bluntly dissected free of subcutaneous tissue, and exteriorized through the skin incision. The urethra was distended, but no calculi were visible or palpable at any point along the 12-cm length of exposed penis. The dorsal caudolateral portion of the penis was attached to the skin at the dorsal end of the incision with a simple interrupted suture of 2-0 monofilament polyglyconate on each side of the urethra. The needle was passed through the layers of the corpus spongiosum without penetrating the urethral mucosa. Once the penis was secured to the skin, it was transected leaving an 8-cm stump proximally and intact attachments of the retractor penis muscles distally. Bleeding was minimal due to occlusion of the corpus cavernosum blood supply by the dorsal sutures. The urethra was then incised longitudinally at the proximal stump. A #8 French urinary catheter was passed through the urethra toward the urinary bladder. After initial resistance, the catheter was passed successfully into the bladder and clear yellow urine flowed through. A urine sample was collected for evaluation, but was not collected steriley for culture. The penis was secured to the skin and the remainder of the skin incision was closed routinely. The urinary catheter was removed.
The carfentanil immobilization was antagonized with naltrexone HCl (Trexonil, Wildlife Pharmaceuticals Inc., Fort Collins, CO; 100 mg/mg carfentanil; 25% i.v., 75% s.c.) and the elk recovered uneventfully. Within 15 min of surgery, the elk voided large amounts of urine through the perineal urethrostomy without evidence of straining. Urinalysis was unremarkable; urine pH was 9.0 and specific gravity was 1.009. No crystalluria was observed.

Mild intermittent bleeding from the perineal urethrostomy site lasted for 10 days after surgery. Antibiotic treatment was initiated at the time of surgery with penicillin G benzathine and penicillin G procaine (Dura-Pen, Durvet, Inc., Blue Springs, MO; 22,000 IU/kg) administered every 48 hr for 2 treatments. Phenylbutazone (Butatabs-E, Burns Veterinary Supply, Rockville Centre, NY; 4 mg/kg) was administered p.o. on postoperative days 1 and 3 for analgesia. *Clostridium* spp. bacterin (Ultrabac 7, Smith-Kline Beecham Corp, West Chester, PA; 4 ml s.c.) was administered at the time of surgery. Abdominal pain and stranguria were not observed after surgery and the elk has continued to void urine through the perineal urethrostomy site 9 mo postoperatively. Although a 10% weight loss occurred over the 2-wk postoperative period, a return to initial body weight occurred over the following 2 mo without dietary changes.

**Discussion**

Urethral obstruction has been reported in many domestic species of artiodactylids and urolithiasis is the most common cause of urinary tract obstruction in these species. The distal aspect of the sigmoid flexure is the most common site for urinary calculus impaction in cattle and is the second most common site for obstruction in sheep and goats. Urinary calculi have been documented in white-tailed deer and elk, although urethral obstruction secondary to urolithiasis has not been reported. Magnesium ammonium phosphate calculi are the most common type of urolith in domestic ruminants while calcium carbonate and calcium oxalate uroliths have been documented in cervids. Phosphatic uroliths are common in animals grazing lush pastures that are high in calcium and oxalate content. Decreased water intake may also be calculogenic. The elk’s ration was not high in calcium or oxalates, and water intake appeared to be adequate. However, the animal’s ration was formulated into wafers, and this condensed food source may have contributed to calculogenesis. Early castration is also thought to influence urethral obstruction as average urethral luminal diameter is smaller in male ruminants that are castrated prior to sexual maturity. This elk was castrated at less than 6-mo-old which may have contributed to a decreased urethral luminal diameter and consequent urethral obstruction.

Captive artiodactylids with urethral obstruction have been euthanatized without specific treatment because of the perception that this condition bears a poor prognosis. Reports on the use of surgical treatment of urethral obstruction in nondomestic artiodactylids are rare, although surgical procedures including perineal urethrostomy, cystotomy and urethral process amputation are used routinely to correct urethral obstruction in domestic ruminants. While the cause of this elk’s urethral obstruction remains undetermined, early diagnosis and treatment have allowed an excellent prognosis in this animal. Minimal postoperative treatment was required and the urinary obstruction has not recurred.
LITERATURE CITED

ENCEPHALOMYELITIS OF UNKNOWN ETIOLOGY IN A COLONY OF JAPANESE MACAQUES (Macaca fuscata)

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Abstract

Between November 1993 and March 1997, 19 cases of encephalomyelitis were diagnosed in a closed colony of Japanese macaques (Macaca fuscata). This syndrome had a high fatality rate (84%), commonly affected weaned juveniles, and was characterized by severe lesions of encephalomyelitis mainly involving the white matter. Two of the nine animals treated with antibiotics recovered completely, and passive immunotherapy was beneficial in two cases. Several infectious agents were investigated, but the etiology of this neurological disorder remains undetermined.

Introduction

An outbreak of fatal encephalitis, thought to be associated with a viridans group Streptococcus, has been recently described in a captive colony of Japanese macaques (Macaca fuscata).12 Since this first report, several similar fatalities have occurred in this colony. However, recent findings do not support the initial proposed etiology. The aim of the present communication is to summarize the knowledge regarding this neurological syndrome.

Case report

Affected animals were members of a group-housed colony of 40 Japanese macaques of the Arashiyama strain used exclusively for behavioral research.3 There had been no new animals nor any major medical problems during the first 10-yr period. In November 1992, the colony was moved to an indoor/outdoor facility adjacent to a research barn housing horses, cows, swine and dogs. The diet consisted of commercial monkey chow, grains, fruit, and vegetables.

Clinical presentation

Between November 1993 and March 1997, 19 cases (84% mortality rate) were observed in the colony (Table 1). The interval between each case was highly variable (from 4 to 282 days, mean: 68 days), and cases were usually clustered. With the exception of a 7-yr-old animal, all affected animals
were juveniles (5-41 mo; mean: 22 mo). Gender, social rank, and season did not appear to be correlated with disease. Clinical signs were similar among the affected animals, lasting from a few days to over a month. Lethargy, reluctance to move, muscular weakness, and loss of balance were the most common initial clinical signs, which were usually followed by progressive paralysis, head tilt, and hemifacial paresis. Attempted treatments and clinical outcome are presented in Table 2. Two of the nine animals treated with antibiotics recovered completely. A third animal with advanced disease (lateral recumbency) recovered following intravenous administration of mixed sera from the two previous survivors. Another animal improved after intravenous injection of mixed sera, but subsequently deteriorated and was euthanatized.

**Pathology**
A complete postmortem examination, as described elsewhere, was performed on 16 animals. Gross lesions were similar in all animals, and consisted of circular yellow areas 2-7 mm in diameter, sprinkled with hemorrhages, located within the white matter of the brain, the cerebellar medulla and the spinal cord. Microscopically, most cerebral lesions were located at the junction of the grey and white matter and were characterized by well demarcated areas of severe neuropile vacuolation with moderate to severe hemorrhages. Necrotic leukocytoclastic vasculitis, characterized by segmental fibrinoid necrosis of the media and severe infiltration of the vascular walls by a dense population of neutrophils, was observed in several cases. Moderate numbers of neutrophils were scattered in the leucomalacic foci and were more numerous in areas adjacent to vessels. Perivascular lymphoid cuffs and mild diffuse gliosis were present in the cerebral tissue surrounding the lesions. Infiltration of leucomalacic foci by Gitter cells was predominant in animals that survived for a longer period. Microorganisms were not observed in these lesions using Gram and periodic acid Schiff stains. Screening for equine, bovine, canine and feline herpesviruses, Listeria monocytogenes, Haemophilus somnus, and Toxoplasma gondii was negative on formalin-fixed brain from two animals using an immunofluorescence technic (Dr. Fabio del Piero, College of Veterinary Medicine, Cornell University, Ithaca, NY). A few colonies of viridans group Streptococcus, similar to S. salivarius, were isolated from a cerebral lesion of the first three animals, and from the lungs of the fourth case. This bacterium was not found in any of the subsequent cases, and microagglutination tests against the same Streptococcus failed to demonstrate the presence of significant titers in the sera of six affected animals. Viruses or bacteria were not detected during electron microscopy examination of glutaraldehyde-fixed cerebral tissues from 4 animals. Viral isolation from frozen brain and cerebellum of two cases yielded negative results (see Hilliard et al. for methodology).

**Serology**
The following 13 agents were confirmed absent from the colony: Coxiella burnetii (0 positive / 10 sera tested), Leptospira sp. 8 serovars (0/10), Borrelia burgdorferi (0/20), western equine encephalitis (0/20), Powassan equine encephalitis (0/20), St. Louis encephalitis (0/20), hantavirus (0/20), poliovirus (0/32), Herpesvirus simplex-1 & 2 (0/32), Varicella zoster virus (0/32), simian immunodeficiency virus (0/4), and simian retrovirus (0/4). Anti-Herpesvirus simiae (B virus) antibodies were found in 20 out of 32 sera tested, but in none of the three survivors. All 32 sera tested for the presence of anti-Epstein Barr virus were positive. However, seroconversion was not observed in the three survivors. Antibodies against
cytomegalovirus were detected in 13 of the 32 sera tested, but were not present in two of the three survivors, and seroconversion was not observed in the third animal. Simian T-cell leukemia virus-antibodies were present in one of the four sera tested. Finally, 50% of the sera tested (16/32) were positive for measles virus. However four of the symptomatic macaques were seronegative, and seropositive clinical cases had either similar or smaller titers that asymptomatic animals.

Discussion

Despite extensive investigations, the etiology of the neurological disorder endemic to this colony could not be determined. Neurological diseases are not uncommon in primates of the genus Macaca, and have been associated with different bacterial, viral, and parasitic pathogens. In Japanese macaques, reported cases of neurological disorders include a case of canine distemper virus infection, and possible coyotillo berry intoxication. Clinical and pathological findings observed in our colony differ from all conditions reported previously in macaques.

The epidemiological pattern of this condition (clustering of cases, predisposition for immature weaned animals) and the benefit of passive immunotherapy favor an infectious etiology. Based on the isolation of the same bacterial species in the first four animals and on the successful treatment of two cases with antibiotics, a bacterial etiology was previously proposed for this syndrome. The failure to isolate this microorganism from the 11 subsequent cases and the ineffectiveness of the antimicrobial treatment on seven affected animals make this hypothesis unlikely.

Histologic lesions observed in the brains and spinal cords shared some similarities with those described in acute disseminated encephalomyelitis (ADEM) in humans, and in experimental autoimmune encephalomyelitis. ADEM is believed to be associated with an acute autoimmune reaction to myelin, and is usually triggered by different viral infections, such as measles, infectious mononucleosis (Epstein-Barr virus), influenza, parainfluenza, and mumps. Both Epstein-Barr and measles viruses were prevalent in our colony. However, the absence of titers and/or seroconversion in several of the affected animals does not support their implication in this syndrome. A search for the presence of antibodies against mumps, influenza, and parainfluenza viruses could not be performed. In addition, the success of the passive immunotherapy and the failure of corticosteroid therapy make diagnosis of post-infectious encephalomyelitis unlikely. Finally, it should be realized that ADEM is extremely rare in humans. An epidemic pattern as observed in this colony would be highly unusual for an autoimmune disorder.

Animals from this group have been exposed to simian T-cell leukemia virus. This virus has been previously reported in free-ranging Japanese macaques, but has never been associated with neurological problems. In addition, no titers were found in three of the four affected animals tested. Despite their prevalence, Herpesvirus simiae (B virus) and cytomegalovirus do not appear to be involved in this condition since seroconversion or titers for these viruses were not observed in several of the affected animals. Finally, the negative serology to the immunosuppressive viruses (simian immunodeficiency virus and simian retrovirus), combined with no previous evidence of these viruses in the colony, makes it highly unlikely they played a role in this disease.
Additional investigations for other potential etiologies, such as spuma virus, retrovirus, and non-B herpesviruses, are still in progress.

ACKNOWLEDGMENTS

We are grateful to Drs. Guy Fitzgeral, Robert Higgins, Daniel Martineau, Khvay Mittal, and André Ravel (Université de Montréal), to Drs. Radmila Mirkovic and Julia Hilliard (Southwest Foundation for Biomedical Research), and to Dr. Timothy O’Neil (Registry of Comparative Pathology, AFIP) for useful discussion. We also thank Dr. Fabio del Piero (College of Veterinary Medicine, Cornell) for the immunochrometry work, Drs. Denise Deniscourt and Harvey Artsob (Health Canada) for the serology, and Paul Vasey, Dr. Jean Prud’homme, and Dr. Guy Fitzgeral (Université de Montréal) for technical assistance.

LITERATURE CITED

Table 1. Encephalomyelitis in 19 Japanese macaques. Distribution of the cases (November 1993 to March 1997)

<table>
<thead>
<tr>
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</tbody>
</table>

○: One case.

Table 2. Treatments and outcomes in Japanese macaques with encephalomyelitis. No treatments were attempted on animals 1, 2, 11, 12, and 14.

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>Outcome</th>
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<tr>
<td>3</td>
<td>Oxytetracycline(a) (25 mg/kg i.m. b.i.d., 10 days)</td>
<td>Euthanatized</td>
</tr>
<tr>
<td>4</td>
<td>Enrofloxacin(b) (5 mg/kg, i.m. b.i.d., 14 days)</td>
<td>Recovered</td>
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<td>5</td>
<td>Enrofloxacin (5 mg/kg, i.m. b.i.d., 5 days)</td>
<td>Euthanatized</td>
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<td></td>
<td>Dexamethasone(c) (0.17 mg/kg i.m. s.i.d., 4 days)</td>
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<td>6</td>
<td>Enrofloxacin (5 mg/kg, i.m. b.i.d., 8 days)</td>
<td>Euthanatized</td>
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<tr>
<td></td>
<td>Penicillin G(d) (20000 IU/kg, i.m. b.i.d., 8 days)</td>
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<td></td>
<td>Dexamethasone (0.2 mg/kg i.m. s.i.d., 2 days)</td>
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<tr>
<td>7</td>
<td>Gentamicin(e) (1.5 mg/kg i.m. t.i.d., 11 days)</td>
<td>Recovered</td>
</tr>
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<td></td>
<td>Ampicillin(f) (83 mg/kg s.c. t.i.d., 11 days)</td>
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<tr>
<td>8</td>
<td>Gentamicin (1 mg/kg i.m. s.i.d., 8 days)</td>
<td>Euthanatized</td>
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<tr>
<td></td>
<td>Ampicillin (83 mg/kg s.c. b.i.d., 8 days)</td>
<td></td>
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<tr>
<td></td>
<td>Enrofloxacin (15 mg/kg, i.m. b.i.d., 6 days)</td>
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<tr>
<td></td>
<td>Dexamethasone (1.7 mg/kg i.m. b.i.d., 6 days)</td>
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<tr>
<td>9</td>
<td>Enrofloxacin (5 mg/kg, i.m. b.i.d., 8 days)</td>
<td>Euthanatized</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone (1.25 mg/kg i.m. b.i.d., 3 days)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Gentamicin (2.6 mg/kg i.m. b.i.d., 4 days)</td>
<td>Euthanatized</td>
</tr>
<tr>
<td></td>
<td>Ampicillin (70 mg/kg s.c. b.i.d., 8 days)</td>
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<td></td>
<td>Dexamethasone (1.4 mg/kg i.m. b.i.d., 4 days)</td>
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<tr>
<td>13</td>
<td>Enrofloxacin (5 mg/kg, i.m. b.i.d., 8 days)</td>
<td>Dead</td>
</tr>
<tr>
<td>15</td>
<td>Serum from animals Nos. 7 and 4 (18 + 9 ml)</td>
<td>Recovered</td>
</tr>
<tr>
<td>16</td>
<td>Serum from animals Nos. 7, 4, and 15 (10 ml)</td>
<td>Improved, then relapsed, euthanatized</td>
</tr>
</tbody>
</table>

\(a\) Liquamycin-LP\(^a\) (Rogar/STB Inc., Pointe-Claire, Quebec)
\(b\) Baytril\(^b\) (Bayvet, Concord, Ontario)
\(c\) Centrazone-5\(^c\) (Central Sales LTD., Brampton, Ontario)
\(d\) Ethacillin\(^d\) (Rogar/STB Inc., Pointe-Claire, Quebec)
\(e\) Gentocin\(^e\) (Schering Canada Inc., Pointe-Claire, Quebec)
\(f\) Ampicillin sodium\(^f\) (Novopharm, Toronto, Ontario)
LEPTOMYXID AMOEBIC MENINGOENCEPHALITIS IN A WESTERN LOWLAND GORILLA (Gorilla gorilla gorilla)

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Abstract

Clinical Presentation

A 14-yr-old male western lowland gorilla (Gorilla gorilla gorilla) housed at the Milwaukee County Zoo experienced a fatal meningoencephalitis caused by Balamuthia mandrillaris. Initial signs (day 0) were myoclonic jerks of the head, neck, and shoulder muscles, and a subtle ataxia. Nonenhanced magnetic resonance imaging (MRI) of the brain performed on day 2 was normal. Standard hematologic and serum biochemical tests were unremarkable, and titers against Epstein-Barr and herpes simplex I and II viruses were considered negative. Progression of encephalitic signs was rapid, with development of dysconjugate ocular deviation, progressive lethargy, severe cerebellar ataxia, and generalized weakness by day 8. A second MRI on day 9 showed multifocal lesions scattered throughout the cerebrum and diencephalon, and extending into the cerebellum. Cerebrospinal fluid was clear, but analysis revealed an elevated protein level of 487 mg/dL (human reference range: 20-45 mg/dL),2 and 45 white blood cells/μL (human reference range: 0-3/μL),2 with 1% neutrophils, 94% lymphocytes, and 5% eosinophils. Fungal and Gram stains were negative for organisms, as were antigen assays for Haemophilus influenza group B, Streptococcus pneumonia, ß-hemolytic Streptococcus group B, and Neisseria meningitidis groups A, B, C, Y and W135. Despite treatment with intravenous metronidazole, broad spectrum antibiotics, and supportive care, the animal became comatose and died on day 10.

Pathology

Necropsy revealed large granulomatous nodules at the base and apex of the heart, as well as smaller nodules within the pancreas and the retropharyngeal lymph nodes. Gross reaction in the cerebral tissue was limited to slight, multifocal, yellowish discoloration and depression. Histopathologic examination revealed multifocal areas of granulomatous cellular proliferation with and without acute necrosis. These foci contained massive neutrophilic and eosinophilic, or mixed eosinophilic and granulomatous infiltrates, accompanying large eosinophilic ovoid to polygonal bodies that resembled amoeba. Lesions often showed a prominent vascular orientation. Cardiac sections contained a similar infiltrate with more fibrosis, and with foreign body giant cells containing amoeba within the mixed inflammatory cell infiltrate. Similar nodules of cellular reaction were seen in the hilar lung, pancreas, and kidney. Tissues were sent to Dr. Visvesvara at the Centers for Disease Control and
Prevention, where immunofluorescent studies confirmed the presence of *B. mandrillaris*.

**Discussion**

*Balamuthia mandrillaris* is a free-living amoeba of the Order Leptomyxida, and is usually thought of as a soil inhabitant. It has been identified as a cause of both acute and granulomatous amoebic encephalitis in humans and animals,\(^1,4,6,9\) with a number of human cases believed to have been infected via open wounds.\(^5\) Serological tests do not exist currently; diagnosis is generally made from biopsies or at necropsy. Pentamidine isethionate has been recommended as treatment for extraneural forms of *B. mandrillaris* infection, however, treatment of the encephalitic form has not been successful.\(^7\)

This is the third reported case of fatal amoebic meningoencephalitis in western lowland gorillas caused by *B. mandrillaris*, and the first to be reported outside the San Diego Zoo.\(^6\) Human cases have been reported worldwide, many in acquired immune deficiency syndrome (AIDS) patients, suggesting that immunosuppression and debilitation may be a factor in susceptibility. However, infection has also occurred in immunocompetent persons with severe trauma or extended exposure to soil and water.\(^5\) The gorilla in this case was a young vigorous animal with no medical history indicative of immunosuppression or pathology of the lymphoid system. However, underlying immunocompromise secondary to social stress remains possible in this animal. Thirty-one months earlier, an older male silverback gorilla had been added to the collection, and changes in family groups occurred periodically in an attempt to find a compatible breeding group. The extensive granulomatous pericardial lesions suggest that infection was present for some time before the rapidly progressive encephalitic disease occurred. The generalized visceral involvement and vascular orientation of the lesions in this gorilla suggest a hematogenous dissemination of the organism, but the source of infection in this animal is unknown. No amoebae were found in samples of water, sand or soil collected from the indoor and outdoor exhibit and holding areas.

Amoebic meningoencephalitis and granulomatous peritonitis is an important cause of disease in Old World primates, and a significant cause of death in lowland gorillas. Given the ubiquitous distribution of this soil organism, infection with *B. mandrillaris* should be considered a possible cause of granulomatous pneumonia, peritonitis, or encephalitis in primates. While the danger of zoonotic transmission from the live animal is unknown, it is presumed to be low. Zoo personnel may be at risk when handling infected tissues during necropsy or biopsy procedures.

**ACKNOWLEDGMENTS**

The authors would like to thank Dr. Visvesvara from the Centers for Disease Control and Prevention, Atlanta, GA, for performing the immunofluorescent antibody tests on the tissues provided, and for attempting to culture amoeba from the soil and water samples supplied.
LITERATURE CITED


PERINATAL EVENTS IN TWO WESTERN LOWLAND GORILLAS

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Abstract

First time pregnancies of two western lowland gorillas (Gorilla gorilla gorilla) resulted in the live births of both neonates. The first case describes a 26-yr-old wild-caught gorilla with vaginal bleeding during the third trimester of its pregnancy. Operant conditioning allowed the transpelvic ultrasonographic assessment of fetal health and placental integrity. The term pregnancy and unassisted parturition resulted with the neonate dead at 4 days of age with a cleft palate and complications from sepsis. The cleft palate was believed to be a sporadic congenital malformation. The second case describes a 10-yr-old captive-born gorilla with a protracted labor requiring immobilization and labor management to vaginally deliver a normal term fetus. The neonate was resuscitated immediately upon delivery and has survived without complications. Both neonates were the only progeny of a 36-yr-old wild-caught male that has since died of an aortic aneurysm.

Introduction

A perinatal team of specialists, including veterinarians, obstetricians, a radiologist, a neonatologist, and anesthesiologists worked together to oversee obstetrical conditions during the pregnancies of two western lowland gorillas. Each pregnancy and potential birth was important for these genetically unrepresented individuals.

Case Report One

A 25-yr-old primiparous western lowland gorilla presented with episodes of vaginal bleeding. Both parents were wild caught with no histories of reproductively related disease. Gestational age at the onset of bleeding was 120 days and continued intermittently through 210 days of gestation. The expected range of gestational period for gorillas is 237-285 days, with a mean of 255 days, or 8.5 mon.6 Urinary progesterones measured by radioimmunoassay (RIA) and chorionic gonadotropin (CG) detection (OvuQuick, Quidel Corp., San Diego, CA 92121 USA) had confirmed the pregnancy. The gorilla had been trained to give daily urine samples upon request by the gorilla keeper. Urinary reagent strips (Hemastix, Bayer Corp., Elkhart, IN 46515 USA) indicated consecutive days of 1+ (slight) to 2+ (moderate) blood in the animal’s urine. Urine cultures were negative for bacterial growth and urinalysis results were not consistent with cystitis. The pair had continued to copulate
throughout the pregnancy and a vaginitis was suspected. Sonographic monitoring (UltraMark 4, Advanced Technology Laboratories, Bothell, WA 98041 USA) was performed to rule out a placental or other cause of bleeding.

The gorilla was conditioned to accept the 2.25-MHz transducer probe against its ventral pelvis for 3-5 min periods while receiving a juice or milk reward. The handheld probe was introduced between the cage bars while the animal sat on a large, end-on-end PVC pipe with an opening cut along the rim to allow the probe to be extended into the center of the PVC pipe. The gorilla was only comfortable having the gorilla keeper manipulating the probe. The sonographer was stationed with the ultrasound monitor 3 m away so that he could view the images and tell the gorilla keeper how to direct the probe placement.

During four separate exams within a 1-wk period, a single living intrauterine gestation was identified in cephalic position. The cranium was fairly low in the pelvic vault and obscured the cervix internal os. The biparietal diameter (BPD) of 7.3 cm was estimated since the cavum septum and thalami were not identified. The placenta was left lateral, fundal and tapered normally making a placenta previa very unlikely. The amniotic fluid volume was normal. The four chambered heart with a rate of 150 bpm was noted. No placental cause for the observed bleeding was determined and no fetal anomalies were detected. The value of the sonography was minimized due to the sonographer not directly performing the examination since many structures were not visualized. The vaginal bleeding resolved without treatment.

The neonate delivered unassisted during an observed parturition. The length of gestation was 262 days. The neonate was assessed as normal and determined to be suckling by experienced veterinary and gorilla keeper observations. The maternal level of care was judged as excellent. The neonate’s condition deteriorated suddenly on day four requiring a physical examination. The mother gorilla was immobilized with 5mg/kg i.m. blow dart injection of ketamine hydrochloride (Ketaset, Fort Dodge, Fort Dodge, IA 50501 USA). The presence of breast milk was confirmed. The 2.2 kg male neonate was removed for examination in a dehydrated, moribund state and died several hours later.

Gross findings at necropsy included a right-sided cleft of the hard and soft palate, subcutaneous and subdural hemorrhage with severe cerebral edema, and a mild myxomatous degeneration of the right atrioventricular valve. The right-sided cleft palate communicated with the nasal cavity as confirmed by the passage of a blunt probe through the right nostril and into the oral cavity. The u-shaped cleft tapered posteriorly to involve the distal edge of the hard palate and proximal soft palate. Postmortem three-dimensional reconstruction of computerized tomographic scans demonstrated a cleft maxilla, asymmetrical inferior turbinates, intact premaxilla, and vomer. No anomalies of the dentition, cranial base, or calvaria were noted. It was felt that the cleft palate defect had prevented any intraoral suction by the neonate during nursing attempts and no breast milk had been ingested.

Histopathology revealed a suppurative omphalophlebitis and focal suppurative encephalitis with cerebral edema. Adipose tissues, hepatocytes, and pancreatic acinar cells were atrophic.
Case Report Two

A 10-yr-old, 129 kg primiparous western lowland gorilla experienced a protracted labor. Urinary progesterone levels measured by RIA and CG levels (Ovuquick) had confirmed the pregnancy and approximate date of conception. Length of gestation was estimated at 275 days. Birth watch observations by experienced gorilla keepers were ongoing from the onset of labor signaled by a watery fluid discharge presumed to be amniotic fluid covering the gorilla’s rump. Contractions and labor activities were light and infrequent (one/15 min). This animal had been unresponsive to attempts at conditioning for elective sonography.

The gorilla was described 24 hr later to be uncomfortable, restless, unable to sleep and taking small amounts of oral fluids. The animal would repeatedly change position, roll over, and would often remain on its back lifting its rump slightly during each contraction. Approximately 36 hr after the onset of labor the animal was immobilized to assess fetal viability and position, amniotic fluid volume, and cervical dilation.

The gorilla was immobilized with a 5mg/kg i.m. blow dart injection of ketamine hydrochloride (Ketaset) and an intravenous catheter was placed. Anesthesia was induced with isoflurane (Isoflo, Abbott Laboratories, North Chicago, IL 60604 USA) in oxygen via face mask, followed by endotracheal intubation. The isoflurane levels required for immobilization varied from 0.5-2.5% depending on the amount of manipulation by the obstetrician. No muscle relaxant was used. The animal was monitored using a pulse oximeter (Model N-100, Nellcor, Hayward, CA 94545 USA), automated blood pressure monitor, and EKG. Complete blood counts and serum chemistries were all within normal limits. Uterine contractions were abolished by the anesthesia.

Transpelvic scanning (UltraMark 4) was obtained using 2.25-MHz mechanical and 3.5-MHz curvilinear array transducers. A 1.5-cm pocket of amniotic fluid was initially present, but disappeared after additional labor. This severe oligohydramnios made the sonographic exam difficult and incomplete. A single viable intrauterine fetus was identified in cephalic, occipitoposterior position. The fetal head was in the vagina which precluded sonographic visualization and measurements of the cranium, orbits and neck. No maternal uterine or ovarian masses were identified.

Initial attempts with vacuum extraction were unsuccessful. The stable condition of the fetus allowed the labor to be augmented with i.v. oxytocin (Syntocinon, Sandoz Pharmaceuticals, East Hanover, NJ 07936 USA) at a starting dose of 6 mU/min and increased by 6 mU every 15 min. The induced uterine contractions were monitored both by palpation and with sonography. The fetal pulse rate measured from the abdominal aorta dropped from 180 bpm at the initiation of the oxytocin to 130 bpm after 85 min. The contraction frequency, strength, and duration increased and the fetal head could be felt to be descending.

After approximately 1.3 min on oxytocin, the head was clearly under the pelvic symphysis and close to the pelvic floor. The vacuum extractor was reapplied without problems and was used for pulling
with the contractions. The gorilla was noticeably contracting from an increase in its respiratory rate and facial grimacing. After approximately three contractions, the widest point of the head emerged through the pelvic canal. The patient sustained a vaginal laceration at this time. With gentle traction the head was delivered in the occipitoanterior position. There was no evidence of any nuchal cord. The shoulders delivered easily. The cord was clamped and cut.

The 2.54 kg anesthetized female neonate required ventilatory support with 100% oxygen. Immediately after birth, the neonate had a low heart rate with no respiratory efforts or muscle tone. The neonate was intubated with a 3.5 mm oral endotracheal tube. The neonate exhibited a markedly compressed anterior ribcage, but had no other physical abnormalities. Auscultation of the lung fields revealed adequate breath sounds, symmetric chest movement, and normal heart sounds. The neonate started to spontaneously breathe after 45 min and the airway was decannulated. The neonate was gavage-fed premature infant formula (Enfamil, Mead Johnson & Company, Evansville, IN 47721 USA) to maintain its blood glucose. At 4 hr of age it was able to take small amounts of formula by nipple and progressed to a full intake during the first 24 hr. The animal has survived to date without complications.

**Discussion**

We describe the observations of a perinatal team of specialists surrounding the events that occurred with the pregnancies of two western lowland gorillas. Fetal growth, development, and health assessment during gestation are monitored routinely in human medicine. These same techniques can be applied to the nonhuman primate patient with expected limitations that arise from working with animals.

With the cleft palate case, the suppurative nature of the inflammation suggested the presence of a bacterial sepsis most likely originating from the inflamed umbilicus. It was not felt that the cleft palate was involved with the sepsis or the cerebral edema. There was no evidence of bronchopneumonia to suggest aspiration pneumonia. The failure of passive transfer due to the functional dysphagia of the cleft palate most likely lead to humoral immunosuppression, with a suppurative omphalophlebitis and encephalitis. The atrophy of fat, pancreas, and liver implied a malnutritional state in the neonate.

Anatomically, the cleft found in the gorilla was identical to those observed in humans with congenital disorders involving the palate. Dietary, teratogenic, and genetic causes of cleft palate are recognized. The prevalence for humans is approximately 0.4-0.8/1000 for liveborn infants in the USA but remains undetermined for nonhuman primates. Oral-facial clefts are among the most frequent congenital anomalies reported in nonhuman primates. Cleft lip and palate have also occurred in fetal macaques following intrauterine exposure to ethanol.
can be particularly useful when animal management changes, such as isolation or intervention, are planned for the periparturient period. Sonography could have potentially detected the cleft palate defect in the fetus, allowing zoo staff and perinatal consultants to intervene at the time of birth. Fetal BPD measurements have been published for the gorilla. Continued measurements of multiple fetal parameters in gorillas will assist future attempts to assess fetal growth and health. Operant conditioning of gorillas for sonography to determine fetal growth measurements needs to be completed as has been reported in other species.

ACKNOWLEDGMENTS

The authors recognize gorilla keepers, Violet Sunde and Judy Sievert, for their efforts in the operant conditioning of the gorillas to voluntarily assist with routine veterinary procedures; the zoo nursery technician staff, Harmony Frazier, Linda Shipe, and Carol Simkins; and the Departments of Neonatology, Obstetrics and Gynecology from Swedish Hospital.

LITERATURE CITED

CLINICAL INVESTIGATION OF HYPOTHYROIDISM IN A WESTERN LOWLAND GORILLA (Gorilla gorilla gorilla)

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Abstract

We describe a case of overt primary hypothyroidism in a 26-yr-old captive-born female western lowland gorilla. This animal presented with a long history of nonspecific clinical signs including weight gain, unsettled appetite, anxiety-like behavior, lethargy, and poor intraspecies interactions. Compared with clinically normal gorilla and human reference values, its thyroid-stimulating hormone (TSH) level (107 mIU/L) was markedly elevated, and its thyroxine (T4) (< 14.0 nmol/L) and free T4 (5.0 pmol/L) levels were significantly lower. Oral supplementation with levothyroxine sodium was associated with weight reduction (-16%), and with an increase in its daily activity and interactions with the other members of the group. Levels of circulating TSH were monitored to access the effect of the levothyroxine supplementation on the thyroid homeostasis. TSH levels estimated by human-TSH assays seem to be a good indicator of thyroid function in gorillas. An initial dosage of 50 µg/day of levothyroxine, with increments of 25 µg/day at monthly intervals up to a dosage of 100 µg/day, is proposed as starting regimen for hypothyroid gorillas.

Introduction

Hypothyroidism, the most common disorder of thyroid function in humans, is associated with a wide range of subtle clinical signs.2 The condition is uncommon in nonhuman primates. The most important diagnostic aid in the clinical evaluation of thyroid function in humans and dogs is the estimation of circulating thyroid-stimulating hormone (TSH).3,13 Even though such assays are species-specific, TSH of some nonhuman primates can be detected by human-TSH assays.1,9 We describe here the use of a human-TSH assay in the clinical investigation of hypothyroidism in a female western lowland gorilla (Gorilla gorilla gorilla).

Case Report

A 26-yr-old captive-born female western lowland gorilla has been housed at the Metropolitan Toronto Zoo since 1974. The gorilla gave birth successfully 13 yr ago, but has not displayed any further breeding activity. Various signs have been noticed by keepers over the last 3 yr including weight gain, unsettled appetite, lethargy, anxiety-like behavior, and poor intraspecies interactions. In addition, a “head-holding” behavior has been observed regularly. No major illness has been recorded since its arrival, but bilateral swellings in the axillary areas were first reported in February 1995. On July 25, 1996, a general physical examination, including thoracic and abdominal...
radiographs, ultrasound, intradermal TB testing, and standard blood work was performed under general anesthesia. The only significant clinical finding was moderate obesity characterized by a 5.5% weight gain in the last 3 yr, a somewhat distended abdomen, and an excessive amount of subcutaneous and abdominal fat revealed by ultrasonography. No fluid nor material could be retrieved from transcutaneous aspirations of the axillary swellings, which were later believed to be associated with the accumulation of fat. Levels of TSH, thyroxine (T₄), and free T₄ were determined (TSH using a microparticle enzyme immunoassay; T₄ using a fluorescence polarization immunoassay; and free T₄ using a microparticle enzyme immunoassay; AxSYM®, Abbott Laboratories, Abbott Park, IL 60064 USA). Frozen sera taken from the same animal in 1993, and from three clinically healthy adult gorillas were also analyzed (Table 1). Compared with the clinically normal gorilla, and human reference values, the TSH level of the affected animal was markedly elevated, and its T₄ and free T₄ levels were significantly lower. Similar changes were also present in the 1993 sample (Table 1). Based on these results, a tentative diagnosis of primary hypothyroidism was made, and oral supplementation with levothyroxine sodium (Synthroid, Knoll Pharma Inc., Markham, Ontario, L3R 6H6, Canada) was started on August 18, 1996 at 50 μg/day. This dose was progressively increased by 50 μg every 2 wk, reaching a total of 200 μg/day on October 1, 1996. To monitor TSH levels, the gorilla was re-anesthetized 10 and 21 wk after the first immobilization. After 10 wk, TSH levels were below the reference range for humans, indicative of oversupplementation. Oral levothyroxine was consequently decreased to 88 μg/day and then 75 μg/day on October 25, 1996, and January 3, 1997 respectively. A 16% decrease in weight was observed during the 21 wk period (from 93 kg on July 25, 1996, to 78 kg on December 20, 1996). This weight reduction was associated with obvious changes in its body conformation, mainly characterized by a decrease in abdominal size and by slimming of facial features. Although its actual food consumption could not be accurately determined, its appetite seemed to increase. Its daily activity, and interactions with the other members of the group also markedly increased, and several new behaviors, such as wrestling, playing and “drumming,” have been reported since the beginning of the replacement therapy. As an unanticipated side effect, its hair coat has become quite rough and thrown up in whorls. Since hair growth anomalies have been described in humans with thyrotoxicosis⁸ this change might have been due to oversupplementation of levothyroxine.

Discussion

In humans, hypothyroidism is usually of thyroid origin (primary hypothyroidism), characterized by a low serum T₄, a high serum TSH, and the associated lack of T₄ negative feedback. Based on the clinical signs, the elevated TSH, the reduced T₄, and the therapeutic response, we believe that this gorilla is affected by overt primary hypothyroidism. TSH levels obtained from the three clinically normal gorillas were comparable to values reported in euthyroid humans, and the TSH values obtained for the hypothyroid gorilla markedly decreased following prolonged administration of oral levothyroxine. These findings indicate that the human-TSH assay used for this study is most likely valid for determination of gorilla-TSH levels. The relatively low T₄ and high TSH levels in the sample taken in 1993 indicate that the investigated animal had been hypothyroid for at least 2.5 yr. The three gorillas used as control had a marginally elevated TSH compared to reference values for humans. However, since variation between species might exist, and since these animals do not show
any clinical signs indicative of an endocrinopathy, the significance of this finding is unknown. Most hypothyroid humans show an increase in serum cholesterol.5 This was not the case in our animal; its serum cholesterol was low (3.31 mmol/L). Oral administration of levothyroxine produced a noticeable improvement of the animal’s physiological and psychological status. Monitoring of the serum TSH and $T_4$ every 6-8 wk is recommended during the establishment of the replacement therapy.11 In order to prevent iatrogenic hyperthyroidism, the goal of the treatment is to target a slightly high TSH level (between 10 and 20 mIU/L). The cause of the hypothyroidism observed in this gorilla remains undetermined. Primary hypothyroidism is not uncommon in older women12, secondary to autoimmune-mediated thyroiditis.2 No reproductive activity has been recorded in this female for several years. Since hypothyroidism has been associated with disturbance of the reproductive cycle,4 it is possible that its thyroid disorder may contribute to this reproductive inactivity. However, other factors such as sexual incompatibility with the present silverback and the presence of its daughter in the same group may have also contributed to its reduced sexual activity.

To our knowledge, cases of primary hypothyroidism have never been reported in gorillas. Primary hypothyroidism seems to be a rare event in nonhuman primates since only one case, described in a chimpanzee (Pan troglodytes), was found in a literature review.6 Considering the relatively high incidence of this condition in humans (between 0.1 to 0.7%),7 we believe that hypothyroidism is possibly underdiagnosed in nonhuman primates. Because of the nonspecific symptomatology of this condition, we advocate that a thyroid profile should be part of routine blood work in adult gorillas. TSH levels estimated by human-TSH assays seem to be a good indicator of thyroid function in gorillas, but results should be interpreted with caution since specificity could vary between different assays, laboratories, and species.10 In order to prevent iatrogenic thyrotoxicosis, slowly increasing dosages of thyroxine and monitoring of the serum TSH are advisable. An initial dosage of 50 µg/day of levothyroxine, with increments of 25 µg/day at monthly intervals up to a dosage of 100 µg/day, is proposed as starting regimen for hypothyroid gorillas.

ACKNOWLEDGMENTS

We are grateful to the keepers of the African Pavilion, and volunteers of the Metropolitan Toronto Zoo. We also thank Dr. Nancy Behme for useful discussion.

LITERATURE CITED


Table 1. Serum T₄, free T₄ levels, and TSH values in one hypothyroid and three euthyroid western lowland gorillas.

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Date</th>
<th>T₄  (nmol/L)</th>
<th>Free T₄ (pmol/L)</th>
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<td>25</td>
<td>9.2</td>
</tr>
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</table>

Reference values for human*: (64 - 162) (10 - 23) (0.4 - 5.0)

*Med-Chem Laboratories Limited, 8150 Sheppard Av E. Scarborough, Ontario, M1B 5K2, Canada

*Animal with clinical signs compatible with hypothyroidism.

*Animals clinically normal (euthyroid).

*Pre-levothyroxine supplementation.

*Excessive levothyroxine supplementation.

T₄: Fluorescence polarization immunoassay: Axsym®, Abbot Laboratories, Abbott Park, IL 60064 USA
Free T₄: Microparticle enzyme immunoassay: Axsym®, Abbot Laboratories, Abbott Park, IL 60064 USA
TSH: Microparticle enzyme immunoassay: Axsym®, Abbot Laboratories, Abbott Park, IL 60064 USA
HYPOTHYROIDISM IN AN ORANGUTAN (*Pongo pygmaeus pygmaeus*): CLINICAL MANAGEMENT AND LONG-TERM MONITORING

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Abstract

A 30-yr-old intact female Bornean orangutan (*Pongo pygmaeus pygmaeus*) was presented as a non-reproductive animal with a sparse haircoat, obesity (104 kg), lethargy, and consistent migration towards warmth. Previous history had included superovulatory drug stimulation without reproductive success. Dietary management did not alter its clinical signs.

Initial evaluation of its clinical status in 1993 revealed normal reproductive tract anatomy (via ultrasound, hormonal values and vaginal cytology) but low values for 3,5,3',5'-tetraiodothyronine ($T_4 = 3.3 \mu g/dL$, range = 4.5-12.0 $\mu g/dL$) and free 3,5,3'-triiodothyronine ($T_3 = 139 \text{ ng/dL}$, range = 230-420 ng/dL) when compared with human normal reference values.\(^1\)\(^,\)\(^2\) Values for thyroid stimulating hormone (TSH) were considered normal (3.28 ng/mL, range = 0.35-5.50 ng/mL), as were values for serum cortisol (2.5 $\mu g/dL$, range = 1.0-4.0 $\mu g/dL$) when compared to human reference normals.\(^1\)\(^,\)\(^2\) The complete blood count and serum chemistry profile demonstrated a fasting hypercholesterolemia (347 mg/dL, range = 100-200 mg/dL) which was also compatible with a clinical diagnosis of hypothyroidism.\(^1\) The rest of the complete blood count and serum chemistries were unremarkable, as were several urinalyses.

The orangutan was placed on synthetic levothyroxine sodium (Synthyroid, Knoll Pharmaceutical Company, Mount Olive, NJ 07828 USA) according to a therapeutic protocol developed for elderly humans (K.A. Alm, personal communication). Basically, a morning synthroid tablet of 0.05 mg for 2 wk, followed by 0.10 mg for 2 wk, then 0.15 mg for 2 wk, and finally its current dose of 0.20 mg is administered p.o. every 24 hr. Side effects noted were an increase in activity levels and mentation.

To monitor thyroid levels, the orangutan care staff has trained the orangutans to utilize a “blood sleeve” whereby the animal sits still for blood sampling from the antebrachium or the back of the hand. In this manner, $T_3$, $T_4$, and TSH values are routinely monitored. Blood samples are taken 6-8 hr and 24 hr after administration of levothyroxine.\(^1\) In addition, complete blood counts and serum chemistries are monitored without immobilization.

The orangutan has lost 60 kg during the 4 yr it has been supplemented with levothyroxine, and reevaluation of $T_3$, $T_4$ and TSH values are within normal limits ($T_3 = 274 \text{ ng/dL}$; $T_4 = 10.76 \mu g/dL$). In addition, its mental status, hair coat and activity level have improved greatly. The cholesterol levels have decreased from a high of 347 mg/dL to current levels of 190 mg/dL. Unfortunately, correction of this underlying problem has not resulted in conception. Further investigation into its reproductive soundness is continuing. In addition, further testing of thyroid function is continuing.
in two orangutans that are clinically normal.

This case represents a true team effort in addressing this orangutan’s problem, and monitoring its progress. Without the access to routine collection blood, it is unlikely that it would be immobilized to monitor thyroid levels. This orangutan continues to do well 4 yr after the initiation of thyroid supplementation.

ACKNOWLEDGMENTS

The author would like to thank the orangutan care staff for their intense efforts in training this animal to allow routine blood sampling.

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2. Roche Biomedical Laboratories, Burlington, North Carolina 27215-2250.
Baylisascaris: A ZOO-WIDE EXPERIENCE

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Abstract

Introduction

Baylisascaris sp., the common roundworm of raccoons and skunks, can cause devastating disease in many captive species of animals due to abnormal parasitic migration.1-5,8,11-14 Baylisascaris is not only a concern for a zoo’s animals, but it is a zoonosis, especially dangerous in a zoo/park setting where children abound.6,7,9,10 In the past 3 yr at the Los Angeles Zoo, we have seen numerous cases of central nervous system disease secondary to Baylisascaris. Major management changes and aggressive action has brought this disease under control at our zoo.

Case Reports

Affected animals were primarily from four exhibits in the zoo and included six species:

1) A family of 2.1 golden-headed lion tamarins (Leontopithecus rosalia chrysomelas). The two wild-caught adults survived, but their 2-yr-old male offspring was euthanatized.

2) A 12-yr-old female Indian fruit bat (Pteropus giganteus), which was euthanatized.

3) 1.1 juvenile yellow-footed rock wallabies (Petrogale xanthopus), which both survived.

4) An 8-yr-old male red-tailed black cockatoo (Calyptorhynchus magnificus), which survived.

5) A 7-yr-old male thick-billed parrot (Rhynchopsitta pachyrhyncha), which survived.

6) 0.3 young adult rose-breasted cockatoos (Eolophus roseicapullus), which were euthanatized.

In addition, multiple necropsies have demonstrated Baylisascaris in the wild fox squirrel (Sciurus niger) population at the zoo. All affected animals showed neurologic impairment consistent with brain dysfunction (ataxia, head tilt, intention tremors, circling). In surviving animals, the diagnosis was based on clinical signs, the elimination of other differential diagnoses, and gradual improvement. Unfortunately, no ante-mortem diagnostic test is available for this disease in animals.

Management Problems
The Los Angeles Zoo sits in the middle of Griffith Park, which is a large wild area. Previous non-management of pests had allowed the zoo to become overrun with these animals, and problems had reached epidemic proportions in 1995. Coyotes living in the zoo were hunting gerenuk and flamingos, skunks were everywhere, and raccoons had free-roam of the zoo. Common problem areas included “roundhouse” exhibits with wire tops which were the staple of 1960's design in our zoo. Raccoons and skunks traveled over the tops of these roundhouses and defecated into the exhibit. Even worse, feces could remain undetected on the top of the exhibit where they would dry up, crumble, and fall into the exhibit. Large wire mesh allowed skunks to run in and out of exhibits at will. A large overgrown botanical collection provided perfect hiding areas and pathways over the roundhouses. Garbage bin tops were broken or missing completely, allowing free access to unwanted visitors.

Management Response

With a change in zoo management came an immediate and aggressive response to this problem. A multi-faceted approach was taken as described below.

1) Current population of resident wildlife were removed. Working with an outside agency, we trapped and eliminated skunks, coyotes, and raccoons from the zoo.
2) Access by future inhabitants was prevented by repairing gaps in the perimeter fence.
3) Tree-trimming and foliage pruning were instituted to eliminate overhead pathways and hiding areas.
4) Animals were prevented from getting into exhibits. Small gauge mesh along bottoms of large gauge mesh exhibits was installed in heavily trafficked areas.
5) Animals were prevented from having easy access to garbage bins containing thrown away food by repairing lids and educating keepers to keep them closed.
6) Infected exhibits were rehabilitated. As much dirt as possible and all foliage were removed and replaced. A protocol for clean-up was issued to involved personnel and incorporated into the zoo’s procedure manual. Personnel cleaning exhibits were required to wear face mask filters capable of filtering ova larger than 53 microns, disposable coveralls, gloves, and shoe-covers or boots. All disposable and organic materials were incinerated. No ground-eating or foraging species were put in these exhibits after rehabilitation.
7) Control measures were instituted. Animal care staff was instructed to check exhibit tops and nooks and crannies routinely for feces or debris. Staff was educated about the importance of removing feces immediately since feces are not infective until 3-4 wk later when the eggs embryonate. Keeper education was paramount to the success in getting them to report raccoons and skunks in the area and maintenance of routine monitoring and trapping.

Conclusion

The tasks outlined above were mammoth but were successful in preventing additional clinical cases. Community and staff education and cooperation were challenging, but were the key to the
implementation of the above steps. Political issues regarding control of wildlife at the zoo had to be overcome, including animal rights objections and the refusal of outside agencies to help. While native wildlife can not be completely eliminated, the population can be controlled by continual trapping and surveillance. Through education of personnel regarding the effects of this parasite, and aggressive, thorough, and continual action, zoological parks can conquer Baylisascaris.

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ADDENDUM: *Baylisascaris* - EVERYTHING A KEEPER SHOULD KNOW

**What is it?**

*Baylisascaris* is a nematode parasite, a roundworm that is carried commonly by raccoons, as well as by badgers, skunks, fishers and martens, and bears (different species for each).

**Why should it be a problem for the animals we care for?**

In its normal host, this parasite is just a GI parasite like any others. The problem lies when the eggs are ingested by an “intermediate host”. The larvae mature and start to migrate through this unfortunate animal’s body, eventually encysting wherever they end up (approx. 5-7% of these are in the brain).

**How would I as a keeper come into contact with this parasite?**

Eggs are shed in feces and an intermediate host (animal or human) becomes infected by ingesting eggs from environmental areas that have become contaminated by these eggs.

**What symptoms would be seen with an abnormal parasite migration?**

Three syndromes are seen: visceral, ocular, and CNS migration, depending on the number of larvae ingested, and their location and behavior in the body. CNS disease (incoordination, head tilt, decreased head control, loss of balance, etc.) is the most serious and characteristic form of infection, however, disease can manifest itself in the retina or other tissues of the body. Clinical signs may never show in a mild infection of muscle, connective tissue, or other noncrucial parts of the body.

**How common is human infection?**

This is difficult to say, as the disease has been documented only relatively recently. Thus far only four CNS cases, two of which were fatal, and about a dozen cases of ocular disease are known.

**How is this disease diagnosed and treated?**

Unfortunately, there is no good test for animals. In people, blood tests available. There is no treatment for CNS disease. In ocular disease, laser therapy of the retina is a potential treatment.

**How can we prevent exposure to this parasite?**

Foremost in importance is proper sanitation. The eggs are only infective after 3-4 wk of sitting in the feces. Prompt cleanup of feces eliminates this problem. As with any infectious disease, careful sanitation habits will prevent transmission. Wash hands frequently, and always wash before eating, smoking, etc. Avoid putting your hands in your mouth (biting fingernails, etc.) during work. Showering and changing of clothes before leaving the zoo is ideal. Animals that have become clinically infected with CNS disease from migration are **NOT** infective to you. The larvae have encysted in the brain and thus they are not shedding eggs in their feces. The other form of control is eliminating the animals that are shedding these eggs. Feral animals must be prevented from entering captive animals’ enclosures.
How do we clean up chronically infected areas?
Unfortunately, there is no easy way. The eggs are resistant to all common disinfectants and most environmental conditions. Large areas of contaminated soil or concrete must be flamed. Topsoil can be removed and buried. Autoclaving and use of a 1:1 mixture of xylene:ethanol can be used for certain surfaces or small cages. Personnel should wear disposable clothes, gloves, a dust mask, and rubber boots that should be washed in hot soapy water to clean organic debris, then rinsed with a foot bath containing bleach.

For further questions, discuss this topic with your veterinarian. Information for this article was taken from “Zoonosis Update: Baylisascaris larva migrans” (J. Am. Vet. Med. Assoc. 1989. 195:7).
THE GENETIC AND BIOCHEMICAL BASIS FOR THE “GOLDEN TABBY” AND “SNOW WHITE” BENGAL TIGERS

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Abstract

Introduction

Records of a breeding colony of Bengal tigers were analyzed, classifying tigers and their offspring according to color (Standard, Golden Tabby, White, and Snow White) and sex, where known. The “Standard,” or wild type, Bengal tiger is a reddish orange with narrow black, gray, or brown stripes.4 The popular “White” tiger is usually a cream y to chalky white with darker stripes.1 The “Snow White” tiger appears to be a variation of the White tiger. Snow Whites are also pale white, but with pale to almost nonexistent stripes. The last type will be referred to as a “Golden Tabby”, or “Tabby”. The Golden Tabbies are orange like the Standard but have darker orange to brown stripes, rather than black.

Hair samples from each of the color variations were analyzed to determine the type of pigment present. The presence of melanin deposits in the hair shaft determines the color of the tiger’s coat. There are two different kinds of melanin: eumelanin and phaeomelanin. Eumelanin absorbs almost all light, thus producing a black color. Phaeomelanin reflects light in the red-orange-yellow range.5

The coloration of the white tiger is due to a single autosomal recessive gene.6 This gene is symbolized as c and appears to have an effect similar to the Chinchilla gene in other species. It acts by drastically reducing or eliminating phaeomelanin, causing the base coat color to change from standard orange to a cream or white color. The eumelanin present in the hair of the black stripes is hardly affected, at most fading to a dark brown.1

Breeding record analysis

The breeding records of this Bengal tiger colony listed 15 litters produced by 5 female and 5 male tigers (Tables 1 and 2). These matings consisted of Golden Tabbies bred to each of the four colorations, and Standards bred to White and Snow White. Based on the hypothesis that there are two autosomal recessive genes involved in the production of these four colorations, the following results were expected assuming all individuals were either recessive or heterozygous at both loci (Fig. 1). Tabbies bred to Standards should produce 3/8 Standard, 3/8 Tabby, 1/8 White and 1/8
Snow White. Tabbies bred to Tabbies would produce 3/4 Tabbies and 1/4 Snow White. Tabbies bred to Snow Whites would produce equal numbers of Tabbies and Snow Whites. Tabbies bred to Whites would produce equal numbers of all four colorations, as would Standards bred to Snow Whites. Finally, Standards bred to White would produce 3/8 Standard, 3/8 White, 1/8 Tabby, and 1/8 Snow White.

The data were analyzed by locus using Chi-square analysis, with the expected ratios based on the stated hypothesis (Tables 3 and 4). None of the results of the crosses deviated from the expected ratios more than that due to chance ($P > 0.05$). The gender ratios were also analyzed to confirm that both genes were autosomal rather than sex-linked. If the Tabby gene were sex-linked, all male offspring of a Tabby female would be Tabbies or Snow Whites, which was not seen. However, as an autosomal gene, the ratios would be expected to be evenly divided between male and female. This was found to be the case, with only some chance deviation, as determined by Chi-square analysis (Table 5).

**Determination of pigment type**

The hair samples were analyzed by high-performance liquid chromatography (HPLC) following chemical degradation. The chemical degradation consisted of permanganate oxidation to produce pyrrole-2,3,5-tricarboxylic acid (PTCA), a specific indicator of eumelanin, and hydriodic acid (HI) hydrolysis to produce aminohydroxyphenylalanine (AHP), a specific indicator of pheomelanin.

Based on the results of chemical degradation and HPLC analysis, the Standard tiger was found to have a pheomelanic base coat with eumelanic stripes, as stated in previous literature. The White tiger has an amelanotic coat, having only a minimal level of pigment, with eumelanic stripes. This agrees with the hypothesis that a “chinchilla”-like gene is acting to dilute pheomelanin in the coat.

The Tabby has a pheomelanic base coat with pheomelanic stripes. This supports the inhibition of eumelanin production as the cause of the color variation. The Snow White has an amelanotic base coat and stripe. This would be expected based on the hypothesis that the Snow White coloration is due to the presence of both homozygous recessives. The Tabby gene changes the stripe pigment from eumelanin to pheomelanin, which is then diluted in both the stripe and the base coat due to the chinchilla gene, to produce the Snow White.

**LITERATURE CITED**


\[
\begin{align*}
1/2 T^+t & \quad \frac{1}{4} C^+C^+ \quad \frac{1}{8} T^+t C^+C^+ \\
1/2 C^+c^{ch} & \quad \frac{1}{4} T^+t C^+c^{ch} \\
1/4 c^{ch}c^{ch} & \quad \frac{1}{8} T^+t c^{ch}c^{ch} \\
1/2 T^t & \quad \frac{1}{4} C^+C^+ \quad \frac{1}{8} tt C^+C^+ \\
1/2 C^+c^{ch} & \quad \frac{1}{4} tt C^+c^{ch} \\
1/4 c^{ch}c^{ch} & \quad \frac{1}{8} tt c^{ch}c^{ch} \\
\end{align*}
\]

3/8 Standard
3/8 Golden Tabby
1/8 White
1/8 Snow White

**Figure 1.** Example of determining offspring ratios Tabby (tt C<sup>+</sup>c<sup>ch</sup>) × Standard (T<sup>+</sup>t C<sup>+</sup>c<sup>ch</sup>) cross.

**Table 1.** Presumed genotype of breeding tigers: based on phenotype, pedigree and progeny information.

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<th>Phenotype</th>
<th>Presumed genotype</th>
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<td>Standard</td>
<td>T&lt;sup&gt;+&lt;/sup&gt;tC&lt;sup&gt;+&lt;/sup&gt;c&lt;sup&gt;ch&lt;/sup&gt;</td>
</tr>
<tr>
<td>Male 4</td>
<td>Snow White</td>
<td>tt c&lt;sup&gt;ch&lt;/sup&gt;c&lt;sup&gt;ch&lt;/sup&gt;</td>
</tr>
<tr>
<td>Male 5</td>
<td>Snow White</td>
<td>tt c&lt;sup&gt;ch&lt;/sup&gt;c&lt;sup&gt;ch&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Table 2. Mating types and resulting offspring phenotypes.

<table>
<thead>
<tr>
<th>Mating-type</th>
<th>Progeny phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard</td>
</tr>
<tr>
<td>Female 1^b</td>
<td>Male 1^a</td>
</tr>
<tr>
<td></td>
<td>Male 1^a</td>
</tr>
<tr>
<td></td>
<td>Male 2^b</td>
</tr>
<tr>
<td></td>
<td>Male 3^a</td>
</tr>
<tr>
<td></td>
<td>Male 3^a</td>
</tr>
<tr>
<td></td>
<td>Male 3^a</td>
</tr>
<tr>
<td></td>
<td>Male 5^d</td>
</tr>
<tr>
<td>Female 2^b</td>
<td>Male 3^a</td>
</tr>
<tr>
<td></td>
<td>Male 3^a</td>
</tr>
<tr>
<td>Female 3^c</td>
<td>Male 2^b</td>
</tr>
<tr>
<td></td>
<td>Male 3^a</td>
</tr>
<tr>
<td></td>
<td>Male 3^a</td>
</tr>
<tr>
<td></td>
<td>Male 3^a</td>
</tr>
<tr>
<td>Female 4^d</td>
<td>Male 4^d</td>
</tr>
<tr>
<td>Female 5^e</td>
<td>Male 5^e</td>
</tr>
</tbody>
</table>

^a Standard  
^b Golden Tabby  
^c White  
^d Snow White  
m-male offspring  
f-female offspring

Table 3. Chi-square analysis of results of matings, segregating at the Tabby locus.

<table>
<thead>
<tr>
<th>Mating</th>
<th>Offspring</th>
<th>Observed</th>
<th>Expected</th>
<th>$\chi^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T't × tt</td>
<td>T't^a</td>
<td>18</td>
<td>17.5</td>
<td>0.0289</td>
<td>0.9-0.7</td>
</tr>
<tr>
<td></td>
<td>tt^b</td>
<td>17</td>
<td>17.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T't × T't</td>
<td>T'^a</td>
<td>9</td>
<td>8.25</td>
<td>0.2727</td>
<td>0.7-0.5</td>
</tr>
<tr>
<td></td>
<td>tt^b</td>
<td>2</td>
<td>2.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tt × tt</td>
<td>tt^b</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>--</td>
</tr>
</tbody>
</table>

^a Standard or White  
^b Tabby or Snow White
Table 4. Chi-square analysis of results of matings, segregating at the chinchilla-like locus.

<table>
<thead>
<tr>
<th>Mating</th>
<th>Offspring</th>
<th>Observed</th>
<th>Expected</th>
<th>$\chi^2$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C^e ch \times c^e ch \rightarrow$</td>
<td>$C^e c^{ch}$</td>
<td>13</td>
<td>10</td>
<td>1.8000</td>
<td>0.2-0.1</td>
</tr>
<tr>
<td></td>
<td>$c^{ch}c^e ch$</td>
<td>7</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C^e ch \times C^e ch \rightarrow$</td>
<td>$C^e_-$</td>
<td>21</td>
<td>20.25</td>
<td>0.1111</td>
<td>0.9-0.7</td>
</tr>
<tr>
<td></td>
<td>$c^{ch}c^{ch}$</td>
<td>6</td>
<td>6.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C^e C^e \times C^e c^{ch} \rightarrow$</td>
<td>$C^e_-$</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>$C^e C^e \times c^{ch}c^{ch} \rightarrow$</td>
<td>$C^e c^{ch}$</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>--</td>
</tr>
</tbody>
</table>

* Standard or Tabby  
* White or Snow White

Table 5. Gender ratios of offspring with Chi-square analysis.

<table>
<thead>
<tr>
<th>Coloration</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>Expected ratio</th>
<th>$\chi^2$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>18</td>
<td>10</td>
<td>8</td>
<td>9:9</td>
<td>0.2222</td>
<td>0.7-0.5</td>
</tr>
<tr>
<td>Golden Tabby</td>
<td>23*</td>
<td>13</td>
<td>10</td>
<td>11.5:11.5</td>
<td>0.3913</td>
<td>0.7-0.5</td>
</tr>
<tr>
<td>White</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>4.5:4.5</td>
<td>0.1111</td>
<td>0.9-0.7</td>
</tr>
<tr>
<td>Snow White</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>2:2</td>
<td>1.00</td>
<td>0.5-0.3</td>
</tr>
</tbody>
</table>

* Does not include two offspring of unknown gender.
CHRONIC FASTING HYPERLIPIDEMIA IN A CHEETAH \textit{(Acinonyx jubatus)} AND NORMAL FASTING LIPOPROTEIN LEVELS FOR THE SPECIES

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Abstract

A severe fasting hyperlipidemia was identified in an 11-yr-old male cheetah immobilized for a two-day history of vomiting, lethargy, and partial anorexia. Compensated chronic renal insufficiency, severe gastric gland atrophy, mild venous occlusive disease, and a parathyroid adenoma were also found in this animal. The hyperlipidemia was characterized by hypertriglyceridemia and hypercholesterolemia. Metabolic causes of hyperlipidemia such as chronic renal insufficiency may have contributed to the condition of this cheetah. The hyperlipidemia spontaneously resolved, but a project was undertaken to document lipoprotein normal values for the species.

Introduction

Lipoproteins consist of triglycerides, cholesterol, and proteins in varying quantities which are divided into four types based on density and electrophoretic mobility. Chylomicrons and very low-density lipoproteins (VLDLs) are primarily triglyceride, the former being dietary in origin and the latter being synthesized by the liver. Low density lipoproteins (LDLs) and high-density lipoproteins (HDLs) transport cholesterol to and from the liver and body tissues.9

Hyperlipidemia refers to an increased concentration of serum lipids. Primary hyperlipidemias result from inherited defects in lipid metabolism, and secondary hyperlipidemias are indicators of underlying metabolic diseases.9,10

Diagnostic approach should first distinguish primary from secondary hyperlipidemias. Primary hyperlipidemias are uncommon and usually recognized in young animals. Depending on the enzyme defect involved, they can have severe elevations in one or more lipoproteins.5 The more common secondary hyperlipidemias cause varying hyperlipidemias. Secondary hyperlipidemias have been associated with diabetes mellitus, nephrotic syndrome, pancreatitis, hypothyroidism, chronic renal failure and hyperadrenocorticism in animals and man.27,10 The type of lipoprotein that is elevated can assist in identifying a metabolic etiology.
Case Report

An 11-yr-old male cheetah was examined for vomiting, lethargy, and anorexia of 2 days duration. Significant findings were moderate dehydration and a severe hyperlipidemia noted during blood sample collection after a 36-hr fast. Laboratory findings showed the cheetah to be azotemic, with blood urea nitrogen (BUN) of 69 mg/dL and creatinine of 4.0 mg/dL (International Species Information System [ISIS] reference ranges: 27-47 mg/dL and 1.6-3.2 mg/dL, respectively). A mature neutrophilic leukocytosis was present. The cheetah was treated daily with Penicillin G Benzathine and Penicillin G Procaine (Duo-Pen, G.C. Hamford MFG. Co., Syracuse, NY 13201 USA) at 20,000 IU/kg i.m., and immobilized every other day for intravenous and subcutaneous fluids for 2 wk. Initial clinical impressions of the animal suggested pancreatitis with prerenal azotemia, though a urine sample could not be obtained to rule out renal insufficiency. Pancreatitis could not be verified due to the lack of elevated pancreatic enzymes on serum biochemical analysis. The cheetah responded to therapy with a return to normal attitude and appetite.

Persistent azotemia, submaximal urine concentration, and hyperlipidemia were found during weekly immobilizations. Chronic renal insufficiency with an idiopathic hyperlipidemia was considered at this time. Over the next 12 wk, the animal had three episodes of mild lethargy and partial anorexia. Immobilizations were performed every 1-2 wk for fluid administration and additional testing to find the cause of the persistent hyperlipidemia.

Other problems identified in this cheetah by biopsy were mild hepatic venous occlusive disease, moderate renal interstitial fibrosis with tubular atrophy, and severe gastric gland atrophy consistent with the chronic gastritis seen in this species. The hyperlipidemia was characterized to be chylomicron in nature by the “refrigerator test”. To subjectively test lipoprotein lipase (LPL) activity, the animal was given intravenous sodium heparin 10,000 IU/ml (Heparin, Elkins-Sinn, Inc., Cherry Hill, NJ 08003 USA) at 80 IU/kg. Post-heparin injection samples were less opaque than the pre-injection sample. Serum samples from the cheetah were submitted for lipid gel electrophoresis (Comparative Nutrition Research Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4474 USA) to identify the different lipoprotein particles. The electrophoresis confirmed the less sophisticated test results of a hypertriglyceridemia that was primarily chylomicron and VLDL in nature (Table 1).

Twenty-four weeks after initial presentation, the fasting hyperlipidemia spontaneously resolved. Compensated chronic renal insufficiency with azotemia, isosthenuria, polydipsia and polyuria remained. Forty-four weeks after the initial illness, the cheetah was euthanatized for uncompensated renal failure that failed to respond to aggressive fluid therapy. Hyperlipidemia was not present at euthanasia.

Significant necropsy and histology findings confirmed mild hepatic venous occlusive disease, severe chronic gastritis, and chronic renal failure with interstitial nephritis, multifocal renal fibrosis, and amyloidosis. Acute renal papillary necrosis was also present. The right parathyroid gland was markedly enlarged and histologically contained an adenoma. The pancreas was small and fibrous.
with no histological evidence of inflammation.

Discussion

A clinical finding of hyperlipidemia is not uncommon in veterinary medicine, with postprandial hyperlipidemia being the most frequent cause. Most common metabolic etiologies such as diabetes mellitus, hypothyroidism, hyperadrenocorticism, and nephrotic syndrome were ruled out either due to lack of clinical laboratory evidence or inappropriate lipoprotein elevation. The exact cause of the persistent severe hyperlipidemia was not determined. Chronic renal failure was a significant finding in this cheetah and could have contributed to hyperlipidemia. In laboratory animals, experimentally-induced renal failure interfered with VLDL removal from the circulation causing hyperlipidemia that was triglyceride in nature. A similar pathogenesis has been suggested for the hyperlipidemia of renal failure seen in man. Increased parathyroid hormone can contribute to hyperlipidemia of renal failure by suppression of LPL. Unfortunately, it was not known whether the parathyroid adenoma in this cheetah was an active neoplasia. Pancreatitides could also have contributed to hyperlipidemia in this animal.

ACKNOWLEDGMENTS

The authors thank Patricia Hawkins, CVT, Angie Burris, CVT, Tammy Mottl, CVT, and the Oklahoma City Zoo, Milwaukee County Zoo, and White Oak Plantation for their assistance and support of this project.

LITERATURE CITED


1997 PROCEEDINGS AMERICAN ASSOCIATION OF ZOO VETERINARIANS

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Table 1. Serum triglyceride and cholesterol concentrations, and percent distribution of lipoproteins in a hyperlipidemic cheetah (*Acinonyx jubatus*), as determined by agarose gel electrophoresis.

<table>
<thead>
<tr>
<th>Lipoprotein Fractions (%)</th>
<th>Affected Cheetah(^a)</th>
<th>Normal Cheetahs(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>25.0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Beta</td>
<td>32.0</td>
<td>19.41 ± 5.59 (9.7-29.2)</td>
</tr>
<tr>
<td>Pre-Beta</td>
<td>23.5</td>
<td>12.56 ± 7.9 (0-22.4)</td>
</tr>
<tr>
<td>Alpha</td>
<td>21.0</td>
<td>68.03 ± 10.0 (51.4-85.2)</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>1417.0</td>
<td>24.77 ± 10.0 (9.5-46.3)</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>549.5</td>
<td>168.56 ± 47.76 (96.9-290.2)</td>
</tr>
</tbody>
</table>

\(^a\)Value for affected cheetah is the average of two samples.
ODONTOIDECTOMY AND ATLANTOAXIAL ARTHRODESIS FOR TREATMENT OF A MALFORMED DENS IN AN AFRICAN LION (Panthera leo)

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1Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 USA; 2International Clinical Scholar in Wildlife and Zoological Medicine, Guatemala City, Guatemala

Abstract

Case report

An 8-mo-old 54-kg castrated male African lion (Panthera leo) was referred for diagnosis and treatment of chronic progressive ataxia. On presentation, the lion had difficulty rising from lateral recumbency and exhibited signs of discomfort when his neck and limbs were manipulated. Incoordination, ataxia, and quadriparesis were observed during the brief periods of ambulation. Physical examination under anesthesia revealed an incipient cataract OD, rear limb muscle atrophy, and mild deviation of the neck to the right that could not be manually straightened. Needle electromyography revealed fibrillation potentials in the platysma muscle adjacent to C1-C2. Survey radiographs of both shoulders and the entire spine did not reveal abnormalities. Myelography by cisternal injection revealed an extradural compressive cervical spinal cord lesion due to dorsal placement of the odontoid process (dens) of the axis. Dorsal and ventral subarachnoid spaces were attenuated at this site. During flexion of the head, the width of the spinal cord was approximately half of the width of the adjacent spinal cord at C2. Following recovery from anesthesia, it was evident that the cervical manipulations had exacerbated the clinical signs. The lion demonstrated quadriplegia with inability to rise and ambulate. Acetylcysteine (Roxane Laboratories, Columbus, OH 43216 USA; 1 g p.o. every 8 hr × 5 days followed by 1 g p.o. every 8 hr every other day for 6 days) was administered until surgery could be performed 72 hr after presentation. With the lion in dorsal recumbency, a midline ventral approach was made to reach the cranial cervical vertebrae. The atlantoaxial articulation was identified and exposed. The dorsally displaced dens was removed using a power bur and a bone rongeur to allow decompression of the spinal cord. The cartilage of the articular facets of the atlas and the axis was removed using a power bur. Subluxation and right flexion of the atlantoaxial articulation were reduced. Cancellous bone screws (6.5 mm) were placed through the facets of the axis and atlas. Cancellous bone was collected from the proximal humerus, mixed with a synthetic bone graft particulate (Consil™, Bioglass®, US Biomaterials Corporation, Nutramax Laboratories, Inc., Baltimore, MD 21236 USA), and packed into the atlantoaxial joint space to encourage fusion of C1-C2. Postoperative radiographs confirmed surgical removal of the dens, proper screw and graft placement, and adequate alignment of the vertebrae. Within 24 hr following surgery, the lion made attempts to stand, began to regain strength in all limbs, and exhibited fewer signs of discomfort during manipulation and movement. Two weeks following odontoidectomy and atlantoaxial arthrodesis, the lion moved easily and playfully with only mild to
Discussion

Treatment centered on surgical decompression of the spinal cord, surgical stabilization to prevent further damage to the spinal cord, and medical therapy with acetylcysteine to reduce free radical and oxidative damage to spinal cord tissue.\(^2\) The thickness of the wings of the atlas in this animal allowed placement of 6.5 mm cancellous bone screws. Unique to this procedure was the utilization of a bioactive ceramic (Bioglass\(^\circledast\)) in conjunction with bone graft to aid regeneration of bone.\(^5\) This synthetic particulate product initiates a rapid chemical bond to bone\(^3,4\) and shows osteoprotective properties. After exposure to blood in the surgical wound, the surface of Bioglass\(^\circledast\) converts to a silica-rich gel layer that mineralizes into a hydroxyapatite layer. Osteogenic stem cells in the bone graft-Bioglass\(^\circledast\) admixture colonize the bioactive surface of the synthetic particulate.\(^6\) Osteoblasts then differentiate and produce new bone throughout the implant site.\(^1\)

LITERATURE CITED

PHARMACOKINETICS OF ENROFLOXACIN AFTER ORAL AND INTRAMUSCULAR ADMINISTRATION IN SAVANNA MONITORS (Varanus exanthematicus)

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¹College of Veterinary Medicine, Auburn University, Auburn, AL 36830 USA; ²Department of Animal Health, National Zoological Park, Washington, DC 20008 USA; ³Department of Anatomy, Physiological Sciences and Radiology, North Carolina State University, Raleigh, NC 27606 USA

Abstract

Introduction

Enrofloxacin is usually given subcutaneously or intramuscularly to reptiles at a dosage of 2.5-5.0 mg/kg at intervals varying from s.i.d. to once every 5 days. Adverse effects reported for injectable enrofloxacin include muscle soreness and local tissue swelling after injection.³ Oral administration of enrofloxacin has recently become popular, although most dosages for oral enrofloxacin in reptiles are empirically derived. If absorbed, orally administered enrofloxacin could prove useful for treatment of very large or very small reptile patients.

Methods

All protocols were approved by the Institutional Animal Care and Use Committee at the National Zoological Park, Washington, D.C. Ten adult savanna monitors (Varanus exanthematicus), weighing from 1.2 kg to 2.0 kg, were used from the Department of Zoological Research at the National Zoological Park in Washington, D.C. The lizards were individually housed (86 × 71 × 58 cm cages), and maintained at 27 ± 1 °C. They were maintained on a diet of whole laboratory mice offered once/wk. The lizards were assessed to be clinically healthy 2 wk prior to the study based upon physical exam, survey radiographs, and routine blood analyses (complete blood count and chemistry panel). The pre-trial blood sample was also used as the “zero sampling” time for administration of the antibiotics. Plasma concentrations of enrofloxacin and its metabolite, ciprofloxacin, were determined by high-performance liquid chromatography (HPLC).¹ The detection limit of quantitation of enrofloxacin and ciprofloxacin using this method was 0.05 µg/ml.

In the first trial, 10 savanna monitors were each fed a laboratory mouse containing 10 mg/kg of injectable (22.7 mg/ml) enrofloxacin (Baytril, Miles Inc., Shawnee Mission, KS 66201 USA). Each lizard was manually restrained for collection of 1 ml of heparinized blood from the caudal tail vein at predetermined intervals following ingestion. Five animals were sampled four times (30 min, 3 hr, 12 hr, and 36 hr) and five were sampled three times (1 hr, 6 hr, and 24 hr).

Two weeks later in the second trial, 10 savanna monitors were manually restrained and given an intramuscular injection of enrofloxacin at a dosage of 10 mg/kg in the left forelimb. One milliliter of heparinized blood was collected from the caudal tail vein at the time intervals described in Trial
1.

Results

The highest measured mean plasma concentration of enrofloxacin was 5.82 µg/ml at 36 hr post ingestion (Fig. 1). The peak plasma concentration of enrofloxacin was 12.47 µg/ml at 6 hr post i.m. administration (Fig. 2). The calculated average half life of enrofloxacin after i.m. injection is 56 hr. It was not possible to calculate the half life after oral administration because plasma levels were continuing to increase after 36 hr. Conversion of enrofloxacin to ciprofloxacin was minimal in both trials, never rising above 0.1 µg/ml.

Discussion

Enrofloxacin has a wide spectrum of antimicrobial activity and a large volume of distribution. The minimum inhibitory concentration (MIC) for common reptile pathogens is lower for enrofloxacin than other commonly used antimicrobials, which results in a favorable margin of safety. This antibiotic has a broad spectrum of activity which includes the common reptile pathogens: *Pseudomonas* sp., *Aeromonas* sp., and *Klebsiella* sp. Pharmacokinetic studies for intramuscularly administered enrofloxacin have been conducted on two species of tortoise (*Gopherus polyphemus* and *Geochelone elegans*), one species of frog (*Rana catesbeiana*), and one species of snake (*Python molurus bivittatus*). No published reports were identified which described the pharmacokinetics of oral enrofloxacin, or any other oral antibiotic, in any reptile species.

Enrofloxacin, and its active metabolite ciprofloxacin, are capable of inhibiting growth of pathogenic bacteria at serum levels of approximately 0.1 µg/ml. In savanna monitors, the plasma concentrations exceeded 0.1 µg/ml 30 min after intramuscular injection and 6 hr after ingestion. These levels were maintained throughout the sampling period (36 hr) in both trials. For both oral and intramuscular administrations, peak plasma concentrations were two to six times higher than those reported for tortoises, bullfrogs, and pythons. Given the long calculated half life (56 hr) following intramuscular enrofloxacin, a dosing interval of every 5 days should be considered.

In comparison to plasma concentrations following i.m. injection, absorption of orally administered enrofloxacin was delayed. Plasma concentrations after 24 hr were similar. Therefore, an initial dose of enrofloxacin (10 mg/kg i.m.) followed by oral administration for continued therapy would be beneficial for acute infections. In future trials, a lower dosage (5 mg/kg) and longer sampling times up to 100 hr post administration will be evaluated to determine peak levels after oral administration.

LITERATURE CITED


Figure 1. Semilogarithmic plot of plasma enrofloxacin and ciprofloxacin concentrations (µg/ml) versus time in 10 savanna monitors (Varanus exanthematicus) following administration of a single oral dose of enrofloxacin (10 mg/kg).
Figure 2. Semilogarithmic plot of plasma enrofloxacin and ciprofloxacin concentrations (µg/ml) versus time in 10 savanna monitors (Varanus exanthematicus) following administration of a single intramuscular dose of enrofloxacin (10 mg/kg).
MANAGEMENT OF A DISTAL METATARSAL FRACTURE IN A GIRAFFE

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Abstract

A distal physeal metatarsal fracture (Salter IV) in a 15-mo-old female reticulated giraffe (Giraffa camelopardalis) was successfully managed by external coaptation using fiberglass walking casts and barn confinement. The animal was immobilized using xylazine and etorphine on four occasions over 20 wk for three cast applications and the final cast removal. It was maintained in barn confinement during this time and for an additional 2 mo because of joint laxity in the affected fetlock after removal of the final cast. Return of the animal to the herd was uneventful although full resolution of soft tissue swelling and laxity in the affected joint required several months.

Case Report

A 15-mo-old female giraffe presented with an acute weight bearing lameness of the right hind limb associated with significant lateral deviation of the extremity distal to the fetlock. The fetlock area was swollen and the degree of misalignment was such that a fracture or luxation was apparent, yet the animal bore considerable weight on the injured limb. It was separated in a stall for immobilization and evaluation the following day. The stall had a quarter circle configuration and a swing gate designed for crowding a giraffe to restrict space during immobilization.

The animal was estimated to weigh 400 kg. It was darted with 25 mg xylazine (Xyla-Ject, Phoenix Pharmaceuticals, Inc., St. Joseph, MO 64504 USA) combined with 10 mg atropine (Atropine Sulfate L.A., Phoenix Pharmaceuticals, Inc., St. Joseph, MO 64504 USA). The squeeze was gradually closed to restrict movement until the animal was confined in a space in which it was unable to turn around. Twenty minutes after the initial injection, 2 mg etorphine (M-99, Lemon Co., currently available from Wildlife Pharmaceuticals, Inc., Fort Collins, CO 80524 USA) combined with 75 mg hyaluronidase (Wydase, Wyeth Laboratories, Inc., Philadelphia, PA 19101 USA) was given by pole syringe. The squeeze gate prevented the animal from falling over backward and recumbency was complete in 9 min. The gate was then opened, the animal was laid in lateral recumbency with the head and neck elevated. Oxygen was administered by nasal catheter and the tongue was extended to prevent it from impeding respiration.

Radiographs revealed a distal physeal fracture of the metatarsal bone and a fracture of the corner of the distal lateral metatarsus (Salter IV) with 10-15% lateral displacement at the physis. Traction was applied to the injured limb for approximately 15 min by means of a rope tied around the base of the foot and attached to a cable ratchet device (“come-along”). The animal was prevented from sliding...
when traction was applied by securing another set of thick cotton ropes through the groin, around the limb and up over the hip to tie against a back wall. Intravenous ketamine (Ketaset, Fort Dodge Laboratories, Inc., Fort Dodge, IA 50501 USA) was given to deepen anesthesia during traction. This effort was unsuccessful and an equine-type walking cast was applied to a level just below the hock without further attempts to reduce the fracture. This was done using fiberglass cast material over custom foam support and stockinette (Orthopedic Products Division/3M, St. Paul, MN 55144 USA).

Total work time required to accomplish the procedures was 132 min. Four supplemental doses of 200 mg ketamine i.v. and two doses of 0.5 mg i.v. etorphine were given during the procedure. The last dose of etorphine was given to position the animal in sternal recumbency for closure of the crowding gate prior to recovery. Reversal was accomplished with tolazoline (Priscoline, Ciba-Geigy Corp., Pharmaceuticals Div., Summit, NJ 07901 USA)) 100 mg i.v. and diprenorphine (M50-50, Lemon Co.) 6 mg i.v. The animal stood 9 min after reversal, but fell back down to a sternal position until successfully standing at 15 min. The crowding gate prevented it from falling on its side.

The giraffe was confined in two adjoining barn stalls measuring 8.6 m × 6 m, and 8 m × 5 m. It was not bearing weight on the affected limb for several days and was given phenylbutazone (Equi-Phar Butazolidin, Vedco, Inc., St. Joseph, MO 64503 USA) 2 g/day on the feed during this time. During a second immobilization 2 wk later, the cast was removed and radiographs were taken which showed the fracture to be stable with possible early callus formation. Swelling of the fetlock was reduced and the fracture site was stable by palpation. A second cast was applied as before with extra reinforcement of the toe to compensate for excessive wear from dragging the cast. The heel area was built up to help promote weight bearing. The time required to complete the procedures was 86 min and additional doses of etorphine and ketamine were necessary as before. The animal stood up successfully on the first attempt 5 min after reversal using 300 mg tolazoline i.v. and 6 mg diprenorphine i.v. The giraffe was walking with improved use of the limb 3 days after the second cast was applied and was fully weight bearing in about 3 wk. By this time, it trotted around the barn stalls showing excellent adaptation to the cast.

The second cast was removed in 8 wk. Radiographs showed solid callus formation with bone remodeling and beginning resorption of the fragment. A third cast was applied since it was judged that at least 16 wk of cast immobilization would be required for complete healing, as is recommended in the equine for this type of fracture. At the time of this procedure, the animal’s body weight was estimated at 450 kg. For the third immobilization, 50 mg xylazine and 3 mg etorphine were given, which provided excellent induction with 42 min of work time. However, respirations were irregular and shallow with breath holding. This was improved by giving doxapram (Dopram-V, Fort Dodge Laboratories, Inc., Fort Dodge, IA 50501 USA) 20 mg i.v., after which respirations were regular at 24/min. The animal stood up with good control 2 min after reversal using 400 mg tolazoline (½ i.v. and ½ i.m.) and 6 mg diprenorphine i.v.

The giraffe was immobilized a fourth time at 20 wk post fracture to remove the final cast and radiograph the limb. Immobilization and reversal doses were the same as for the previous procedure and provided a smooth induction and recovery (28 min of work time) with doxapram given as before
to stimulate respiration. Radiographs showed complete calcification and remodeling of the fracture site with slight lateral angulation distal to the fracture. There was significant concern about instability of the fetlock because of marked flexor tendon laxity in the unsupported joint after 20 wk in a cast. The animal was confined in the immobilization stall with the crowding gate positioned to restrict space (21 m²) for 2 wk to allow the supporting tendons and ligaments to strengthen and tighten while avoiding a destabilizing force from overexertion. The animal remained calm and compliant while in this confinement. Phenylbutazone was given for 3 wk and access to additional space was gradually permitted.

The giraffe was released from the barn into the exhibit 6.5 mo after the injury. It bolted out of the barn and galloped around the exhibit for about 15 min without resting. There were no signs of lameness or favoring of the injured limb although there was still a slightly perceptible lateral deviation of the extremity distal to the fracture. The laxity of the fetlock gradually reduced over the next several months and there have been no further complications in the 2 yr since the original injury.

This injury almost certainly resulted from the animal wedging its foot under a gap beneath a gate while it was recumbent with its limbs extended posteriorly. It then stood up before retracting the limb. All gates and barriers were carefully inspected to identify and eliminate such gaps. The successful management of this fracture was based on several factors, but among the most important were: 1) having a tractable patient; 2) having an adequate immobilization stall with a crowding gate to allow for controlled induction and recovery with reduced risk of injury to the unstable fracture site or to the animal; 3) having spacious barn facilities for separation of the patient on a clean dry substrate for several months; and 4) allowing adequate time for healing prior to return of the animal to the exhibit and to other herd members.

LITERATURE CITED

Abstract

Nearly every contact with other living organisms, whether it be with humans or other animals, carries some risk of disease transmission. Diseases that are spread from animals to humans are called zoonoses (i.e., zoonotic diseases). Responsible zoos should and do make reasonable attempts to limit the risk of the spread of disease from the animals in their care to their employees and to the general public.4,9 For the general public, the risk of contracting disease from most zoo animals is minimal to nonexistent due to their distance and isolation from the animals. However, contact areas for the general public can present increased risks that can be controlled with reasonable precautions. For this paper, contact areas refers to those areas in which there is direct physical contact between animals and people. These precautions are most effective when they are part of an overall preventive medicine program for the zoological park.5,8

Risks of infection from zoonotic disease can be markedly reduced by avoiding direct animal contact. However, this forgoes many valuable educational experiences and the establishment of a direct relationship between animals and the public. A reasonable alternative is adequate hand washing for those in whose direct contact with the animals is touching. Hand washing is perhaps the single most effective personal hygiene procedure for reducing the risk of infection.4 Given that fact, all areas should have access to hand washing facilities that are in the immediate vicinity of the contact (or an equivalent, e.g., bacteriocidal hand-wipes).

As outlined by the American Zoo and Aquarium Association (AZA) and the Animal Welfare Act (7 U.S.C. 2131 et. seq.) administered by the U.S. Department of Agriculture’s Animal and Plant Health Inspection Service, animal contact areas should always be supervised by a trained zoo representative. Obviously, animals that are ill, should not be used. Human food consumption should not occur in the immediate area of contact. In addition, zoological institutions should be aware that the Centers for Disease Control and Prevention (CDC) standards advise additional precautions may be necessary for humans who are classified as being at increased risk of disease, including those who are immunocompromised. When a reportable disease is identified, all appropriate local, state, and federal regulatory officials should be contacted.

More detailed information on zoonotic diseases may be obtained from a variety of veterinary and medical textbooks and journals,1,6 and from public health officials. In addition, the AZA’s
Quarantine Protocol provides further testing recommendations. Also referenced at the end of this report is a review of some of the risks associated with animals and immunocompromised humans. Following is a list of disease considerations and control programs recommended for animals commonly used in contact programs. Depending on the disease and history of the animals, testing protocols may vary from an initial or incoming quarantine test, to yearly repetitions. This protocol should be used at the discretion of the institutional veterinarian.

**Reptiles and Amphibians**

Most notable among the disease risks presented by reptiles is the transmission of *Salmonella* sp. Salmonellosis is a common and often nonpathogenic infection of reptiles (in one survey, depending on the species, the infection rate ranged from 3-55%). Diagnosis may be difficult. A cloacal swab or other sample positive on culture for *Salmonella* sp. is diagnostic for infection. However, due to intermittent fecal shedding of these organisms, false negative cultures frequently occur. So it is difficult, if not impossible to ascertain with certainty that an animal is *Salmonella* “negative”. Therefore, all reptiles should be treated as *Salmonella* carriers. Attempts to eliminate *Salmonella* carriers with antibiotic therapy have been unsuccessful and may be contraindicated as they can lead chronic carrier states and increased resistance of these bacteria to antibiotics. Risks of transmission can be reduced in two ways: 1) avoid all direct contact with reptiles or surfaces with which they have come in contact, or, 2) allow only supervised contact followed by hand washing as previously described.

Reptiles can also transmit a variety of other organisms, mostly gastrointestinal in origin, and the same procedures described above should be effective in reducing the risks of transmission to those in contact. These other risks include other gram-negative bacterial infections. Reptiles used in contact areas should be free of snake mites and pentastomids (e.g., *Armillifer* sp.).

Amphibians may present several of the same zoonotic risks as reptiles, so again, contact should be followed by hand washing.

**Birds**

Birds used in contact areas should be free of chlamydiosis and zoonotic parasites (e.g., *Giardia*). Chlamydiosis testing is appropriate for members of the Orders Psittaciformes, Galliformes, and Columbiformes. As in reptiles, salmonellosis can be present and difficult to diagnose and so, birds should be treated as suspects. In the general human population, avian tuberculosis is generally considered to have very low zoonotic potential, however, it can present significant risks for immunocompromised individuals. Care should be taken to avoid public contact with known infected flocks.

**Mammals - General**

All mammals are considered at risk for infection with rabies. Current rabies vaccines are licensed.
for use in only six domestic species: dogs, cats, ferrets, sheep, horses and cows. For wild caught individuals of most species, a prolonged (3-6 mo) quarantine is necessary to reduce the risk that they are infected with the virus. Even then, some species, such as skunks, foxes, raccoons and bats may still represent a greater risk.

Any skin lesions compatible with dermatomycosis ("ringworm") should be carefully evaluated in order to prevent transmission to those in direct contact with them.

**Mammals - Primates**

Unless extensive testing has been performed for a variety of viral, parasitic and bacterial diseases, all direct public contact with primates should be avoided. Public contact also places the primates at considerable risk of contracting diseases from humans.

**Mammals - Small Ruminants/Neonatal Ruminants**

All small ruminants, (e.g., pygmy goats, sheep, dwarf cattle, llamas, etc.) that are greater than 6-mo-old and used in contact areas should be tested for tuberculosis, brucellosis and leptospirosis. Obviously any animals with lesions compatible with sarcoptic mange (*Sarcoptes scabiei*) should be removed from contact. Any animals with lesions compatible with contagious ecthyma ("orf" in man) should be tested and removed from contact until proven negative. Calves should be checked and found free of *Cryptosporidium* sp. and other infections with protozoa. Other diseases of a potential zoonotic nature include infection with *Coxiella burnetii* (Q-fever) in endemic areas. Additionally, recent reports indicate that infection with Johne’s disease (*Mycobacterium paratuberculosis*) may present zoonotic concerns, primarily in goats.

**Mammals - Swine**

These animals should be checked for gastrointestinal infection with *Balantidium* sp. efforts made to control this infection. Additionally, consideration should be given to regular vaccination for the bacterial disease, *Erysipelothrix rhusiopathiae* ("diamond skin disease").

**Mammals - Small Carnivores**

In general, due to the potential for bites, small carnivores should be used in contact areas only with extreme caution. Due to the risk of bites, small felids are generally not used in direct contact. If they are, care must be taken that such animals are negative for infection with *Toxoplasma gondii*. All carnivores should be tested for and be free of zoonotic species of roundworms such as *Baylisascaris* sp. Small carnivores (e.g., raccoons and skunks) obtained from the wild may present a greater risk of rabies and their use should be avoided in contact areas.

**Mammals - Rodents and Lagomorphs**

When using rodents and lagomorphs in contact areas, consideration should be given to the risks of
bites, past history and exposure to hantavirus, *Salmonella* and tularemia.

**Mammals - Chiroptera**

At the present time, CDC regulations effectively prohibit the use of bats in direct contact areas.

**Fish and Aquatic Tanks**

Due to the potential for infection with atypical mycobacteria, *Vibrio* sp., *Erysipelothrix rhusiopathiae* and variety of gram-negative bacteria, contact with fish or touch tanks should also be followed by hand washing.

**Summary**

It is important to evaluate the risks of zoonotic diseases in a rational context. Contact animals can provide a valuable educational experience for visitors and participants in public programs to zoological parks and aquariums. Most zoonotic diseases of concern in public areas can be prevented with reasonable testing and quarantine programs and proper hand-washing techniques.

These are intended to be general guidelines and the risk of diseases can vary by area, so zoological institution should develop its own zoonoses control program. Two excellent resources for reviewing testing and preventive procedures for many of these diseases are the American Association of Zoo Veterinarian’s *Infectious Disease Notebook*¹ and the American Veterinary Medical Association’s *Zoonoses Updates*.⁶ In summary, the most effective method for disease prevention is a complete and thorough veterinary program and common sense sanitary measures.

**LITERATURE CITED**

NONTRADITIONAL ANIMALS FOR CONTACT WITH IMMUNOSUPPRESSED PEOPLE: PRECAUTIONS AGAINST ZOONOTIC DISEASE TRANSMISSION

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Abstract

Veterinarians are concerned with preventing the transmission of zoonotic diseases. Special attention to prevention of disease transmission must be paid to those who are immunosuppressed, or those in convalescent centers or schools, particularly if they participate in “hands-on” demonstrations or exhibits at or from zoological parks. This discussion considers diseases potentially transmissible between selected common nontraditional companion animals and immunosuppressed humans.

Introduction

Under the right circumstances, an animal infected with a potential zoonosis may transmit this disease to a human. Physicians and public health authorities must work to minimize the human health risks of zoonotic disease. The veterinarian is increasingly asked not only to screen animals for any and all known zoonotic organisms, but also to counsel those involved in animal programs that have contact with immunosuppressed members of the public. Veterinarians and physicians understand that diagnostic tests for and treatments of potential zoonotic diseases are not 100% effective. Not all potential zoonoses are easily diagnosed. Where organisms are difficult to culture or are shed intermittently (such as Chlamydia, Salmonella, or Campylobacter) repeated testing may be necessary to assure physicians and public health authorities that an animal is under routine disease surveillance. The veterinarian must ensure that the public understands that “negative” is not equivalent to “disease-free”, and that certain precautions, such as proper hand-washing immediately after touching an animal, are essential. It is only through complete disclosure that veterinarians will minimize their liability, and the immunosuppressed person will be able to make the decisions that best balance a psychological need for animal contact with the risks of disease.

Together public health professionals, veterinarians, and physicians can effectively educate the public regarding potential zoonoses. Recognizing the importance of the human-animal bond, it is inappropriate for the human health professional to recommend the elimination of a given animal from the human’s environment without consideration of treatment of disease in the animal as an alternative. In some species, specific diagnostic testing may be necessary. Some species are inappropriate for contact with immunosuppressed persons due to a lack of adequate diagnostic testing, a lack of disease-eliminating treatments, or a lack of knowledge of carrier states, disease conditions, or “normal” microflora of the animal.

Ideally, animal companions should screened for the known potential zoonotic diseases they may harbor. If appropriate, therapies should be instituted and complete follow-up diagnostics performed.
to determine whether the disease agent has been eliminated. With the immunosuppressed person’s permission and knowledge, diagnostic results and recommended treatment plans, including diagnostic or therapeutic follow-up, should be shared with the physician, as necessary. The physician should be encouraged to discuss concerns with the veterinarian. The veterinarian is usually responsible for educating the client in proper sanitation techniques and other safeguards needed during routine animal care. Disinfectants appropriate for home use for “animal clean-up” and recommended by the veterinarian should be made available to the client. A good management program should meet both the physician’s and veterinarian’s criteria for maintaining the health and well-being of both human and animal patients.

Immunosuppressed persons should be provided sufficient zoonotic disease information to allow appropriate contact (if any) with various animals during visits to friends, farms, zoos, etc. The approach should be one of common sense: washing hands well after handling any animal is one example. Educational materials should be available that consider both individual species and their potential diseases, as well as guidelines for safeguarding human health. A copy of this information should probably be supplied to the physician. Open communication between the physician, veterinarian and client should be encouraged.

Discussion

Potential zoonotic diseases of selected nontraditional companion animals are listed below:

**Domestic ferret**
- *Giardia*
- *Listeria*
- *Microsporum canis: Dermatomycosis*
- *Salmonella*
- *Ctenocephalides*
- *Listeria*
- *Sarcoptes scabiei*
- *Ear mites: Otodectes*
- *Salmonella*
- *Dirofilaria immitis: Heartworm*
- *Cryptosporidia*
- *Dipylidium caninum, Ancylostoma caninum, Toxocara sp."

With the exception of some of the gastrointestinal nematodes, the infected or infested ferret will usually be clinically symptomatic. Although the risk of rabies is extremely low and there is a rabies vaccine licensed for use in ferrets (Imrab 3, Rhone Poulenc, Athens, GA), some public health authorities do not recognize the vaccine’s efficacy and will require sacrifice and testing of the animal in the event of a bite. Precautions for prevention of disease transmission from ferrets are essentially the same as those recommended for dogs and cats. It is suggested that immunosuppressed persons should not clean litter boxes.

**Companion domestic rabbit**
- *Campylobacter sp.*
- *Psoroptes sp., Cheyletiella sp., Sarcoptes sp."
- *Salmonella*
- *Pasteurella*
Dermatomycosis · Bacterial infections from bites, scratches

While wild rabbits may carry Tularemia, Taenia taeniaeformis, and Multiceps serialis, these are more likely a risk to hunters in contact with raw rabbit meat than to pet owners.

**Companion small rodents (mice, rats, hamsters, gerbils)**

- Salmonellosis (rare)
- Lymphocytic choriomeningitis (LCM)
- Acinetobacter
- Allergies to rodent antigens (dander, urine)
- Dermatophytosis
- Hymenolepis nana, Taenia taeniaeformis

**Guinea pigs**

- Dermatophytosis: *Trichophyton mentagrophytes*
- Mange mites: *Trixacarus caviae* (burrowing);
  - sarcoptic mite
- Salmonellosis (rare)
- Yersinia pseudotuberculosis
- Allergic responses to guinea pig allergens
- Lymphocytic choriomeningitis (LCM)
- Acinetobacter
- Hymenolepis nana, Taenia taeniaeformis

Animals are usually symptomatic; diagnostic and treatment regimens are documented.

**Chinchillas**

- Dermatophytosis: *Trichophyton mentagrophytes, Microsporum canis*
  - *M. gypseum*
- Lymphocytic choriomeningitis (LCM)
- Listeria monocytogenes
- Fleas (*Ctenocephalides*)
- *Baylisascaris procyonis* (will be symptomatic cerebral nematodiasis but will not shed infective oocytes)
- Bacterial infection from contaminated bites or scratches

Chinchillas infected with LCM should be euthanatized as there is no specific treatment that will eliminate the virus.

**Prairie Dog**

- Yersinia pseudotuberculosis, *Y. pestis, Y. enterocolitica* (can be acute, subacute, chronic or latent; agent ingested, shed in feces. Found in wild-caught/exposed to wild-caught).
- Listeria monocytogenes
- Pasteurella multocida
- Ectoparasites (mites, fleas, lice)
- *Baylisascaris procyonis* (will be symptomatic cerebral nematodiasis but will not shed infective oocytes)
- Bacterial infection from contaminated bites or scratches
- Hantavirus (wild-caught)
- Topical irritation to quill pricks

Risk potential is dependent upon number of generations away from the wild, or contact with wild-caught adults. Accuracy of diagnostics and treatments in these animals has yet to be determined.

**African pygmy hedgehogs (Atelerix albiventris)**

- Foot and Mouth Disease (wild-caught/exposed wild-caught)
- *Salmonella serotype* Tilene (asymptomatic carriers, standard culture/serotyping)
- Topical irritation to quill pricks
Brushtail possums “Phalangers” (*Trichosurus vulpecula*)¹²
- *Mycobacterium bovis* (imported animals/exposure to imported)

Efficacy of ante-mortem diagnostic testing and treatment is unknown.

Domestically bred and raised raccoons and skunks may carry *Baylisascaris (procyonis* and *columnaris*, respectively). Bacterial and parasitic diseases common to other carnivores may also be carried by raccoons and skunks. Without an approved rabies vaccine, any bite from an individual of either of these species usually results in euthanasia and testing, per public health guidelines. Efficacy of diagnostic tests and disease treatments for these species has not been established.¹⁴

Nonhuman primates (NHPs)⁷,⁸
- *M. tuberculosis*
- *Herpes B, Marburg virus, Filovirus, Retroviruses, LCM*
- *Salmonella, Shigella, Campylobacter*
- *Balantidium, Entamoeba, Giardia, Cryptosporidia, Strongyloides stercoralis, Trichuris sp., etc.*

NHP's carry the greatest potential for zoonotic disease transmission because of their close genetic relationship to humans.

**Birds**²⁸
- *Chlamydia psittaci*
- *Mycobacterium avium*
- *Campylobacter: Undetermined, possible C. laridis (diarrhea in children)*
- *E. coli*
- *Erysipelothrrix*
- *Listeria (conjunctivitis)*
- *Caryospora (9 species) (pyogranulomatous dermatitis)*: *Entamoeba histolytica, E. polecki*

*Chlamydia psittaci* is the most common of the avian zoonoses; diagnostic tests continue under development. Treatment regimens are considered effective in relieving clinical signs in many species, but true clearance of the organism is debated. *Mycobacterium avium* usually causes disease in the infected bird; treatment efficacy is debated.

**Reptiles**⁸,¹⁰
- *Aeromonas spp., Yersinia enterocolitica, Pseudomonas spp.*
- *Campylobacter spp., Citrobacter spp., Enterobacter spp., Klebsiella spp., Proteus spp., and Serratia spp. Erysipelothrrix rhusiopathiae*
- *Q fever (Coxiella burnetii) from reptile ticks*
Zygomycosis, other mycoses
WEE (from reptile ticks)
Pentastomiasis (Armilliferiasis)
Cestodes: Spirometra (Sparganosis), Diphyllobothrium, Mesocestoidiasis
Salmonella spp. - Treatment aimed at eliminating *Salmonella* from reptiles is often difficult, as antibiotics may merely suppress the excretion of detectable organisms. Following antibiotic treatment, *Salmonella* organisms may not be excreted for up to 8 wk. Because of intermittent organism excretion, it may be difficult to determine if treatment has been effective. Treatment failure may promote the development of drug-resistant strains. Gloves and mask should be worn during cleaning of the habitat.

Invertebrates kept as companion animals or for display and educational purposes include tarantulas, scorpions, hermit crabs, exotic roaches, and others. Little has been documented regarding invertebrate zoonoses. The author recommends culturing invertebrates fed live prey or insects for *Salmonella* and other potential bacterial pathogens. Feed colonies (e.g., meal worms, crickets or mice) should be maintained under hygienic conditions. Uneaten foods should be removed promptly. Water containers need to be sanitized regularly. Gloves and mask should be worn during cleaning of the habitat, as with reptiles. The most frequently noted problem in humans handling tarantulas is skin irritation caused by reaction to the “hair”. An intense pruritus and inflammation may occur on skin surfaces touched by the tarantula. Tarantula bites should be treated as other animal bites and promptly cleaned and disinfected. Scorpion stings should be seen by the physician.

**Recommendations and Conclusions**

Given the increase in immunosuppression among the general population, an increase in immunosuppressed pet owners and zoo visitors is to be expected. It may be advisable to have information available for participants of contact/visitation programs prior to the visitation. The best way to ensure that immunosuppressed people are not excluded from animal contact by is education of the general public, as well as public health professionals, physicians and veterinarians. With proper education and communication, keeping of nontraditional pets need not pose zoonotic risks to pet owners. Communication and education between human and veterinary health care providers and the immunosuppressed person is necessary to best prepare immunocompromised individuals for safe contact with nontraditional animals.

No veterinarian can guarantee that an animal is absolutely safe for contact with immunosuppressed persons. The veterinarian can recommend as contact species animals that present minimal risk of zoonotic disease. A physical examination and appropriate diagnostic testing should be performed on animals intended for contact with immunosuppressed persons. For pets, a post-purchase certificate of examination should be provided to the client, with a copy to the client’s physician. The exam record may contain a statement of release from liability with the explanation that negative test results do not guarantee freedom from pathogens, but rather indicate that the animal has been examined for zoonotic diseases, by acceptable diagnostic methods. Examinations should be completed prior to placement of the animal in the client’s home or in known contact with immunosuppressed people.
Animals not recommended as pets or for other contact with immunosuppressed persons include: invertebrates, reptiles, brushtail possums, nonhuman primates, skunks, raccoons, and wild-caught, non-domesticated or exotic species bred for the pet trade. NHP’s probably pose the greatest risk to immunosuppressed humans. The potential for bacterial infection, as well as for serious bite and scratch wounds from NHPs is great. In light of known transmission of retroviruses between macaques and humans, and hepatitis A between chimpanzees and humans, it would be extremely unwise to house an NHP with a human carrying the HIV virus.

Animals recommended, with reservations would include African pygmy hedgehogs, sugar gliders, wallabies, ringtail possums, short-tailed possums, prairie dogs (domestically bred only), and exotic rodents such as duprasi and degus.

Animals the author considers appropriate pet or contact species for immunosuppressed persons include domesticated animals such as cats, dogs, rabbits, ferrets, chinchillas, guinea pigs, rats, mice, hamsters, gerbils, and domestically-bred birds. Domesticated ruminants, equids, and suids would also be appropriate as the diseases affecting these species are well-known, as are appropriate precautionary measures. All animals should have routine veterinary care and receive appropriate vaccinations. They should also be adequately socialized to humans and of even temperament. Minimal contact with young animals prior to completion of deworming protocols should be emphasized. Education covering appropriate methods of care, sanitation, and contact (e.g., don’t kiss the animal on the mouth!) is essential.

It is suggested that immunosuppressed persons who attend fairs, livestock shows, rodeos, etc., a masks and eye protection, and that they refrain from handling the animals, equipment, feed, or litter. At a zoo or public aquarium, contact with other humans is probably presents greater risk than does contact with animals, but it should be recommended that immunocompromised persons refrain from participating in hands-on exhibits (petting zoo, tidal pools). Handling of pet store animals should also be discouraged. It is recommended that the patient decline visits to aviaries, animal breeding facilities, and animal processing plants.

LITERATURE CITED
FOMITE TRANSMISSION OF Salmonella enteritidis INVOLVING KOMODO DRAGONS (Varanus komodoensis) AT THE DENVER ZOOLOGICAL GARDENS

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Abstract

This report describes fomite transmission of Salmonella enteritidis to visitors of the Denver Zoological Gardens during a temporary exhibition of Komodo dragons (Varanus komodoensis). The zoo cooperated with state health department officials and the Centers for Disease Control and Prevention (CDC) in conducting an epidemiological investigation of the incident. Thirty-three confirmed cases of S. enteritidis and 17 suspect cases were reported in Denver and surrounding counties. Visitor behaviors that were found to be significant in disease transmission included touching the exhibit barrier, and failure to wash hands prior to eating or drinking after visiting the exhibit.

Introduction

Between 13 January and 21 January of 1996, the Denver Zoological Gardens hosted a temporary exhibition of its four resident Komodo dragons (Varanus komodoensis). The event known as “Komodo Days” was kicked off with a VIP party on 11 January 1996. The purpose of the event was to help raise funding necessary to build a permanent exhibit for the Komodos. Approximately 15,000 people visited the zoo during the 7-day exhibition.

The exhibition was set up in the Denver Zoological Garden’s Tropical Discovery building. The building contains a large indoor plaza referred to as the Discovery Center. It was in the Discovery Center that the temporary Komodo exhibit was located. A temporary barrier made of painted plywood, 2 ft in height, was constructed to separate the animals from the visitors. Bark mulch was placed on the floor as substrate for the animals. The plywood barrier was surrounded by decorative plants and a chain and post barrier, creating a space of approximately 2-3 feet between the public and the animals. Also located in the Discovery Center was an education department station, typically manned by docents to allow visitors contact with biofacts and a variety of reptiles; this station was closed during the exhibition.

Case Report

On 26 January 1996 the Animal Health Department of the Denver Zoo Hospital (DZH) was notified by the Colorado Department of Public Health and Environment (CDPHE) that seven children from the Denver Metro area were infected with Salmonella enteritidis, with a characteristic rough colony morphology. The children had all visited Tropical Discovery during the “Dragon Days” event. Eventually, 33 cases were confirmed by culture; 17 other cases were considered to be suspect.
At the time of the initial report, all animals in the building with the potential for public contact were considered possible sources of the infection. The majority of the exhibits in Tropical Discovery are inaccessible to the public. Some exhibits do have open tops which the public, with some difficulty, can physically access. The Komodo dragon exhibit and the educational animals were considered the most likely sources of *S. enteritidis* for the following reasons: first, these exhibits afforded visitors the greatest access to animals and second, the timing of the human outbreak coincided with the dates of the Komodo exhibition.

Samples were obtained for culture from the skin and cloaca of animals known to have been in contact with the public: four Komodo dragons and education animals including a corn snake (*Elaphe guttata*), a blue tongued skink (*Tiliqua scincoides*), and a common boa (*Boa constrictor*). A preserved skin from a Malayan water monitor (*Varanus salvator*) used as a biofact was also cultured. Additional samples for culture were obtained from two rats intended to be fed to the Komodos. *S. enteritidis* with rough colony morphology was isolated from the skin of one Komodo and the cloaca of another. *Salmonella arizonae* was isolated from the corn snake and another Komodo. *Salmonella blukwa* was isolated from the blue tongued skink. No *Salmonella* species were isolated from the other culture samples.

On 27 January 1996, officials from the CDPHE inspected the Tropical Discovery building. Cloacal and some fecal samples were obtained for culture from all of the animals previously tested. *S. enteritidis* with rough colony morphology was isolated from the cloaca of one of the two Komodo dragons that previously tested positive for this organism.

On 1 February 1996, investigators from the CDC arrived in Denver at the invitation of the CDPHE. The goal of this visit was to identify modes of transmission of this strain of *S. enteritidis* and to determine appropriate prevention measures for exhibits of this kind. Initially an environmental investigation was performed. *S. enteritidis* was isolated from the wooden barrier.

The CDC investigation indicated that the following factors likely contributed to infection with this species of *Salmonella*. The majority of people infected, reported touching the exhibit barrier. Failure to wash hands after visiting Tropical Discovery or failure to wash hands before consuming food or drink were also considered to be significantly associated with illness. Among those who had touched the barrier surrounding the Komodo exhibit, not washing hands either at the zoo, after visiting Tropical Discovery, or before the next meal was significantly associated with illness.

**Discussion**

The outbreak of *S. enteritidis* in visitors led to the formulation of a policy regarding public contact with reptiles at the Denver Zoological Gardens. This policy is based on the AZA Guidelines for Animal Contact With the General Public. The following should be considered in the formulation of a reptile contact policy:
1. All reptiles should be handled as though they are *Salmonella* spp. positive.

2. Cultures positive for *Salmonella* may identify carrier animals. However, failure to isolate *Salmonella* does not provide the animals’ *Salmonella* status.

3. Treatment with antibiotics has not been shown to be beneficial in the elimination of *Salmonella* carrier states.

4. Visitors should not be allowed access to reptile holding areas, as they may be contaminated by animal waste products.

5. If visitors are allowed to handle reptiles under controlled situations, the animal should be wiped down with an antiseptic solution prior to handling.

6. Under no circumstances should reptiles be allowed to crawl or perch on visitors.

7. Visitors should not be allowed contact with species that may be considered to have a greater likelihood of carrying *Salmonella* species, such as iguanas, Varanid lizards, and aquatic turtles.

8. All persons handling reptiles should be required to thoroughly wash their hands when finished.

9. The risks of handling reptiles should be explained to parents or guardians and signed waivers from them should be received prior to allowing children to contact or handle reptiles.

10. Eating and drinking should be prohibited in areas in which reptiles are being handled.

11. Individuals considered at high risk (e.g., pregnant women, children less than 5-yr-old, and immunocompromised persons) should not handle reptiles.

This incident demonstrates the importance of serotyping isolates of *Salmonella*, especially in the face of a disease outbreak. Serotyping helped to establish the relationship between the human infections and the *Salmonella* cultured from the Komodo dragons. It is suggested that all isolates of *Salmonella* be submitted for serotyping regardless of human involvement. This incident also provides evidence of the intermittent nature of *Salmonella* shedding by reptiles.

**LITERATURE CITED**

Abstract

Mycobacterial infections are common among humans. Of these, infection with *Mycobacterium tuberculosis* (TB) is the most common and of the greatest concern. Non-tuberculous species of mycobacteria may also cause infections in man, especially among immunosuppressed individuals. Human TB is typically acquired by inhalation of aerosols carrying tubercle bacilli following exposure to a person with active pulmonary infection; non-tuberculous species of mycobacteria are acquired from environmental sources. Since zoonotic transmission of TB does occur, the identification of acid fast bacilli (AFB) in clinical specimens from animals is a cause of concern, unease, and occasionally misconception for animal care handlers and zoo personnel.

*M. tuberculosis* Complex

1. Epidemiology, Pathogenesis, and Diagnosis

*M. tuberculosis* complex organisms include *M. tuberculosis* and *M. bovis*, as well as *M. africanum* and *M. microti* and infection with any of these species is appropriately termed as “tuberculosis”. These organisms are genetically related, grow slowly on artificial media, and have no known environmental niches.

*M. tuberculosis* and *M. bovis* infection is limited to warm blooded animals, although there is one report of *M. tuberculosis* infection in a turtle. In general, while both *M. tuberculosis* and *M. bovis* should be considered equally pathogenic, animal related differences in susceptibility to the two species have been reported. Thus, the incidence of infection parallels the frequency of exposure.

Mammals, excluding humans, are most commonly infected with *M. bovis* although there have been numerous cases of human disease. Humans are more commonly infected with *M. tuberculosis*, which may represent a “humanosis” with infection documented in elephants, rhinoceros, dogs, and nonhuman primates. Further, *M. tuberculosis* may also infect birds, especially parrots and other common pet species.

Primary infection is usually acquired via tubercle laden droplets generated by coughing or sneezing. The droplets settle into the alveoli where the bacteria begin to multiply. Macrophages engulf the bacteria and while most of the organisms are killed, some survive intracellularly. Groups of macrophages form granulomas around the bacteria that may grow large enough to be detected visually. Active disease occurs in two situations: (1) immediately following primary infection; or,
more commonly, by (2) reactivation from a latent source of infection. Clinical disease is primarily pulmonary although extra-pulmonary infection may occur alone or concurrent with pulmonary disease. In both humans and other animals any organ system may be infected.

In both humans and other mammals, skin testing detects only TB infection, NOT active disease. Active disease in humans is diagnosed either by 1) recovery of the organisms by culture or smear, or 2) identification of thoracic radiographic lesions consistent with active TB. Polymerase chain reaction (PCR) and other amplification methods detect organisms and can thus be used to diagnose active disease. Other indirect methods, such as antibody detection (ELISA) and lymphocyte transformation (Blood Tuberculosis [BTB] test), cannot discriminate infection from active disease.

2. Zoonotic Transmission of Tubercle Bacilli

The primary route of transmission of TB to humans is primarily through aerosols. The most common source of these aerosols is coughing, which generates small droplets that can settle in the alveoli. Only animals with active disease are at risk to spread infection to humans and other animals. The major risk factors for animal to human transmission are the same as those for human to human spread of the disease and are listed below.

Risk factors for TB transmission.

<table>
<thead>
<tr>
<th>Number of bacilli being actively shed</th>
<th>Droplet size carrying tubercle bacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total time of exposure</td>
<td>Proximity to an infected animal</td>
</tr>
<tr>
<td>Immune status of the person exposed</td>
<td>Poor ventilation</td>
</tr>
</tbody>
</table>

Other sources of exposure include aerosols created 1) from tuberculous abscesses, 2) during necropsy, and 3) during handling of infected carcasses. Fecal-oral spread is not as important for humans as for animals. The fecal-oral route should be considered when children or animal workers are exposed to infected animals and their excreta. Because their cell wall lipids protect mycobacteria from drying they remain viable in the environment for prolonged time spans. In contrast, sun-exposed bacteria are rapidly killed (see section on control measures).

3. Zoonotic Outbreaks of *M. bovis* and *M. tuberculosis*

Transmission of *M. bovis* from cattle to humans via infected milk accounted for ~20% of the cases of tuberculosis in the early part of the century. In large part due to pasteurization and TB control programs in domestic hoofstock, milk has been eliminated essentially as a source of infection in the United States. Unfortunately, the same cannot be said for other countries.

*Elephants.* In August 1996 two circus elephants died of *M. tuberculosis*. A third elephant has subsequently been diagnosed with active disease by culture. The state health department is currently conducting skin testing of the animal handlers and other workers for evidence of recent PPD skin conversions. *M. tuberculosis* was documented in an elephant in this herd in 1983 and thus highlights the risk of possible animal to animal spread of *M. tuberculosis*.
Fennec foxes. In the mid 1970's, two of three foxes housed in the primate building at the Duluth Zoo died of *M. bovis*. All 13 primates housed in the same building subsequently tested skin test positive. Two animals were euthanized because of wasting, and at autopsy were discovered to have disseminated TB. Of 34 animal handlers tested, 4 (11%) were skin test positive. Skin testing of the public was also performed; of 674 individuals tested, 23 (3.4%) were PPD-positive. It is unknown whether any of the positives in the public represented new conversions.

Rhinoceros. In 1991 a male white rhinoceros died at the Audubon Zoo of *M. bovis*. Postmortem examination of the animal was performed “on a dark and stormy night” in a closed building adjacent to the rhinoceros compound. Subsequently, two colobus monkeys housed downwind from the building were skin test positive; both were later euthanatized and found to have disseminated *M. bovis* infection. Of 24 animal handlers exposed to the rhinoceros, seven demonstrated PPD conversions: six were zoo-keepers exposed during routine husbandry; one person converted following exposure during the necropsy.

Seals. In 1986 three seals died at a marine park in Western Australia of *M. bovis*. Three years later, one of the seal trainers developed active pulmonary tuberculosis with *M. bovis* of the same strain as that cultured from the seals. No data was reported for the other trainers.

Elk. After an epizootic of *M. bovis* was identified in domestic elk herds in Alberta, Canada, testing of exposed humans was commenced. Of 446 human contacts, 391 had skin tests. Of these, 81 (20.7%) tested positive, 50 with known contact with infected animals. One person developed active pulmonary infection with *M. bovis*.

Abattoir workers. *M. bovis* infections between 1953-1988 among 87 persons from Queensland Australia were reviewed. Of 87 cases, isolated pulmonary disease occurred in 67 individuals, extrapulmonary disease was found in eight; in 12 cases both pulmonary and extrapulmonary disease were identified. Work-related exposure to cattle was documented for 57 persons including 40 meatworkers. Thirteen persons were exposed by drinking unpasteurized milk. One person developed disease after exposure to an *M. bovis* infected human.

4. Control Measures to Minimize and Prevent TB Exposure and Transmission

The most important factor in preventing exposure to animals with TB is a high index of suspicion for infection. In many cases, the diagnosis is considered only when caseous lesions are detected at necropsy. Since the signs and symptoms of TB infection are nonspecific, TB should be included in the differential diagnosis of ALL animals, regardless of exposure history, that present with wasting, poor feeding, and especially respiratory signs, such as coughing.

All mammals should be considered as potential sources of *M. bovis*. Ideally, all captive mammals maintained in groups or in close proximity should be evaluated for TB. Unfortunately, TB testing has not been validated for many animal species. Furthermore, differentiating latently infected from actively shedding animals is difficult. For animals considered at high risk for infection, contact time
should be limited and personnel should wear masks (HEPA masks, if possible). Protective covering (disposable coveralls, boots) and foot baths are beneficial in limiting potential spread to other animals which may ingest infected particulate matter. Gloves should be used for routine care to minimize fecal-oral transmission to humans. Careful handwashing is mandatory, regardless of glove usage. Showering is not necessary.

Other measures to decrease exposure risks to humans include limiting contact with infected animals to well-ventilated areas (such as open pens), and areas with access to ultraviolet (UV) radiation (direct sunlight or UV ceiling lights). The latter exploits TB’s exquisite sensitivity to UV radiation. If possible, rooms used for animal care should be HEPA filtered with >6 air exchanges/min.

To put these recommendations into context, humans admitted to the hospital with a suspicion of TB (cough, sputum production, and consistent chest radiogram) are immediately placed into isolation. Isolation rooms are under negative pressure and have 6 air changes per min through a HEPA filter. No non-filtered air is vented to the outside. Patients are removed from isolation when three successive sputum exams are negative for AFB. If the sputum is acid fast positive, isolation is canceled after 2 wk of treatment if no AFB are detected on repeat sputum exams.

**Non-tuberculous Mycobacteria**

Infection with any of the non-tuberculous mycobacteria should not be considered “tuberculosis”. While any of these organisms may cause granulomatous, TB-like disease, the risk and mode of transmission from animal to animal and from animal to human differs drastically from that for TB. Further, labeling an animal as having “tuberculosis” immediately raises the specter of exposure to handlers and is a cause for alarm.

*M. avium* complex

Organisms of the *M. avium* complex (MAC -- *M. avium* and *M. intracellulare*) are the second most common mycobacterial species to cause infection in both animals and man. Because of a preference of these bacteria for higher growth temperatures, warm blooded animals are at risk. Marsupials are especially susceptible to disseminated *M. avium* infection. DNA typing of isolates has not been able to show animal to animal spread between tree kangaroos, although ducks housed together have been found to be infected with common strains (J Maslow, unpublished data). Animal to animal spread of *M. avium* may be possible through the fecal-oral route, especially when it is considered that *M. avium* may survive desiccation for periods greater than 1.5 yr (J Maslow, unpublished data). DNA typing of isolates has not been able to show animal to animal spread between tree kangaroos, although ducks housed together have been found to be infected with common strains (J Maslow, unpublished data).

Human disease due to MAC infection includes lymphadenitis (scrofula), osteomyelitis, and a TB-like pulmonary infection. Disseminated disease is common among individuals with defects in cell mediated immunity, especially AIDS patients. Animal disease due to MAC, similar to that in humans, includes lymphadenitis, abscesses, hepatic and splenic infections, and osteomyelitis. To date...
there have been no documented cases of transmission from animals to humans. It is unknown whether isolates pathogenic for animals are pathogenic to humans. Further, environmental exposure is a more likely source of disease for humans because *M. avium* is ubiquitous in the environment.

*M. fortuitum-M. chelonae* complex

*M. fortuitum* is a common mycobacterial infection among fish causing abscesses, scale disease, and gill infections. *M. chelonae* has been observed among reptiles including snakes and turtles.

Disseminated disease, with *M. fortuitum* has been reported for patients with AIDS.\(^{16}\) Immunocompetent hosts can develop skin abscesses and tendonitis following puncture injuries\(^{17}\) and may occasionally develop disseminated infection.\(^{18}\) No documented cases of animal to human transmission have been recorded.

*M. marinum*

Human infection with *M. marinum* is limited to cooler body sites (such as the hands and feet) because of the growth requirements of the organism. The best described infection is “fish handler’s disease”, a localized nodular swelling that develops at the site of trauma (puncture wounds and abrasions).\(^{19}\) Modes of inoculation include puncture by fish spines and abrasions sustained while cleaning fish tanks. Affected animals include fish and snakes. Disease may be disseminated in both species.

Snakes housed together may be infected with the same strains, that may represent either animal to animal spread through the environment or common source exposure (R Wallace, J Maslow, unpublished data).

*M. ulcerans*

*M. ulcerans* can cause an indolent, necrotizing skin disease endemic in some tropical countries. One report documents osteomyelitis, gangrene and subsequent dissemination of *M. ulcerans* infection in a West African child following a snakebite (unknown species of snake).\(^{20}\)

*M. xenopi*

*M. xenopi*, a cause of mycobacterial infections among amphibians that is found in many aquatic environments, has been documented as a cause of disseminated infection among patients with AIDS.\(^{21}\)

Conclusions

Zoonotic transmission of mycobacteria is well described, although essentially limited to organisms of the *M. tuberculosis* complex. While *M. bovis* is the most common species to be transmitted from
animals to humans, *M. tuberculosis* may also be transmitted zoonotically. For the non-tuberculous mycobacteria, only *M. marinum* in animals has been documented as an immediate infectious disease risk to humans. Any measure that limits the time and level of exposure to infected animals will decrease the risk of transmission of TB and other mycobacteria.

**LITERATURE CITED**

TUBERCULOSIS IN CAPTIVE ELEPHANTS

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Abstract

Introduction

Recent findings of *Mycobacterium tuberculosis* in captive elephants have been a source of great concern to the public, zoo and circus communities, and federal/state authorities. The following is a synopsis of recent cases and the actions that have been taken to address this problem.

Background

In August 1996, two female Asian elephants, aged 46 and 26, died while on the road with a circus. One, “Joyce,” had exhibited marked weight loss for several months and had been tentatively diagnosed with a dental malocclusion. She was anesthetized for a dental procedure but never recovered from anesthesia. Upon necropsy, widespread granulomatous lesions involving 80-90% of the lung tissue were found. Acid-fast organisms were appreciated on histopathology; the organisms were identified on culture as *M. tuberculosis*.

Since there was no established procedure to deal with tuberculosis in elephants, the U.S. Department of Agriculture (USDA) and the American Association of Zoo Veterinarians assembled a joint panel to review the situation and make recommendations for action. In September 1996 the panel met and observed the affected elephant herd at its home facility. During this meeting, the panel learned of the previous history of tuberculosis (TB) in this herd: one elephant had died of confirmed TB in 1994, and yet another had died of TB in 1983. Before testing was initiated, an adult Asian breeding bull died suddenly at the facility. Necropsy showed no signs of TB and trunk cultures were negative. Of the 18 remaining elephants, 16 appeared clinically normal; two exhibited weight loss.

Methods and Findings

Since many zoo veterinarians felt that the tuberculin skin test alone was an unreliable indicator of disease status in elephants, multiple test methods—including skin testing, trunk cultures, blood tuberculosis (BTB), and serum ELISA assays—were utilized in order to gather as much data as possible from the animals tested. In addition, all animal handlers were tested by the local health department. Many (50%) had positive skin tests; one had a positive sputum culture and chest radiograph.

Most of the elephants in this affected herd were positive on two or more assays; one that had exhibited significant weight loss was culture-positive for *M. tuberculosi*. Since there were little
historical data on the accuracy of these assays in elephants, it was decided to classify individual animals as “high-risk” or “low-risk” based upon both test results and level of exposure to known-positive animals. Fourteen elephants were classified as “high-risk” and four as “low-risk” (Table 1).

Affected animals were treated using protocols based on those prescribed for treatment of human tuberculosis. Sensitivities showed that the \textit{M. tuberculosis} cultured was susceptible to most of the commonly used human anti-tubercular drugs. For high-risk group animals, multi-drug therapy with isoniazid (INH), rifampin, and pyrazinamide (PZA) was chosen because of its effectiveness in humans and because all the drugs could be given orally. The low risk group was placed on preventive treatment with INH and rifampin for twelve months. PZA was added for the first two months of therapy. Vitamin B\textsubscript{6} (pyridoxine) was also prescribed to counter the effects of INH and prevent possible peripheral neuropathy.

During treatment body weights, liver function, bacterial trunk cultures, and diagnostic blood tests were monitored for each risk group. In addition, travel restrictions were instituted to safeguard the health of the animals and prevent further spread of disease.

**Results**

By February 1997, the one culture-positive elephant had converted to culture-negative and was gaining weight. As of July 1997, all elephants were tolerating chronic medication with no ill effects reported. INH tends to be unstable in solution and may bind with glucose to become inactive. As a result, some difficulty was encountered when INH was mixed with a syrup. Questions remain as to what drug levels (serum or dosage) can be considered therapeutic in elephants.

**Other Cases**

CA-1. Texas - In January 1997, a 26-yr-old African female died after a long history of respiratory illness and weight loss. Skin test was negative in November 1996. Acid-fast bacteria were identified on histopathology. Necropsy revealed consolidated, “wooden-like” lungs. \textit{M. avium} was cultured from lungs at necropsy. Handlers and stablemate were skin test negative.

CA-2. California - In March 1997, a 30-yr-old Asian female died of \textit{Salmonella}. Necropsy was incomplete, but both lungs were possibly infiltrated with firm granulomata. Cut surface of the lung was calcified. \textit{M. tuberculosis} was isolated from a calcified mesenteric lymph node. BTB assay was positive for \textit{M. avium}.

CA-3. California - June 1997, a 29-yr-old Asian female previously housed with Case #2 was trunk culture positive for \textit{M. tuberculosis}. Skin testing was negative, BTB was positive for \textit{M. bovis}, and two separate serum ELISAs (one performed at Colorado State University and one at Iowa State University) were positive. Therapy was initiated with the three drug regimen described above. Difficulty was encountered in gaining acceptance of oral medications. This elephant had been exposed to at least seven other elephants (four at a private exhibitor, and three at a public zoo) during
the 12 mo prior to testing. Testing and treatment were initiated for each of these in-contact elephants. The four elephants at the private exhibitor were dosed by oral syringe with INH and rifampin, and exhibited side effects ranging from poor appetite and lethargy to colitis. The serum drug concentrations in these animals showed were much higher than were obtained in previous studies and the dose was reduced by half. After dose reduction, no further adverse side effects were appreciated. New drug level trials are ongoing. An attempt at medicating by suppository is also being conducted.

CA-4. California - In July 1997, a 30-yr-old Asian female that was exposed to Case #3 was trunk culture positive for *M. tuberculosis*. Skin testing was negative and BTB positive for *M. bovis*. This elephant was exhibiting a trunk discharge.

Table 2 presents a summary of all test results from all *M. tuberculosis* culture-positive elephants (as of 1 August 1997).

**Analysis**

In June 1997, the draft TB protocol utilized in the first outbreak was reviewed by members of the National TB Working Group for Zoo & Wildlife Species. Given the information that had been gathered since the protocol’s inception, the response from the group was that more work needed to be done to validate diagnostic tests such as the BTB, ELISA and skin tests before they were used to make decisions regarding treatment and travel restrictions. As this is being written, a new approach to the management of the disease in elephants is being considered, involving culture as the key diagnostic procedure. Given the great expense of treatment and the possible adverse side effects, it was felt that until other tests have been validated only a definitive test, such as culture, along with level of exposure to known positive cases should be used to determine which animals should undergo drug therapy.

Results from drug trials have been inconsistent and more work remains to be done to finalize drug therapy regimens. At this time it is recommended that at least two drugs to which the organism is sensitive be used for treatment. In most cases isoniazid and rifampin would be the initial drugs of choice. A third drug, such as pyrazinamide, would be beneficial as well, especially for animals known to be shedding the organisms. Using humans as a model, current recommendations are to treat culture-positive cases for at least 12 mo with isoniazid and rifampin, with PZA added during the first 2 mo of treatment. Exposed animals should also be treated either prophylactically or therapeutically, depending on the risk factors involved.

The proposed testing and treatment protocol discussed by the TB Working Group is undergoing further revision at this time. Until it is finalized, any new outbreaks will be handled on a case-by-case basis.
Table 1. Classification of 18 members of an elephant herd as at low or high risk for *M. tuberculosis* infection, based upon results of tuberculin skin testing, BTB and ELISA testing, and level of exposure to known-positive animals.

<table>
<thead>
<tr>
<th>Elephant</th>
<th>October Skin test result</th>
<th>December Skin test result</th>
<th>October BTB result</th>
<th>December BTB result</th>
<th>ELISA CSU</th>
<th>ELISA ISU</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neg</td>
<td>Neg</td>
<td>No data</td>
<td>Avian</td>
<td>Neg</td>
<td>Neg</td>
<td>Low risk</td>
</tr>
<tr>
<td>2</td>
<td>Neg</td>
<td>Neg</td>
<td>No data</td>
<td>Avian</td>
<td>Neg</td>
<td>Neg</td>
<td>Low risk</td>
</tr>
<tr>
<td>3</td>
<td>Neg</td>
<td>Neg</td>
<td>No data</td>
<td>Bovine</td>
<td>Neg</td>
<td>Neg</td>
<td>Low risk</td>
</tr>
<tr>
<td>4</td>
<td>Neg</td>
<td>Neg</td>
<td>Bovine</td>
<td>Equivalent</td>
<td>Neg</td>
<td>Neg</td>
<td>Low risk</td>
</tr>
<tr>
<td>5</td>
<td>Neg</td>
<td>Neg</td>
<td>Bovine</td>
<td>Bovine</td>
<td>Neg</td>
<td>Neg</td>
<td>Hi risk</td>
</tr>
<tr>
<td>6</td>
<td>Neg</td>
<td>Pos</td>
<td>Bovine</td>
<td>Bovine</td>
<td>Neg</td>
<td>Neg</td>
<td>Hi risk</td>
</tr>
<tr>
<td>7</td>
<td>Pos</td>
<td>Bovine</td>
<td>Bovine</td>
<td>Neg</td>
<td>Neg</td>
<td>Hi risk</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Pos</td>
<td>Bovine</td>
<td>Avian</td>
<td>Neg</td>
<td>Neg</td>
<td>Hi risk</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Pos</td>
<td>Bovine</td>
<td>Avian</td>
<td>Neg</td>
<td>Neg</td>
<td>Hi risk</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Pos</td>
<td>Bovine</td>
<td>Avian</td>
<td>Neg</td>
<td>Neg</td>
<td>Hi risk</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Pos</td>
<td>Pos</td>
<td>Equivalent</td>
<td>Bovine</td>
<td>Neg</td>
<td>Neg</td>
<td>Hi risk</td>
</tr>
<tr>
<td>12</td>
<td>Neg</td>
<td>Pos</td>
<td>Equivalent</td>
<td>Equivalent</td>
<td>Neg</td>
<td>Pos</td>
<td>Hi risk</td>
</tr>
<tr>
<td>13</td>
<td>Neg</td>
<td>Pos</td>
<td>Equivalent</td>
<td>Equivalent</td>
<td>Neg</td>
<td>Pos</td>
<td>Hi risk</td>
</tr>
<tr>
<td>14</td>
<td>Pos</td>
<td>Neg</td>
<td>Bovine</td>
<td>Neg</td>
<td>Pos</td>
<td>Hi risk</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Pos</td>
<td>Pos</td>
<td>Bovine</td>
<td>Bovine</td>
<td>Neg</td>
<td>Pos</td>
<td>Hi risk</td>
</tr>
<tr>
<td>16</td>
<td>Neg</td>
<td>Neg</td>
<td>Bovine</td>
<td>Bovine</td>
<td>Pos</td>
<td>Pos</td>
<td>Hi risk</td>
</tr>
<tr>
<td>17</td>
<td>Neg</td>
<td>Neg</td>
<td>Avian</td>
<td>Bovine</td>
<td>Neg</td>
<td>Neg</td>
<td>Hi risk</td>
</tr>
<tr>
<td>18</td>
<td>Neg</td>
<td>No Data</td>
<td>Pos</td>
<td>Neg</td>
<td>Positive</td>
<td></td>
<td>Positive</td>
</tr>
</tbody>
</table>

1. #15 had a negative chest x-ray (young animal -3-yrs-old).
2. #17 & 18 were clinically thin.
3. 0.1 ml PPD Bovis injected intradermally in the posterior aspect of the pinna.
4. bovine (avian) - reacted to bovine (avian) mycobacterial antigen on the lymphocyte transformation portion of the BTB assay.
5. an equivalent response is one in which the test sample responds equally to both the bovine and avian antigens.
6. serum ELISA assays performed at Colorado State University (CSU) and Iowa State University (ISU).

Table 2. Summary diagnostic testing of all culture-positive cases of *M. tuberculosis* in elephants (current outbreak, as of 1 August 1997).

<table>
<thead>
<tr>
<th>Elephant ID</th>
<th>Skin test</th>
<th>BTB test</th>
<th>ISU ELISA</th>
<th>CSU ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Negative</td>
<td>Bovine</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>CA-2 (dead)</td>
<td>Unknown</td>
<td>Avian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA-3</td>
<td>Negative</td>
<td>Bovine</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>CA-4</td>
<td>Negative</td>
<td>Bovine</td>
<td>Positive</td>
<td></td>
</tr>
</tbody>
</table>

1. ISU = Iowa State University; CSU = Colorado State University.
POTPOURRI FROM CDC: BATS, RATS, SNAKES, AND MONKEY BITES

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Abstract

There are public health issues related to the importation and secondary distribution of several species of that are of particular public health concern. Megachiropteran species are of renewed interest due to recent isolations of morbilli- and lyssa- viruses in Australian species. There is reported interest in commercial trade in Pteropus species for human consumption. Traffic in exotic rodent importations for zoologic and commercial uses is being examined more closely in association with recent outbreaks of leptospirosis (Central America), plague (Central Africa), and arenavirus diseases (Central Europe, Latin America). Large and venomous exotic reptiles and arachnids (snakes, scorpions, and large lizards) can cause human injury and death. Zoologic and laboratory species of primates have been reported to the Centers for Disease Control and Prevention in association with “pet-animal” bite injuries to private citizens.
Abstract

Since 1988, Texas has experienced the onset of an expanding epizootic of canine rabies (678 cases) in South Texas and gray fox rabies (779 cases) in West Central Texas. To contain these rabies epizootics, the Texas Department of Health’s Zoonosis Control Division, Texas Animal Damage Control Service, Texas National Guard, and a group of enthusiastic volunteers from all over the state completed the largest distribution of vaccine-bait units in the world. It was also the first of its kind for coyotes and gray foxes. Over 5.9 million edible baits containing an oral rabies vaccine were airdropped over 98,000 square miles from 1995 through 1997.

Vaccine-bait combinations contained 2.0 ml Raboral V-RG vaccine at a minimum field dosage of $10^{7.4}$ virus particles in a plastic container (sachet) within a hollow extruded bait. Coyote baits were produced using a fish meal-based formula, while gray fox baits were dog food-based. The baits were manufactured by Rhone-Merieux, Inc., Athens, GA, and contained tetracycline at a level of 150 mg/bait as a biomarker.

The Ontario Ministry of Natural Resources provided three Twin Otter aircraft outfitted with automated bait distribution equipment. Aerial distribution of bait was conducted over the entire target area at a bait density of 70 baits/square mile. Bait placement occurred in January and early February of each year. Distribution during winter is critical to program success, as the reduction in natural food availability makes the vaccine/bait units more attractive to the target animals.

Post-vaccination surveillance programs began with the collection of coyotes and gray foxes from the baited area 45 days after completion of each drop. Blood samples were sent to the US Army Veterinary Laboratory at Fort Sam Houston, San Antonio, Texas for RFFIT (Rapid Fluorescent Focus Inhibition Test) rabies viral antibody testing. Canine tooth samples were sent to Dr. David Johnston of Ontario, Canada for aging and biomarker analysis.

The goals of Oral Rabies Vaccination Program -- including creating zones of vaccinated coyotes and gray foxes along the leading edge of the epizootics and containing the expansion of the epizootics-- have been met to date. Of all rabies cases statewide, 29% were the Texas fox variant of rabies virus in 1996 versus 41% in 1995. Of all rabies cases statewide, 6% were the canine variant in 1996 versus 24% in 1995. These cases included spillover of both the Texas fox and canine variants to a wide variety of species, such as domestic cats, raccoons, livestock, and bobcats.
DISTRIBUTION OF NONHUMAN PRIMATES IN THE UNITED STATES BY ACCREDITED ZOOLOGIC INSTITUTIONS, 1975-1995: PRELIMINARY FINDINGS

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Abstract

The importation, use, and secondary distribution of nonhuman primates has been restricted by the Foreign Quarantine Regulations of the Public Health Service Act (42 CFR 71.53; Nonhuman Primates) since October 10, 1975. This paper represents the first effort to review regulatory compliance by the North American accredited zoologic community. A retrospective analysis of American Zoo and Aquarium (AZA) studbook records was performed for 59 nonhuman primate (NHP) species, utilizing a total of 32,348 individual animal records.

A total of 2,730 (32%) of 8,596 NHPs in the current (living) population, as listed in the most current studbook for each species as of 1995, were permanently lost to species survival plans through transfers from accredited institutions to dealers or to private individuals. The percent of NHP lost to follow-up varied by species from 0% to 98%, with a median value of 30%. For several species, more animals were lost to follow-up than were retained in AZA institution populations. During the 20-yr period of the study, 59% (99 of 167) of AZA accredited institutions listed in the 1995 AZA directory completed at least one transfer event in which an NHP was lost to follow-up. Animals retained in zoo populations moved almost exclusively from accredited zoo to accredited zoo, rather than through dealers.
AMDUCA AND EXTRA-LABEL DRUG USE: RIGHTS AND RESPONSIBILITIES FOR THE ZOO AND WILDLIFE VETERINARIAN

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Abstract

Prior to October 1994, section 512 of the Federal Food, Drug, and Cosmetic Act provided that a new animal drug (NAD) was deemed unsafe unless it was subject to an approved application and the drug, its labeling and its use conformed to such approved application. Therefore, use of a new animal drug without an approved application or in a manner different from that set forth in an approved application resulted in the drug being unsafe under this act. The Animal Medicinal Drug Use Clarification Act (AMDUCA) for which regulations were published in the Federal Register in November 1996 and for which implementing regulations took effect in December 1996, allows veterinarians to prescribe extra-label uses of FDA-approved animal drugs and approved human drugs for animals.

Zoo species and free-ranging wildlife species which are not harvested for human food fall into the non-food animal arena; therefore, the AMDUCA allows veterinarians to use whatever drug (approved human or animal) they need to effectively practice. Extra-label use (ELU) of an approved human or animal drug is permitted when there is no animal drug approved for the intended use; when there is an animal drug approved for the intended use, but the approved drug is not in the required dosage form or concentration; or when an approved drug has been found to be clinically ineffective when used as labeled. Furthermore, when the intended use involves administration to a nonfood animal, an approved human drug can be used.

Free-roaming wildlife species which may be hunted are viewed differently. The agency understands that some of these animals may be harvested for human food, and, therefore, they are considered to be food animals. When considering ELU in these animals, veterinarians must be in conformity with the provisions of the regulations applicable to food animals.

In food animals the “first resort” for ELU is an approved animal drug rather than an approved human drug. ELU that doesn’t require compounding is the “first resort” over ELU that does. When compounding is appropriate for use in food animals, “first resort” is to an approved animal drug rather than an approved human drug. All other requirements of the regulations must also be met (e.g., adequate record keeping, appropriate withdrawal time, etc.). For example, certain drugs are prohibited for use in food animals. These include chloramphenicol, clenbuterol, diethylstilbestrol, dimetridazole, ipronidazole, other nitroimidazoles, furazolidone (except for approved topical use), nitrofurazone (except for approved topical use), and sulfonamide drugs in lactating dairy cattle (except approved use of sulfadimethoxine, sulfabromomethazine, and sulfamethoxypyridazine).
Fluoroquinolones and glycopeptides are in the process of being added to the list of prohibited drugs.

In addition, the timing of ELU should take into consideration periods of harvest (e.g., hunting seasons). Determination of an appropriate withdrawal time is the responsibility of the veterinarian, taking into account the species, their range, published withdrawal times in approved species, and local hunting seasons.

The agency believes that Congress intended that veterinarians be responsible for overseeing the extra-label use of drugs. The agency recognizes the unique applicability of the veterinary-client-patient (VCP) relationship to free-ranging wildlife. With respect to use of drugs by non-veterinarians, such as wildlife biologists who are typically state or Federal employees, it is noted that they are usually under the general supervision of a veterinarian, who may also be a government employee. Such relationships fall within the scope of a valid VCP relationship.

The preceding comments are general. There are limitations in the AMDUCA prohibiting extra-label use of certain drugs under specific conditions and other special circumstances. Interested parties are encouraged to contact the Center for Veterinary Medicine for further information.
ANIMAL WELFARE ACT REGULATIONS AND POLICIES

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Abstract

There are significant changes occurring in regulations and policies that will affect the manner in which U.S. Department of Agriculture (USDA) inspectors will evaluate facilities such as zoos. The responsibilities of licensed exhibitors and their attending veterinarians will be affected by these changes; it is imperative that they are understood by both parties.

Proposals for changes that are likely to be nearing completion or in effect by October 1997 are as follows:

1. Risk-based facility inspection. Facilities with good compliance records will be given low priority for repeat inspections, with perhaps one unscheduled USDA visit each 2 yr. High priority will be given to those institutions with repeated compliance problems.
2. Guidelines for tuberculin testing of elephants. Failure to follow this protocol in high-risk cases may place an institution in violation of the veterinary care section of the Animal Welfare Act (AWA).
3. Changes in Subpart F (standards for wild or zoo animals) that will consider such issues as
   * space requirements for large felines, bears;
   * guidelines for enclosure heights;
   * guidelines regarding chaining of elephants
   * guidelines regarding wire gauge, double-entry doors, electric fencing, etc.;
   * guidelines relating to nutrition and husbandry, for example considering presentation of food on the ground and use of bakery products or “road kill” in the diet; vitamin supplementation;
   * guidelines for minimum/maximum temperatures based upon species requirements; and
   * guidelines for behavioral enrichment of various species or groups.
5. Changes in the roles/responsibilities of the attending veterinarian.
ANESTHESIA OF NONDOMESTIC HORSES WITH CARFENTANIL AND ANTAGONISM WITH NALTREXONE

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Abstract

Safe and effective anesthesia of nondomestic horses is challenging. Narcotic agents (carfentanil or etorphine) alone or in combination with other drugs (e.g., xylazine, acepromazine, detomidine) have been used to anesthetize nondomestic horses. The purpose of this report is to (1) summarize published carfentanil dosages for anesthesia of the Mongolian wild horse (*Equus przewalskii przewalskii*) and the Hartmann’s mountain zebra (*Equus zebra hartmannae*); (2) summarize unpublished preliminary carfentanil dosages for anesthesia of the Persian onager (*Equus hemionus onager*), eastern kiang (*Equus kiang holdereri*), and Somali wild ass (*Equus africanus somalicus*). Planning for safe and effective anesthesia includes, but is not limited to, the following: (1) accurate bodyweight, (2) experienced support staff, (3) precise dart placement, (4) advantages/disadvantages of premedications, (5) advantages/disadvantages of supplemental drugs used to prolong anesthesia, and (6) prevalence of renarcotization.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>CARFENTANIL (mg/kg)</th>
<th>CARFENTANIL (mg/100 lbs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mongolian wild horse</td>
<td>0.020</td>
<td>1.0</td>
</tr>
<tr>
<td>Hartmann’s mountain zebra</td>
<td>0.011</td>
<td>0.5</td>
</tr>
<tr>
<td>Persian onager</td>
<td>0.055</td>
<td>2.5</td>
</tr>
<tr>
<td>Eastern kiang</td>
<td>0.044</td>
<td>2.0</td>
</tr>
<tr>
<td>Somali wild ass</td>
<td>0.046</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Naltrexone: In each case, give naltrexone all i.v. at a 50:1 ratio (mg naltrexone to mg carfentanil). Of the species listed above, the Somali wild ass is the most likely one to renarcotize.

PHARMACEUTICALS

1. Wildnil (carfentanil), Wildlife Pharmaceuticals, Fort Collins, CO 80524 USA.
2. Naltrexone, Wildlife Pharmaceuticals, Fort Collins, CO 80524 USA.
3. Ketaset (ketamine), Aveco Co., 800 5th Street N.W., Fort Dodge, IA 50501 USA.
4. Glyceryl Guaiacolate Powder, Western Medical Supply, Arcadia, CA 91006 USA.
5. Diprivan (propofol), Stuart Pharmaceuticals, Wilmington, DE 19897 USA.
6. Dormosedan (detomidine), Pfizer Inc, West Chester, PA 19380 USA.
ETORPHINE-ISOFLURANE-O₂ ANESTHESIA FOR OVARIOHYSTERECTOMY IN AN INDIAN RHINOCEROS (Rhinoceros unicornis)

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Abstract

Most previous reports of anesthesia in the rhinoceros describe the use of injectable agents for capture and translocation or for short clinical procedures. One recent case report documents the use of etorphine followed by guaifenesin, thiopental and isoflurane to provide surgical anesthesia for abdominal exploration through a flank incision in a white rhinoceros.¹ That animal was euthanatized while under anesthesia, therefore, the nature of recovery could not be determined.

This report describes anesthesia for ventral midline celiotomy, ovariectomy, and partial hysterectomy in a 22-yr-old 1955 kg female Indian rhinoceros (Rhinoceros unicornis) with a diagnosis of multiple leiomyomas involving the vagina and uterus.

Etorphine, 2.4 mg, was injected into the muscles on the medial aspect of the hind limb, using a rifle and plastic projectile syringe (Vario System, Telinject, Inc., Saugus, CA 91350 USA). After a brief period of mild hypermetria and ataxia the rhinoceros stood quietly. It was approached 8 min after darting, whereupon it walked into a corner of the paddock and head-pressed. Respiration rate at that time was 12 BPM and pulse rate, taken from the tail, was 76 BPM. Web slings, 9 ft long x 4 in wide (Mill Valley Splicing Co., Inc., Belchertown, MA 01007 USA) were then placed, one around its abdomen and one around its thorax and in front of one shoulder, after which it laid down. Respiration rate had decreased to 4 BPM, but it increased again when it was pulled out of the corner using the slings and a 16,400 lb capacity fork lift (Caterpillar, Inc., Peoria, IL 61629 USA). Leg movement occurred during positioning and the rhinoceros was given 0.2 mg etorphine i.v. An infusion of 12 g guaifenesin with 0.45 g thiopental was given i.v. through an 18-ga 2 in catheter placed in an auricular vein to eliminate chewing motions, and to relax its jaw for intubation. A 35-mm i.d. cuffed silicone tube (Bivona, Inc., Gary, IN 46406 USA) was introduced over an adult equine nasogastric tube which had been placed manually through the glottis into the trachea. Swallowing was present during intubation, but the rhinoceros did not cough. Additional guaifenesin, 22 g, and thiopental, 0.75 g, were given over the next 10 min while the rhinoceros was further positioned and anesthesia equipment was brought into the paddock. Two 9 French 15-cm wire guided catheters (Cook Veterinary Products, Bloomington, IN 47404 USA) were placed in the left (dependent) cephalic vein. The location of the vein was clearly visible, but a surgical cut-down was
necessary to penetrate the thick skin overlying the vessel. A 16-ga 24 in catheter (Intracath, Becton Dickinson Vascular Access, Sandy, UT 80470 USA) was later placed in the opposite cephalic vein. Intravenous fluids (Ringer’s lactate solution) were given through large animal i.v. administration sets (Stat IV-1000 large animal, International Win, LTD., Kennett Square, PA USA 19348). A total volume of 80 L was administered.

A 22-ga 1 in catheter was placed in a right external auricular artery for measurement of arterial pressure (MAP) and blood gases.

At the time of initiation of isoflurane-O₂ anesthesia, (55 min. after induction with etorphine) heart rate was 60 BPM, respiratory rate was 8 BPM, and mean arterial pressure (MAP) was 76 mm Hg. Isoflurane-O₂ was given using a large animal circuit (Narkovet E, North American Drager, Telford, PA 18969 USA) with a 30 L rebreathing bag. Initial delivered isoflurane concentration was 5% in an O₂ flow of 15 LPM. After approximately 10 min, those settings were reduced to 2-3% and 10 LPM where they remained for approximately 1.5 hr. End tidal (ET) CO₂ was monitored using a sidestream capnometer (Normocap 200, Datex Medical Instrumentation, Inc., Tewksbury, MA 01876 USA).

Electrocardiogram (base-apex lead configuration), direct arterial pressure, nasal temperature and relative O₂ saturation (SpO₂) were monitored continuously using a portable monitor (Propaq 106, Protocol Systems, Beaverton, OR 97005 USA). For SpO₂ monitoring, a reflectance transducer, (RS-10, Nellcor, Inc., Hayward, CA 94545 USA) was placed on the gingiva or between the endotracheal tube and hard palate. During placement of the i.v. lines, instrumentation, initial surgical scrub, and stabilization of the anesthetic level a structure was erected to hold a tarpaulin over the anesthetized rhinoceros, and a 14 in wide × 8 ft long × 6 in deep trench was dug in the paddock parallel to its back. Using the fork lift to pull leg ropes, the rhinoceros was rolled into dorsal recumbency, with its midline in the trench, approximately 1.33 hr after etorphine induction. Mattresses and hay were placed alongside the trench to further secure its position. The arterial catheter had been dislodged during positioning, and a second 22-ga 1 in catheter was placed in an artery on the inside surface of the pinna. Mean arterial pressure was found to have decreased to 56 mm Hg, and a dobutamine (Dobutrex, Abbott Laboratories, North Chicago, IL 60664 USA) infusion was begun at a rate of 1.0 μg/kg/min. Within 5 min, heart rate increased to 80 BPM with only a modest increase in MAP to 62 mm Hg, and a phenylephrine infusion (phenylephrine HCl, USP, Elkins-Sinn, Inc., Cherry Hill, NJ 08003 USA) was added at a rate of 0.03 to 0.06 μg/kg/min. The phenylephrine, combined with dobutamine, 0.125-0.25 μg/kg/min, increased mean arterial pressure to 90 mm Hg with a heart rate of 65-70 BPM.

Surgery began 2.2 hr after etorphine induction. The rhinoceros did not show any clinical signs of arousal in response to surgical stimulation, but arterial blood pressure increased dramatically during traction on the ovaries, and MAP varied between 70 and 150 mm Hg during the surgical procedure. Dobutamine and phenylephrine were discontinued and not used again until ovariectomy and partial hysterectomy were completed, and closure of the abdominal incision was begun, at which time MAP decreased to 65 mm Hg.
Although spontaneous respiratory rate was 8 BPM and SpO₂ readings had remained above 95%, ET CO₂ had risen to 74 mm Hg 15 min after the rhinoceros had been placed in dorsal recumbency. An arterial blood gas determination at that time indicated that PaCO₂ was 99 mm Hg. Arterial PO₂ was only 84 mm Hg (Table 1) despite the presumed inspired O₂ concentration of > 90%. Controlled intermittent positive pressure ventilation (CV) was initiated using a Bird Mark 9 respirator (Bird Products, Palm Springs, CA 92262 USA) driven by O₂ at a cylinder outlet pressure of 65 PSI, and a 30L reservoir bag in a 38-L “barrel”. Respiratory rate was controlled at 9-10 BPM and plateau airway pressure, measured at the endotracheal tube was ~25 cm H₂O. An arterial blood gas determination 45 min after initiation of CV indicated that PaO₂ had risen slightly to 97 mm Hg, and PaCO₂ had decreased to 55 mm Hg. Forty-five minutes later, PaO₂ had decreased again, and an attempt was made to provide positive end expiratory pressure (PEEP) at ~10 cm H₂O. Arterial O₂ tension decreased further to 60 mm Hg during PEEP, and it was discontinued (Table 1). Tidal volume was increased by increasing plateau airway pressure to ~34 cm H₂O, and PaO₂ increased to 72-74 mm Hg.

After the first 1.5 hr of inhalation anesthesia, O₂ flow to the breathing circuit was lowered to 3 LPM. This flow was maintained for the remaining 4.5 hr and appeared to be equal to metabolic O₂ requirements for the rhinoceros. The soda lime was changed once during the procedure, because inspired CO₂ rose above 0 mm Hg. Ventilation was continued during the soda lime change using a 160 LPM demand valve (LSP model 063-050, Allied Health, St. Louis, MO 63110 USA) and it was possible to keep ETCO₂ at 45 mm Hg using this device.

Ambient temperature was measured at 24°C at the midpoint of the anesthetic period, and the rhino’s nasal temperature remained at 35.3°C during the last 4 hr of anesthesia.

Isoflurane delivery was discontinued at the beginning of abdominal closure, 5.25 hr after etorphine induction. Blood pressure increased gradually over the next 1.5 hr, but no signs of arousal from anesthesia were noted. Surgery was completed 6.5 hr after etorphine induction. The rhinoceros was put in lateral recumbency and naltrexone HCI (INADA 6277, Wildlife Pharmaceuticals, Fort Collins, CO 80524 USA) 240 mg i.v. and 240 mg i.m. were given 7 hr after the initial etorphine injection. The endotracheal tube was pulled as it rolled to a sternal position, and the rhinoceros stood 1.5 min after naltrexone administration. The rhinoceros stood quietly, and seemed aware of auditory, but not visual stimuli for approximately 10 min. At 15 min, it could apparently see and was walking with some ataxia, and at 17 min it was trotting and charged a member of the staff, running him out of the paddock. Other than mild ataxia it appeared to have no gait abnormalities.

Although the rhinoceros recovered well initially, unfortunately, it died approximately 32 hr later as a result of hemorrhage from the left ovarian pedicle. The rapid recovery from 7 hr etorphine-isoflurane anesthesia demonstrates that this technique can be used to safely anesthetize adult rhinoceros in dorsal recumbency for long periods of time, provided that cardiovascular and respiratory monitoring and support are available.
LITERATURE CITED


Table 1. Arterial blood gas determinations.

<table>
<thead>
<tr>
<th>Anesthesia Time</th>
<th>1H 40'</th>
<th>2H 30'</th>
<th>3H 15'</th>
<th>4H 07'</th>
<th>5H 04'</th>
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<tr>
<td>pH</td>
<td>7.17</td>
<td>7.35</td>
<td>7.36</td>
<td>7.42</td>
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<td>PaCO₂ mmHg</td>
<td>99</td>
<td>55</td>
<td>56</td>
<td>48</td>
<td>48</td>
<td>49</td>
</tr>
<tr>
<td>PaO₂ mmHg</td>
<td>84</td>
<td>97</td>
<td>88</td>
<td>60</td>
<td>72</td>
<td>74</td>
</tr>
<tr>
<td>BE mEq/L</td>
<td>+3.1</td>
<td>+3.5</td>
<td>+5.0</td>
<td>+6.3</td>
<td>+6</td>
<td>+6</td>
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<tr>
<td>SaO₂% (Calc)</td>
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<td>97</td>
<td>96</td>
<td>91</td>
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</tr>
<tr>
<td>Respiration</td>
<td>spontaneous</td>
<td>CV⁺</td>
<td>CV</td>
<td>CV⁻</td>
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</tr>
</tbody>
</table>

⁺Controlled intermittent positive pressure ventilation
⁻Positive end expiratory pressure
ADVANCES IN PSITTACINE ANALGESIA

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Abstract

Very little research has been conducted to objectively determine relief of pain for birds. Synthetic opioids are frequently used in veterinary medicine, including companion avian medicine, in an attempt to control pain and provide analgesia. We have developed a model to study the effects of opioids in awake psittacines. Response to a noxious electrical stimuli was compared before and after intramuscular administration of butorphanol or saline. Based on preliminary data, using African grey parrots, butorphanol is effective at 1.0 mg/kg in 50% of the birds.

Introduction

It is easily accepted that birds are able to feel pain, but very little research has been conducted to objectively determine ways to relieve pain in birds. In addition to the humane reasons to relieve pain, pain has many secondary physiological consequences which may impede patient recovery. Reliable information on analgesia for psittacines is needed by veterinarians, bird owners, and research scientists who are concerned with the palliation of painful conditions in these companion animals. Early studies with birds have primarily used chickens or pigeons and were not intended to correlate with clinical application. The lack of information detailing dose-response characteristics and dosing intervals for analgesic agents has forced veterinarians to extrapolate information obtained from studies performed in dogs, cats and humans.

Opioids are a diverse group of natural and synthetic drugs with morphine-like actions. These drugs combine reversibly with specific receptors in the brain and spinal cord, modifying the transmission and recognition of pain. The analgesic effect of opioids varies widely among animal species. This may be due to the distribution, number and type of opioid receptors within the brain. Opioid receptors are classified into 5 major types and drugs that affect the specific receptors, mu and kappa, are the most common analgesics used. In general, distribution of opioid receptor types is conserved across species in brainstem and spinal cord areas but varies significantly in the forebrain. In mammalian species such as the guinea pig, monkey and human, kappa opioid receptors represent only a third of the total opioid receptor population of the forebrain while in the pigeon forebrain 76% of the total opioid receptors are kappa.

Butorphanol is a mixed agonist-antagonist opioid drug. Mixed agonist-antagonist opioids are the most common drugs used in veterinary medicine for prolonged pain relief. Mixed agonist-antagonists are characterized by agonist activity at opioid kappa receptors and minimal or antagonist effects at opioid mu receptors. If other species of birds are like the pigeon, having a high percentage of kappa opioid receptors, then these drugs may be more effective than mu opioid
agonists.15

Limited studies in chickens suggest that pain perception is mediated by neural pathways and neurotransmitters that are similar to mammals.9 Studies to evaluate pain in birds (primarily gallinaceous birds) have been based upon changes in heart rate, increases in blood pressure, vocalizations, attempts to escape, and behavioral changes.1,7-11,19,21 Studies performed in pigeons and chickens suggest that opiate receptors are present in birds.13,16 Early behavioral studies with pigeons concluded that birds could not discriminate between mu and kappa agonists and therefore both opioid receptors were thought to have a similar mechanism of action.13 Psittacine physiological parameters such as heart rate, mean arterial pressure, arterial pCO₂ and pO₂, tidal volume, inspiratory, and expiratory time are easiest to measure while the bird is anesthetized. If an opioid is administered concurrently with an inhalation anesthetic such as isoflurane, and the concentration of inhalant anesthetic can be reduced, this indicates the opioid may be providing analgesia to the animal. This method to assess analgesic effects of opioids by reduction of inhalant anesthetic concentration has been studied and accepted in mammalian species14,17,18 turkeys20 and chickens.6 A recent study tested both a mu and kappa opioid agonist and found that both drugs effectively decreased isoflurane anesthetic concentrations in a dose-dependent manner when administered to chickens.6

Results and Discussion

The first studies of analgesic agents in psittacines were done at the University of Wisconsin using butorphanol in anesthetized parrots. Our laboratory studied the effects of butorphanol in cockatoos4 African grey parrots and blue-fronted Amazon parrots.5 Studies with African grey parrots showed a significant 11% reduction in the effective dose for 50% of the birds in a population (ED₅₀) of isoflurane following administration of butorphanol 1 mg/kg i.m. In the cockatoo and African gray parrot, butorphanol did not cause any significant changes in physiological parameters. To validate the technique of isoflurane reduction as a reliable method to evaluate avian analgesia, preliminary studies in awake psittacines have been completed.

The effects of electrical current on nociception thresholds and tolerance were evaluated in non anesthetized parrots. A perch was designed to deliver an electrical stimulus to the foot of the bird. Response to the noxious stimuli was compared before and after administration of butorphanol or saline. In the African gray parrot, butorphanol significantly decreased the response to a noxious electrical stimulus. Seven of 14 parrots given butorphanol 1.0 mg/kg i.m. required a higher electrical stimulus to generate an aversion response.

Conclusions

These results from the awake parrot studies suggest 1.0 mg/kg is the ED₅₀. This dosage is found in most avian medicine textbooks and formularies and is the same dosage tested in the study using anesthetized birds to evaluate butorphanol’s analgesic properties. Further studies are in progress to evaluate butorphanol at higher dosages and to find a clinically effective dosage greater than 1 mg/kg,
to recommend for psittacines.

LITERATURE CITED

A POTENT ANESTHETIC COMBINATION WITH LOW CONCENTRATED MEDETOMIDINE IN ZOO ANIMALS

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Abstract

Since medetomidine and atipamezole first were used in non-domestic species in Finland and Sweden, more than 10 yr have passed. Publications from all over the world on the use of these compounds in zoo and wild animals are numerous. Domitor® (medetomidine 1 mg/ml) and Antisedan® (atipamezole, 5 mg/ml) first became available on the U.S. market in October 1996. Most researchers have had access to a concentrated solution of medetomidine (now available in some countries as Zalopine®, 10 mg/ml), and have used relatively high doses of it. These publications therefore will be of limited value to the North American veterinarians as long as only Domitor® is available. Some dosages and practical tips on how to improve the immobilization and anesthesia in zoo and wild animals with the use of low concentrations of medetomidine are provided here.

Materials and Methods

Tiletamine and zolazepam became available in Scandinavia in 1990, initially as Telazol® (Fort Dodge) and since 1993 as Zoletil® (Laboratories Reading, F-06516 Carros); these products have primarily been used in wildlife species such as brown bear (Ursus arctos arctos), Baltic grey seal (Halichoerus grypus) and wild boar (Sus scrofa). From 1987 to 1990, approximately 140 bears were immobilized with medetomidine in combination with reduced doses of ketamine (MK) in association with the Scandinavian Brown Bear Project. The extremely quick and unforeseen recovery after these MK combinations caused a number of unpleasant situations. Since 1990, Telazol® has been used on brown bears at the doses used by North American scientists (i.e., 8-10 mg/kg). Though effective and safe, the disadvantages with the long and sometimes stormy recovery period prompted us to try to combine medetomidine with tiletamine + zolazepam (MTZ) in 1991.

In 1994 the use of etorphine in Sweden was limited to zoo and wildlife projects, even though over 10,000 farmed fallow and red deer had been successfully immobilized with a standard combination containing etorphine + xylazine (EX) in a ratio of 1:40 since 1975. In the exotic farmed species, the demand arose to find an effective and inexpensive non-opioid combination. Studies have been conducted with MK, xylazine + ketamine ([XK]; Hellabrunner Mischung), MTZ, xylazine with tiletamine + zolazepam (XTZ), and most recently with medetomidine or xylazine + butorphanol. In the veterinary work at the Kolmarden Zoo, medetomidine and atipamezole were introduced for clinical evaluation in 1986. The introduction of Telazol® and Zoletil® (TZ) took place in 1991. The use of MK and XK-mixtures were routine and the “new” TZ-products initially were used to replace ketamine in large carnivore species.
Results with MTZ in 450 Zoo and Wild Animals

Experiences using TZ in combination with medetomidine or xylazine indicate that the dosages of TZ given in U.S. literature could be significantly reduced when combined with xylazine and especially with medetomidine. By using Domitor® instead, as the solvent for Telazol®, a potent and stable mixture (MTZ) containing 1 mg medetomidine, 50 mg tiletamine, and 50 mg zolazepam/ml is achieved. This relationship between medetomidine and TZ (1:100) has been found to be clinically satisfactory, reversible and relatively affordable in zoo species such as chimpanzee, lynx, brown bear, polar bear, wild boar, fallow deer, ostrich, Nile crocodile and American alligator. A relatively increased dose of medetomidine (1:25-50) has been used in species such as lion, tiger, wolf, grey seal, fur seal, capybaras, domestic pig and axis deer. This use provides the possibility to reverse the immobilization with atipamezole with only minimal residual effect of TZ, thus avoiding excitement and risk for injuries. In the species mentioned above, an M: TZ ratio of 1:100 would also work fine, but a quick and smooth recovery would be possible only soon before spontaneous recovery by the reversal of medetomidine with atipamezole. The cost and volume increase by adding medetomidine above the optimal 1:100 ratio.

Mortalities

Experiences with MTZ-mixtures have been most successful for immobilizations of 240 wild brown bears; no mortalities occurred. Of the 210 immobilizations in 18 zoo species (Table 1), two mortalities occurred. One large female chimpanzee with a chronic fibrinous pleuritis suffocated in a sitting position during the 25 min before it could be handled. One young 30 kg male axis deer immobilized with M 0.03+TZ 1 mg/kg, died in sternal position with bronchi and trachea filled with foamy fluid and no other lesions that would indicate overexertion or stress could be observed. In 10% of young axis deer, respiration suddenly has stopped. After doxapram, 1-2 mg/kg i.v., and some initial external chest compressions, they have all recovered normally. No pulse oximetry has been carried out in axis deer. In other species, the MTZ combinations have been uneventful, and in primates and carnivores pO₂ between 85-97% has been found.

Practical Recommendations in Zoo and Farmed Animals

When compared with published dosages of Telazol® in zoo animals, these dosages might be reduced by at least 50% by adding medetomidine at 1 mg/100 mg of Telazol®. This corresponds to 10 to 40 µg/kg of medetomidine in most zoo species. The mixture seems to be potent and safe for several weeks. The immobilization can, after 30-40 min, be reversed by atipamezole administered at a 5:1 ratio to medetomidine. If atipamezole is administered earlier, the animal is still sedated, immobilized, and/or excited by the Telazol®. If administered intramuscularly after more than 30-40 min of immobilization, most species will have a smooth recovery within 15 min. If the animal must be immobilized longer than 40-50 min, more anesthetics, either ketamine, 2 mg/kg, or Telazol®, at half initial dose, should be administered.
Table 1. Recommended dosages of M+TZ in some zoo species. The doses are based on the subjective experiences during routine clinical zoo veterinary work in Kolmarden Zoo 1991-1997.

<table>
<thead>
<tr>
<th>Species</th>
<th>Body weight</th>
<th>Dosage in mg/kg</th>
<th>Remarks</th>
<th>Documentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chimpanzee</td>
<td>40-80</td>
<td>M 0.03 + TZ 3</td>
<td>Rapid induction, good relaxation and anesthesia. Quick recovery after atipamezole i.m.</td>
<td>2</td>
</tr>
<tr>
<td>Gorilla</td>
<td>170</td>
<td>M 0.006 +TZ 1.5</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Lion</td>
<td>140-180</td>
<td>M 0.015 + TZ 1.0</td>
<td>or M 0.02 + TZ 0.6. Repeat TZ after 45 min.</td>
<td>2</td>
</tr>
<tr>
<td>Tiger</td>
<td>130-180</td>
<td>M 0.02 + TZ 0.8</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Lynx</td>
<td>18-20</td>
<td>M 0.05 + TZ 0.5</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Brown bear (zoo)</td>
<td>140-160</td>
<td>M 0.01 + TZ 2.0</td>
<td>or M 0.03 + TZ 0.5</td>
<td>2</td>
</tr>
<tr>
<td>Brown bear (wild)</td>
<td>20-30</td>
<td>M 0.04 + TZ 5.0</td>
<td>M 1 + TZ 125 mg in darts for 1.5-yr-old cubs</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>40-60</td>
<td>M 0.02 + TZ 5.0</td>
<td>M 1 + TZ 250 mg in darts for 2.5-yr-old animal</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>80-140</td>
<td>M 0.02 + TZ 4.0</td>
<td>M 2 + TZ 500 mg in darts for adult females</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>200-250</td>
<td>M 0.015 + TZ 3.0</td>
<td>M 2.5 + TZ 750 mg in darts for adult males</td>
<td>2</td>
</tr>
<tr>
<td>Polar bear</td>
<td>250-300</td>
<td>M 0.01 + TZ 1.0</td>
<td>or M 0.015 + TZ 0.5</td>
<td>2</td>
</tr>
<tr>
<td>Badger</td>
<td>5-10</td>
<td>M 0.04 + TZ 2.5</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Wolf</td>
<td>35-45</td>
<td>M 0.04 + TZ 1.0</td>
<td>Repeat after 45 min.</td>
<td>2</td>
</tr>
<tr>
<td>Grey seal</td>
<td>70-100</td>
<td>M 0.02 + TZ 1-1.2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>S. African fur seal</td>
<td>40100</td>
<td>M 0.04 + TZ 1-1.2</td>
<td>Smooth induction, spontaneous recovery after 45-60 min, or 5 min after i.v. atipamezole</td>
<td>2</td>
</tr>
<tr>
<td>Capybara</td>
<td>40</td>
<td>M 0.03 + TZ 1.2</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Wild boar (zoo)</td>
<td>60-100</td>
<td>M 0.03 + TZ 3.0</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Wild boar (wild)</td>
<td>40-80</td>
<td>M 0.03 + TZ 6.0</td>
<td>TZ alone required 8-12 mg/kg in stressed animals</td>
<td>2</td>
</tr>
<tr>
<td>Mini-pig</td>
<td>30-50</td>
<td>M 0.04 + TZ 2.0</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Domestic pig</td>
<td>60-100</td>
<td>M 0.03 + TZ 1.0</td>
<td>Younger animals M 0.06 + TZ 3.0 mg/kg</td>
<td>2</td>
</tr>
<tr>
<td>Fallow deer</td>
<td>45-55</td>
<td>M 0.02 + TZ 2.0</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Axis deer</td>
<td>30-55</td>
<td>M 0.03 + TZ 1.5</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Moose</td>
<td>180-400</td>
<td>M 0.03 + TZ 0.8</td>
<td>Insufficient immobilization</td>
<td>1</td>
</tr>
<tr>
<td>Moose</td>
<td>120-130</td>
<td>M 0.02 + TZ 2.0</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Alligator</td>
<td>30-70</td>
<td>M 0.04 + TZ 5.0</td>
<td>Slow recovery, atipamezole + biperidin should be given for quick recovery</td>
<td>3</td>
</tr>
</tbody>
</table>

Documentation: 1-low reliability, few or inconsistent immobilizations  
               2-consistent but relatively few immobilizations  
               3-high reliability due to numerous and consistent results

Dosage in mg/kg: M=medetomidine   TZ=tiletamine/zolazepam
LONG DISTANCE IMMOBILIZATION OF FREE-RANGING CHAMOIS (*Rupicapra rupicapra*)

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Abstract

As part of a chamois (*Rupicapra rupicapra*) monitoring project in the National Park Berchtaagaden an immobilization protocol using a combination of 65 μg/kg medetomidine and 1.5 mg/kg ketamine and its subsequent reversal with 0.4 mg/kg atipamezole has been used. Immobilization was induced with a modified CO2 dart gun that allowed application distances of up to 80 m. In our view this method seems to be a viable time-saving alternative to more traditional trapping methods. The use of a specific antagonist allows for very individual anesthesia configuration and safe release in the steepest alpine environment.

Introduction

Free-ranging chamois in the past have been captured using various leg snares and box traps.1,6 These methods have some important disadvantages. Use of snares in the winter increases the risk of digital frostbite and boxtraps are prone to mechanical defects caused by icing up of the various triggers.5 Both snares and boxtraps require a lot of manpower for constant monitoring purposes. Both methods are also highly “visible” and could have had a negative impact on the large numbers of visiting tourists.

Methods

Because of these disadvantages it was decided to rely on chemical immobilization applied by a CO2 dart gun. After hardware testing, came up with the following combination was developed. A high pressure CO2 dartgun (Daninject IM 25 bar, Smith GmbH, Gelsenkixchen Germany) and low weight single use 1 cc plastic-aluminum darts with barb (Pneudart, Williamsport, PA USA). Accurate determination of the shooting distance is of utmost importance and therefore a scope with integrated laser distance measurement (Swarovski LRS, Abaam/Tirol, Austria) was used. Extensive testing in the pre-project phase allowed us to reduce the impact velocity and therefore allow for dart hits on practically the entire body and thus minimize the danger of trauma due to dart impact. The reduction of the impact velocity however goes hand-in-hand with a decrease in the accuracy of the system due to the greater curve of the dart and its lower speed.

The basic requirements postulated for the chemical immobilization were an extremely rapid and smooth induction (short flight distance), a small drug volume (longer possible dart trajectories), good
sedation and muscle relaxation (better manipulation) and an antagonist (allows for shorter downtime and reversal of the drug effects should a problem arise). Various chemical immobilization techniques for chamois are described in the literature. One of the most frequently used methods is the use of the “Hellabrunnar mixture” (125 mg xylazine + 100 mg ketamine/ml). This combination allows a good immobilization using a very small drug volume (0.04-0.08 ml). Other combinations that have been used are etorphine-acepromazine and tiletamine-zolazepam.

The pioneering work of Jalanka with medetomidine, ketamine and atipamezole has added a very useful supplementary drug combination to the zoo and wildlife immobilization palette. In the past years this combination has been tested on a large number of exotic species. Medetomidine is an alpha-2 adrenergic agonist similar to the frequently used xylazine and the more recently developed detomidine. Its action is characterized by a dose dependent sedation to anesthesia, good muscle relaxation and analgesia. The main side effects are a marked bradycardia with subsequent hypotension and hypothermia. More detailed descriptions of the pharmacological and physiological properties of medetomidine and atipamezole have been reported. Though an immobilization is possible using medetomidine alone, Jalanka and Roken had recommended its use in combination with low doses of ketamine. Medetomidine is available in the European market in two concentrations: 1 mg/ml (Dormitor Farmos Turku Finland) and 10 mg/ml (Zalopine Farmos Turku Finland). The alpha-2 adrenergic antagonist atipamezole is available at 5 mg/ml (Antisedan Farmos Turku Finland). Using the work of Jalanka and Roeken7 and Berthier and a pre-field test as a basis, the field work was started using an estimated initial dose of 70 µg/kg medetomidine and 1.5 mg/kg ketamine. Anesthesia was monitored in the field using sequential rectal temperature measurements, heart rate and oxygen saturation with a pulsoximeter (Nellcor NP-20).

**Results**

Medetomidine was used in an average dose of 65 µg/kg and ketamine at a dose of 1.5 mg/kg. As an antagonist, atipamezole was used intravenously at an average dose of 0.4 mg/kg was used. In all cases reversal using atipamezole was calm, complete and extremely rapid (<2 min). Induction time to lateral recumbency is dependent on accurate body weight estimation. Underestimation of body weights later in the capture season due to increased weight gain of animals in the summer caused some problems. When the weight estimation was fairly accurate and the animal could be observed, induction time was extremely short (4-6 min). To date, it has been possible to successfully dart and collar 11 individuals (4.7). A total of 16 direct hits were recorded with an average application distance of 65 m (range 3-85 m). Five animals could not be found post application (darkness, dense forest) and one animal had to be killed after a headlong fall. An average of 25 hr work per animal captured has been necessary.

**Discussion**

After the first capture season this method seems to be a viable time-saving alternative to more traditional trapping methods. During pre-immobilization, special attention must be given to the geographical location (avoidance of rock faces and precipices) and ample consideration to the
problems of animal recovery post induction (use of hunting dog, etc.). The use of a specific antagonist allows for very individual anesthesia configuration and safe release in the steepest alpine environment.

LITERATURE CITED

CARDIOPULMONARY EFFECTS OF MEDETOMIDINE-KETAMINE-ISOFLURANE ANESTHESIA IN THE GORILLA (Gorilla gorilla) AND CHIMPANZEE (Pan troglodytes)

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Abstract

Alpha2-agonists produce profound sedation and analgesia by virtue of their ability to modulate neurotransmitter release throughout the brain and spinal cord. The highly selective alpha2-agonist, medetomidine, has recently become available for use in the United States. Medetomidine alone has been used effectively as a sedative-analgesic in a variety of species, but has proven inadequate as a complete immobilization agent. The combination of medetomidine (30-100 µg/kg) and ketamine (1-8 mg/kg depending on species) has proven to be quite effective in a variety of nondomestic mammals. In addition to their central nervous system (CNS) effects, alpha2-agonists have significant effects on the cardiovascular system, particularly in carnivores. In the dog, intramuscular medetomidine results in rapid and intense vasoconstriction followed by a profound compensatory bradycardia. A 50-75% decrease in heart rate is not uncommon and may be accompanied by severe hypotension and respiratory depression. The addition of ketamine does not necessarily prevent these effects. Little is known about the physiological effects of medetomidine-ketamine in nondomestic species.

The purpose of our study was to determine the cardiopulmonary effects of intramuscular medetomidine (40 µg/kg) and ketamine (2 mg/kg) when used as an induction combination prior to isoflurane anesthesia in gorillas and chimpanzees. A previous report had indicated that in chimpanzees medetomidine-ketamine provided rapid induction, stable immobilization, excellent relaxation, and calm recovery. We chose to extend these findings by monitoring blood pressure, hemoglobin saturation, and end-tidal CO2 during medetomidine-ketamine-isoflurane anesthesia.

Six chimpanzees and six gorillas undergoing routine physical exams at the North Carolina Zoological Park were included in the study. Medetomidine (10 mg/ml) and ketamine (200 mg/ml) were purchased separately (Wildlife Laboratories, 1401 Duff Drive, Fort Collins, CO, 80542 USA) and mixed in the same syringe prior to injection. The combination was administered either by hand syringe or by dart (Telinject USA, Inc., 9316 Soledad Canyon Rd., Saugus, CA 91350 USA) and allowed at least 10 min to take effect. Animals were then masked with 2-3% isoflurane (Aerrane, Anaquest, 2005 W. Beltline Highway, Madison, WI 53713 USA) in 100% oxygen to permit endotracheal intubation. Following intubation, animals were maintained on a non-rebreathing circuit with 0.5-1.5% isoflurane for the duration of the exam (approximately 1 hr). To assess the cardiovascular effects of anesthesia, indirect arterial blood pressure and heart rate were measured oscillometrically (Dinamap Model 8300, Critikon, 4710 Eisenhower Blvd., Tampa, FL 33614 USA).
at 3-min intervals beginning at the point where animals lost their righting reflexes. To assess respiratory effects, hemoglobin saturation was measured indirectly by pulse oximetry (Model N20, Nellcor, 25495 Whitesell St., Hayward, CA 94545 USA), and end-tidal CO₂ and respiratory rate were measured by mainstream capnography (Model 1260, Novametrix, 3 Sterling Dr., Wallingford, CT 06492). Arterial blood gases were measured with a pH and blood gas analyzer (StatPal SP2-A, PPG Ind., 11077 N. Torrey Pines Rd., La Jolla, CA 92037 USA). At the end of the procedure, isoflurane was discontinued, and either a full dose of atipamezole (Antisedan, Pfizer, Exton, PA 19341) was injected i.m. or a partial dose was given i.v. and the remainder i.m.

Medetomidine-ketamine provided sedation within 3-5 min and complete immobilization within 10-15 min of initial injection in both chimpanzees and gorillas. We found it to be very important that animals be left alone for the initial 10 min, as attempts to move them prior to this could result in rapid arousal. Once the medetomidine-ketamine had taken effect, only 3-5 min of 2-3% isoflurane by mask was required for intubation. Once intubated, an adequate plane of anesthesia was readily maintained with 0.5-1.0% (chimpanzees) or 1.0-1.5 % (gorillas) isoflurane. Both chimpanzees and gorillas had elevated blood pressures immediately following induction. Average systolic, mean, and diastolic blood pressures were 154, 121, and 87 mm Hg, respectively. Heart rates immediately after induction ranged between 60 and 90 beats/min and remained steady throughout the procedure. Blood pressure decreased steadily over the first 10-15 min and generally stabilized to systolic, mean, and diastolic pressures of 125, 95, and 75 mm Hg, respectively. Beyond the first 15 min, blood pressures remained within normal limits, varying only with the depth of isoflurane anesthesia. Spontaneous respiratory rates ranged between 20-40 breaths/min for both chimpanzees and gorillas. Hemoglobin saturation levels were consistently between 95-100%, and end-tidal CO₂ levels ranged between 30-50 mm Hg. Arterial blood gas values were within normal limits in those animals tested (2 chimpanzees and 2 gorillas). After atipamezole injection, first signs of recovery occurred within 8-10 min following injection and all animals were fully recovered (standing, vocalizing, climbing) within 10-13 min.

Our results suggest that intramuscular medetomidine and ketamine provides a rapid and safe method of induction in both chimpanzees and gorillas, and allows for a smooth transition to inhalation anesthesia. Of particular benefit was the decreased volume of drug required for initial injection and the decreased concentration of inhalation anesthetic required for maintenance. Cardiovascular effects of the combination proved to be minimal. Modest increases in blood pressure soon after induction were not accompanied by significant decreases in heart rate as has been reported in some carnivores. The transient nature of the increase in blood pressure suggests that this may be an effect of the ketamine. Maintenance of hemoglobin saturation and end tidal CO₂ partial pressures within normal limits indicate that the combination does not significantly depress ventilation. The increased respiratory rates that we observed were most likely the result of using a non-rebreathing system. The prolonged effect of medetomidine allowed for a smooth transition from inhalation anesthesia to recovery, as the animals remained heavily sedated until given the reversal agent. Reversal in all cases was rapid, smooth and complete.
ACKNOWLEDGMENT

We thank Bill Lance of Wildlife Laboratories for supplying us with concentrated medetomidine and ketamine solutions.

LITERATURE CITED


ABSORBANCE SPECTRA OF FELINE HEMOGLOBINS IN THE VISIBLE AND NEAR INFRARED REGIONS

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Abstract

Anesthesia of rare exotic felids in captivity carries the added risk that exhaustive validation of monitoring techniques is not possible. One such technique, pulse oximetry, as an index of arterial oxygen saturation (SpO2), is part of minimum monitoring requirements in human anesthesia. It can provide a continuous, noninvasive indication of blood oxygen saturation when access to arterial blood or blood gas analysis is unavailable. Although, validation in vivo is preferred, in vitro confirmation of basic principles upon which the technique is based is a viable alternative to repeated and often invasive studies on valuable animals. This study was initiated to determine if the absorbance spectra of hemoglobins from exotic felids were sufficiently different from those of human hemoglobin to affect the applicability of pulse oximetry to zoo cats presented for anesthesia.

Pulse oximetry is based on the Beer-Lambert law which states that the concentration of a pure substance can be determined from the spectral absorbance of that substance (e.g., oxyhemoglobin) at a given wavelength if the pathlength across which the incident light travels is known. In vitro, the measurement is made across a sample of lysed red blood cells in a cuvette of known dimensions. In order to apply the technology in vivo, it is assumed that the pulsatile component of the signal represents the arterial blood, and that all other light absorbing moieties are not important. This condition results in a nonhomogeneity of the absorbing system which necessitates empirical calibration of instrument algorithms. Most monitors currently in use employ calibration curves obtained from human volunteers; which, in theory, can affect the application of pulse oximetry to nonhuman species. Since arterial oxygen saturation is determined by algorithms that analyze plethysmographic waveforms of light absorption measured at both the visible red (660 nm) and near infrared wavelengths (940 nm), mM extinction coefficients for a given species at these wavelengths should be comparable to human extinction coefficients if the derived SpO2 values are to be valid. Such comparisons have already been made between human adult, human fetal, and canine hemoglobin. Using the dog as a model for arterial oxyhemoglobin desaturation accuracy, the absorbance spectra of canine hemoglobin was demonstrated to be almost identical to that of human hemoglobin. This is not unexpected, since the absorbance range used by pulse oximetry affects only the heme moiety of hemoglobin which is virtually conserved among vertebrates. We have determined the light absorbance characteristics of hemoglobins that are relevant to pulse oximetry for those animal species commonly used for biomedical research purposes or presented for anesthesia in veterinary medicine (e.g., dog, cat, horse, cow, pig), and compared them to human
hemoglobin. Comparative absorbance data for exotic feline hemoglobin has not been reported. The need exists to further characterize and validate pulse oximetry in exotic species.

Heparinized blood samples were obtained from adult, nonsmoking human volunteers and domestic cats (F. catus) as controls. Blood samples of exotic felids Sumatran tiger (P. tigris sumatrae), lion (P. leo), lynx (L. lynx), leopard (P. pardus), serval (F. serval), bobcat (F. rufus) were collected at the Columbus Zoo under anesthesia initiated for other medical reasons. Blood samples were immediately shipped on ice after collection. Hemoglobin crude lysates are prepared by lysis of washed erythrocytes in ice-cold distilled water and cleared by centrifugation. Purified hemoglobin was obtained by ion exchange chromatography (DEAE cellulose) followed by gel filtration column chromatography (Sephadex). Total hemoglobin, methemoglobin, and carboxyhemoglobin of the eluted hemoglobin fraction were then determined by in vitro oximetry (OSM-3 Hemoximeter) prior to storage under liquid nitrogen. For spectrophotometric analysis in the 600-1000 nm range (region of interest to pulse oximetry), the sample was diluted to about 1 mM based on total hemoglobin concentration determined by multiwavelength spectrophotometry. The precise concentration of the diluted sample was then determined by a standardized cyanmethemoglobin assay. The supernatants were then analyzed after oxygenation was achieved by aeration with 100% oxygen for 10 min (HbO2), and after deoxygenation with sodium dithionite (Hb).

Oxyhemoglobin was also analyzed at 350-750 nm after further dilution (about 0.017 mM) to minimize the effects of light scattering by impurities in solution. Final concentrations of the dilute samples were calculated from the maximal absorbance of the Soret band (415 nm). Spectral analysis of the samples was performed using a scanning spectrophotometer (Cary-14, Varian Instruments, Sunnydale, CA) in a 1-cm pathlength cuvette. The apparent mM extinction coefficients were calculated using an OLIS 3820 on-line data system (Jefferson, GA). The mM extinction coefficients at 415, 541, 576, 660 and 940 nm for the felids were compared with those of human controls and of accepted human values.

Minor differences in the absorbance of both oxyhemoglobin and deoxyhemoglobin were found initially between human hemoglobin and that of the felids (data not shown). No differences were seen in Hb or HbCO content of the samples that would affect extinction coefficients. Examination of dilute samples in the strongly absorbing Soret region, where the effects of light scattering are minimized, revealed no major differences in absorbencies. Adjustments for the effects of light scattering by purification of the hemoglobin solutions and careful sample handling resulted in extinction coefficients that were consistent with both human controls and with literature values (Table 1).

The results of our studies demonstrate that the hemoglobin absorption spectra for the cats are consistent with that of human use of pulse oximetry in these species. Small quantitative differences in the apparent optical density between human hemoglobin and those of the cat became insignificant when the effects of light scattering were taken into account. These data are in agreement with other studies supporting the conservation of spectral properties among the mammalian hemoglobins. It should be noted, however, that if centrifugally cleared, hemoglobin solutions can produce light scattering artifact, then non-hemolyzed blood from species with different erythrocyte morphology
may affect the accuracy of SpO₂ determinations. Non-hemolyzed blood does not obey the Beer-Lambert law.¹ The effects due to scattering cannot be reliably separated from those due to absorption; thus, the necessity for the empirical calibration curve. It is not unreasonable to assume that given differences in erythrocyte morphology, concentration and aggregation properties, nonhuman species may present challenges to human calibration algorithms. However the clinical applicability of these systems does not appear to be compromised.

ACKNOWLEDGMENTS

This study was supported by a cooperative research grant from the Columbus Zoo and The Ohio State University.

LITERATURE CITED


Table 1. Apparent millimolar (mM) extinction coefficients for oxyhemoglobin (HbO₂) and deoxyhemoglobin (Hb) of felids compared to those of humans. Literature values are indicated in parentheses.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>HbO₂ ε₆₆₀</th>
<th>HbO₂ ε₉₄₀</th>
<th>Hb ε₆₆₀</th>
<th>Hb ε₉₄₀</th>
<th>HbO₂ ε₄₁₅</th>
<th>HbO₂ ε₅₄₁</th>
<th>HbO₂ ε₅₇₆</th>
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<tr>
<td>Human</td>
<td>.08 (.08)</td>
<td>.28 (.30)</td>
<td>.87 (.80)</td>
<td>.21 (.20)</td>
<td>125 (125)</td>
<td>13.8 (13.8)</td>
<td>14.6 (14.6)</td>
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<td>0.81</td>
<td>0.2</td>
<td>125</td>
<td>13.5</td>
<td>14.4</td>
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<td>0.8</td>
<td>0.2</td>
<td>125</td>
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<td>125</td>
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<td>0.8</td>
<td>0.2</td>
<td>125</td>
<td>13.6</td>
<td>14.6</td>
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</tbody>
</table>

¹ εₜ₇₆ = extinction coefficient, which is defined as the optical density of the absorbing substance at wavelength λ in a concentration of 1 mmol/L and a pathlength of 1 cm.
APPLICATIONS OF THE INTERNET AND WORLD WIDE WEB IN CLINICAL
ZOOLOGICAL VETERINARY MEDICINE

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Abstract

Over the past few decades, computers have evolved from a novelty item to essential pieces of equipment. While their use in keeping animal and medical records is well-known in zoos, recent explosive growth of the Internet and World Wide Web has led to numerous new applications. These applications include everything from improving animal husbandry, to computer use as a research tool, to a facilitated sharing of information among the international zoo veterinarian community.

Zoo veterinary medicine first used improved technology communications approximately 3 yr ago when the International Species Information System (ISIS) bulletin board system was initiated in Minnesota. Bulletin boards are comprised of a computer with a modem hookup that allows people to call in and access information through their computer and modem. The secure system was structured for zoo veterinarians to type in messages that other zoo veterinarians could later read and post responses. It was a primitive email system that could be accessed by any other zoo veterinarian. For example, if someone wished to obtain comments on a clinical case, diets for a particular species, or discuss husbandry issues, they would post their question on the boards. Over the following week, other zoo veterinarians would read the posted message and submit their responses. However, due to limitations in the number of people who could simultaneously access the site and a difficult interface, the next logical move was to use the World Wide Web (WWW).

Within the past few months, many zoo veterinarians who used the ISIS bulletin board have been using the new WWW-based forum system identified below.

http://www.worldzoo.org/
http://www.worldzoo.org/forums.htm
The new site allows multiple users to access it at the same time and it saves on long distance charges incurred with the Minnesota bulletin board site. The WWW forum also has an easy point and click interface. European and Australian zoo veterinarians are also joining this forum, thus providing international participation. The primary use of the site is for secure communication among peers, much like the Minnesota system.

The American Association of Zoo Veterinarians also has a presence on the WWW (http://www.worldzoo.org/aazv). The AAZV site includes two separate areas: one for public access and one secure site for professional members only. The public access area includes links to other sites such as the American Zoo and Aquarium Association (AZA), the American Veterinary Medical Association (AVMA), Wildlife Health Information Partnership (WHIP), and ISIS home pages. It also includes an area for veterinary students to access information on the profession,
student manuscript judging guidelines, and the externship and residency manuals. The secure area contains information such as the AAZV newsletters, necropsy protocols, the infectious disease notebook, and an email directory for AAZV members. This area is meant to be a central access site of information. For example, if you wish to send an email note to a colleague or one of your institution’s penguins has died and you don’t have the necropsy protocol, this Web site can be accessed to gain the appropriate information.

Online services are also emerging rapidly. America Online was the first, with the Veterinary Information Network and Compuserve followed, with Network of Animal Health (NOAH). These services require payment as you use them and average cost is approximately $250/yr. The services they provide are astounding. The veterinarian has access to board certified advice and experiences in every discipline of veterinary medicine from veterinarians who have, in many instances, seen dozens to hundreds of similar cases. At our zoo, we use this service for topics that require special expertise, such as dermatology. Smaller zoos with one veterinarian or consulting veterinarians would benefit by subscribing to one of these services as their respective experiences and caseloads in zoo and exotic animal medicine may not be as extensive as colleagues in larger zoos with multiple veterinarians and a variety of experiences. (It is impossible to be an expert in every area of veterinary medicine, much less be an expert on every species, so communication among peers is essential for zoo animal clinical medicine.) The cost is reasonable, considering the value of our animal collections. Hopefully, the two companies will eventually join and make their services available through the WWW. Professional continuing education is also available through each service, thus alleviating inconveniences and costs of traveling to various seminars for continuing education credit.

Several useful online service Web sites are listed below.
**NOAH:** http://www.avma.org/network.html
**VIN:** http://www.vetinfonet.com/
http://www.vetinfonet.com/vincours.htm

Besides online services, there are a number of Web sites that offer almost instantaneous access to expert advice in a variety of areas. The pioneer in this area is Ken Boschert, DVM (Washington University, St. Louis, MO). His Web site acts as a central clearinghouse (link site) for massive amounts of information on everything from animal husbandry issues to medical issues. One of the most interesting areas of his site is the electronic zoo. At this site, you click on the picture of the species you are interested in and it automatically gives you a list of Web sites that contain information in that area. You can access these sites on the Web at the addresses listed below.
**http://netvet.wustl.edu**
**http://netvet.wustl.edu/e-zoo.html**

Continuing education is available in many forms on the Web. This includes everything from Armed Forces Institutes of Pathology (AFIP) histopathology slides offered over the Web to many “virtual” veterinary centers. The WWW is also where the “First International Virtual Conference on Infectious Diseases of Animals,” hosted by the National Animal Disease Center (NADC), took place in April 1997. The conference included everything from opening remarks to poster sessions all presented through the Web site. Several of AFIP’s sites and the international conference can be
The federal government has begun to use electronic communications and databases. A Web site was recently set up by the U.S. Department of Agriculture’s (USDA) Animal and Plant Health Inspection Service (APHIS) which includes all of the current requirements for importation of animals into various states. Zoo veterinarians can now access this site and obtain up-to-date information on tests required to ship out or bring in animals. This is another example of an attempt to centralize information in order to better communicate with the people who need access to it. There is also information at this site regarding international animal trade and how to obtain the necessary permits. Two of USDA’s sites are listed below.
http://www.aphis.usda.gov
http://www.aphis.usda.gov/vs/sregs/

In recent history, education has become one of the top priorities of AZA institutions. This also is true for zoo veterinary medicine. The WWW is one of the best mechanisms for disseminating information to the public regarding what we do, how we do it, and what it takes to follow a similar career path. The following are some of the sites that explain which schools offer veterinary degrees, what zoo veterinarians do, and how zoo veterinarians elect to go into the profession in the first place.
http://vet.futurescan.com/
http://vetpath1.afip.mil/CLDavis/zooprogram2.html
http://www.memphiszoo.org/vet/index.html

What does the future hold in terms of clinical zoo veterinary medicine and the Web? I feel there will be increased sharing of diagnostic imaging through the Internet. For example, last year our zoo’s male jaguar exhibited hundreds of small mineral densities throughout its body. A local ultrasound expert posted the radiograph on the Web and asked for potential diagnoses. We received dozens of thoughts and comments from human and veterinary radiologists from all around the country. I feel this will become standard practice in the future, including the use of radiograph machines that relay images directly into a computer (currently being used in human medicine). Some sites for this type of information include several listed below.
http://radserv.med-rz.uni-sb.de/isapi/query.dll/eunknown/1/548
http://www-ipg.umds.ac.uk/~acd/
http://www-ipg.umds.ac.uk/~acd/xrayfiles/cases/frcr/frcr.htm

Also expected to evolve rapidly are computerized specialties known as telemedicine and telesurgery. The field of telemedicine is currently being extensively developed by federal, state, and independent agencies in 40 states while telesurgery is being developed by National Aeronautics and Space Association (NASA) and the U.S. Army. Telemedicine involves a nurse in the patient’s home with
a video-equipped laptop and standard telephone lines hooked up to the hospital where the doctor is able to conduct the workup. The nation’s largest hospital company (HCA) is currently refining the technique. The cost of this system has decreased from about $50,000 to $12,500 over the past year and should continue to fall as the technology is developed. Unlike telemedicine, telesurgery is still in its infancy. It involves the use of remote robotic surgical equipment along with high definition video cameras and hookups for surgeries to be performed in remote locations. NASA is exploring this technique for applications in space (such as on space stations or shuttles) while the U.S. Army is considering applications in the battlefield where immediate treatment is essential. Once this technology becomes more refined, there should be no reason not to use it, especially in situations such as the mountain gorilla population in Rwanda or other remote locales with dwindling native wildlife populations. Relevant WWW sites include the following:
http://naftalab.bus.utexas.edu/nafta-7/tmpage.html
http://www.op.dlr.de/FF-DR/dr_rs/MEDICINE/hi.html
http://netvet.wustl.edu/top10.htm
LAPAROSCOPIC STERILIZATION IN TWENTY-SIX BABOONS (*Papio hamadryas*) AND ONE HYBRID GIBBON (*Hylobates lar* × *Hylobates pileatus*)

Jacques Kaandorp, DVM1* and Harry A.M. Vervest, MD, PhD2

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Abstract

To control the number of baboons (*Papio hamadryas*) in Safari Beekse Bergen, Hilvarenbeek, The Netherlands, 26 out of 35 female baboons were sterilized by laparoscopic tuba-coagulation. One hybrid gibbon (*Hylobates lar* × *Hylobates pileatus*) was sterilized the same way to prevent the development of a hybrid line of gibbons.

Introduction

In zoos, successful reproduction in a significant number of exotic species is no longer a problem. Veterinarians first praised for their success in reproduction, are confronted with the problems associated with birth control. Implants, PZP-vaccines and long-acting-progestogen-injections are used as alternatives to more invasive surgical methods as castration or sterilization.

In Safari Beekse Bergen, Hilvarenbeek, The Netherlands, surgical methods of contraception such as vasectomy of a male lion (*Panthera leo*), ovariohysterectomy of lionesses and an African wild dog (*Lycaon pictus*) and castration of a hybrid male gibbon (*Hylobates lar* × *Hylobates concolor*), were performed previously. Although surgical methods of birth control are invasive and permanent, they do not have the potential side effects of contraceptive drugs.

In order to control the population of the 70 baboons (*Papio hamadryas*) there was an urgent need for some method of birth control. These baboons, living together with 5 African elephants (*Loxodonta africana africana*), had always been a very stable social group, existing of 35 males and 35 females. To prevent overpopulation a solution of laparoscopic sterilization of the females was chosen. This method of sterilization was performed by laparoscopic tubo-coagulation as performed in humans. The intention was, that sexual behavior would still occur, the social ranking within the group wouldn’t change, nursing behavior wouldn’t be lost and the total number of baboons wouldn’t increase. Maintaining a stable social group would hopefully be achieved with this method.

Former DNA work on the gibbons of the park showed one female to be a hybrid (*Hylobates lar* × *Hylobates pileatus*). To prevent the development of a hybrid line of gibbons, birth control was necessary. Instead of doing an ovariohysterectomy, sterilization was preferred, so sexual behavior would still be present.
Methods

The baboons and the gibbon were first sedated with ketamine (Dopharma, Raamsdonkveer, The Netherlands, 100 mg/ml), 10-15 mg/kg b.w. using Telinject blowdarts through a Telinject blowpipe. They were then checked by ultrasound (Sonorex RK 100, Bandelin Berlin, Germany, Animed Barneveld, The Netherlands) for pregnancy, because in late gestation the procedure is difficult to perform, due to the enlargement of the uterus. In total 26 out of 35 female baboons were brought one after the other, to an improvised surgical area to be sterilized. Intubation was necessary, because despite using atropine HCl (AUV, Cuyk, The Netherlands) 0.1-0.2 mg/kg b.w, salivation was a problem.

After intubation, maintenance of anesthesia with oxygen and Halothane was uneventful. Pulse oximetry (Nellcor N 180, 's-Hertogenbosch, The Netherlands) and capnography (Lameris, Utrecht, The Netherlands) were used to monitor anesthesia.

The monkeys were put in dorsal recumbency, clipped, cleaned and draped. A 1-cm incision was made in the right lateral abdominal wall for a disposable surgical trocar (Endopath, Ethicon Summerville, NJ USA) through which the coagulator could be inserted into the abdomen. A second incision was made in the umbilical area for the laparoscope (Storz, Tilburg, The Netherlands) with mini-camera. A television monitor at the end of the operation table was used to follow the intra-abdominal procedure.

In humans the abdomen is filled with carbon dioxide to obtain better visualization. This frequently demands that machine-controlled respiration be performed. To prevent this, a laparofan was used to create space in the abdomen to work in. The laparofan was also used as a trocar through which the laparoscope is brought into the abdomen.

The oviducts were coagulated by means of a unipolar coagulator, which also functions as a forcep. One or two stitches (Vicryl 3-0, Instruvet, Amerongen, The Netherlands) were used to suture the incision in the abdomen, followed by an uninterrupted subcutaneous suture. All the monkeys where microchipped during the procedure (Trofan, The Netherlands), and received a long acting antibiotic ampicillin (Albipen L.A., Mycofarm, The Netherlands) 40 mg/kg b.w. and 0.2 mg/kg b.w. (Dectomax, Pfizer, Capelle a/d IJssel, The Netherlands) for deworming.

Results

All monkeys survived and no behavioral problems were observed after the procedure. Since there were no stitches to be removed, no complications or infections were observed. One year later the group is stable, there is no fighting and there are no changes in the social ranking.

Discussion

The goal of not interfering with the social behavior of this large group of baboons was achieved with
laparoscopic sterilization. Sexual behavior was assumed to be normal and nursing behavior was not lost.

The laparofan proved to be very helpful in this procedure. The avoidance of inflating the abdomen with carbon dioxide is a practical advantage. Respiratory problems due to anterior pressure of the diaphragm are avoided this way.

Laparoscopic sterilization is a very efficient contraception technique taking 5-10 min to perform. The alternative exploratory laparotomy would be a longer surgical procedure and potentially have more post operative complications. Recovery time after laparoscopy also was observed to be quicker.

In humans, clips or small rings are often used for transfixing tissue, however because baboons and gibbons have thinner oviducts these were not used. The thinner oviducts of baboons and gibbons are difficult to grasp with the larger bipolar coagulator, so a unipolar coagulator was used in these monkeys.

A definitive form of contraception like surgical sterilization is not a problem as long as there are enough females in the group that remain fertile to stabilize the number of animals in the group. In fact the procedure might be necessary again in the future, since nine adults females are still fertile and five female infants will become fertile in the future.

ACKNOWLEDGMENTS

The authors wish to thank individuals of St. Elizabeth Ziekenhuis: Mr. Van Beurden, fa Storz; Mr. Van Dijk, TD Medical; Mrs. Omtzig, gynaecologist; Mrs. Wessels, surgical technician; Mr. Schraffordt Koops, assistant gynaecologist; Mr. Lugtigheid, DVM; Mrs. Van Drunen, vet. technician; Mrs. Heeren, vet. technician; and the zookeepers of Safari Beekse Bergen.
THE USE OF TRANS-ESOPHAGEAL ECHOCARDIOGRAPHY TO ASSESS CARDIOVASCULAR HEALTH IN CAPTIVE LOWLAND GORILLAS (Gorilla gorilla gorilla)

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Abstract

Complete cardiac diagnostic testing, even without evidence of clinical cardiac disease, is warranted due to the high prevalence and acute nature of cardiac disease in captive gorillas. Antemortem diagnostic techniques previously described in gorillas include electrocardiography, echocardiography, blood pressure measurements, oximetry, radiology, cardiac catheterization and coronary angiography. Annual cardiac examinations of the gorillas at the Franklin Park Zoo include a 12 lead electrocardiogram, a thoracic radiograph, doppler flow imaging, trans-thoracic and trans-esophageal echocardiography. Trans-esophageal echocardiography (TEE) with a Hewlett-Packard echocardiograph machine model SONOS 2000/2500 with an OMNIPLANE TEE transducer (5 MHz), has allowed enhanced imaging of the heart and great vessels in an attempt to monitor and hopefully intercede in the development of any cardiovascular diseases.

Introduction

The incidence of cardiovascular disease as a cause of mortality in adult, captive lowland gorillas has been reported to be as high as 41% in the Species Survival Plan (SSP) population. These statistics have elucidated the need for further investigation into cardiovascular disease as a cause of death in captive lowland gorillas (Gorilla gorilla gorilla). The most commonly used cardiovascular diagnostic techniques done in gorillas to date have included electrocardiograms, trans-thoracic echocardiographs (TTE), thoracic radiographs, and blood pressure measurements. The use of TEE presents an additional diagnostic tool to aid in the early detection of cardiovascular disease in large apes.

Discussion

Previously described cardiovascular diseases of gorillas include; aortic dissection, fibrosing cardiomyopathy, congenital defects, and coronary artery disease. Many of these conditions are diagnosed post mortem due to the acute nature of the final stages of these diseases. The increasing use of TEE as part of the annual physical examination of captive lowland gorillas may be a way to detect some of the more subtle cardio-vascular changes that occur early on in the progression of these diseases. The more commonly used diagnostic tool is trans-thoracic echocardiography. This involves sending ultrasonic sound waves across the chest wall, in between the ribs and through the air in the lungs. All of these sound wave interfaces can interfere with the quality and definition of the image.
Another limiting factor of TTE is the compromised view of the aorta. In humans, TTE provides 4-5 windows in which the heart can be visualized. Because of the massive chest and more cranial - dorsal positioning of the heart in the thoracic cavity observed in gorillas, the view is limited to two windows and the sound waves have to travel further before returning to the transducer, thereby creating a less defined image. These limited windows provided by TTE allow only a small portion of the ascending aorta and aortic root to be visualized. Trans-esophageal echocardiography allows for unobstructed imaging of the heart, the entire aorta including the ascending, transverse and descending aorta, the aortic root, the abdominal aorta, the left coronary artery, any small valvular discrepancies, and the left atrial appendage. The TEE transducer actually scans from the back of the heart in 180 degree sweeps and the sound waves do not travel through the lungs and the ribs like they would with a trans-thoracic echocardiograph, thereby creating a clearer, more complete picture. By having a sharper image with an unlimited viewing window, subtle changes in cardiac musculature, cardiac function, and vasculature function and integrity may be apparent at an earlier stage in the disease process.

Conclusion

Human cardiologists are now routinely using TEE to further diagnose and treat cardiovascular disease in cardiac patients. Trans-esophageal echocardiography, where available, should be considered as part of a normal annual cardiac examination on captive gorillas. When used in conjunction with trans-thoracic echocardiography, electrocardiograms, and other diagnostic techniques, a complete data base can be established on the cardio-vascular health of captive gorillas. This may lead us to a better understanding of the factors leading up to these diseases and hopefully a means of prevention or early treatment.

ACKNOWLEDGMENTS

This work was made possible by the generosity of New England Baptist Hospital, Hewlett-Packard, and the cardiology and ultrasound staff of the New England Baptist Hospital.

LITERATURE CITED


CONTINUOUS INTRA-ARTERIAL MONITORING OF BLOOD GASES USING THE PARATREND 7™: APPLICATIONS IN NONDOMESTIC SPECIES

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Abstract

The Paratrend 7™ intravascular blood gas monitoring system (Biomedical Sensors, Pfizer Inc., New York, NY 10017-5755 USA) utilizes fiberoptics and electrochemical sensing elements to provide continuous blood gas monitoring (PO2, PCO2, pH, temperature, base excess, bicarbonate, O2 saturation) of patients. Monitoring of invasive blood pressures via an external transducer can be performed simultaneously with the Paratrend 7™. Currently, the most widely used method of blood gas monitoring is based on intermittent arterial blood gas sampling: this method only provides historical blood gas data. The main advantage of continuous intra-arterial blood gas monitoring (CBGM) is the ability to take immediate action based on the present blood gas status of the patient. Applications in human medicine have focused on high risk, critically ill, decompensating patients; particularly in pediatric medicine and with hypoxic and/or poorly perfused patients in which pulse oximetry is unreliable. The cost of the Paratrend 7™ ($25,000 for the unit and $300/single use sensors) will prohibit the routine use of this system in zoological medicine. However, the Paratrend 7™ would be invaluable for zoological veterinarians during anesthesia, drug, and procedural studies in which trends (Fig. 1) and effects (Fig. 2) of these variables on the blood gas status of patients should be recorded. Surgical procedures on “high profile and/or high risk patients” could also be more safely performed using CBGM of the patient’s status. In this talk, we present the design and function of the Paratrend 7™ and applications in non-domestic species.

ACKNOWLEDGMENTS

The authors thank Mr. Ron Bobele for information on the Paratrend 7™ system.

LITERATURE CITED

Figure 1. Paratrend TM three parameter trend display of cheetah blood gases during Telazol/medetomidine anesthesia study. A fall in the PO2 value is shown during a few minute period.

Figure 2. Paratrend TM single parameter trend display of cheetah PO2 during Telazol/medetomidine anesthesia study. Starting at 10:02, 2 L of supplemental oxygen were delivered by endotracheal tube. A rise in PO2 is shown shortly after oxygen supplementation was begun.
TELEMETRIC MONITORING OF BLOOD PRESSURE, HEART RATE, AND ACTIVITY IN A WOOLLY MONKEY (Lagothrix lagotricha) IN A ZOO SETTING

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Abstract

Radio telemetry technology was applied in a zoo setting to evaluate blood pressure in woolly monkeys (Lagothrix lagotricha), a species with a high incidence of hypertension.1

Radiotelemetry implants (TA11PA-D70, Data Sciences International, 4211 Lexington Avenue North, St. Paul, MN 55126-6164 USA) were placed in two woolly monkeys at the Pittsburgh Zoo to measure blood pressure, heart rate, and activity using procedures previously described.2 For both monkeys, the catheter was placed into a branch of the femoral artery and positioned so the catheter tip was in the femoral artery near the dorsal aorta. The body of the implant in the first monkey was placed subcutaneously over the caudo-lateral abdomen. The body of the implant in the second monkey was sutured to the body wall inside the abdominal cavity.

The implant in the first monkey failed due to impact when the monkey was netted for evaluation of lameness in the catheterized leg. The implant in the second monkey became occluded with fibrin about 30-40 days after placement. Catheter occlusion occurred because the catheter tip was in the femoral artery rather than the larger aorta.

An array of receivers (RLA 2000, Data Sciences International, 4211 Lexington Avenue North, St. Paul, MN 55126-6164 USA) was placed in the woolly monkeys’ holding area to allow for monitoring the animal in its normal setting. A computer and software program (Dataquest IV version 2.2, Data Sciences International, 4211 Lexington Avenue North, St. Paul, MN 55126-6164 USA) provided data collection 24 hr/day at 20 sec intervals (Fig. 1). Data was collected while the second monkey was under no treatment for hypertension and under propranolol treatment for hypertension (5, 10, 15, 20, 35, 30, and 35 mg p.o. b.i.d.). Propranolol doses were increased at 2-day intervals.

During 40 days of data collection in the second monkey, circadian rhythm of blood pressure, heart rate, and activity was demonstrated using a time series auto-correlation analysis and an independent two tailed student’s t test (SPSS 6.1 for Windows, SPSS Inc., 444 N. Michigan Avenue, Chicago, IL 60611 USA). No significant reduction of blood pressure with propranolol treatment at doses of was observed. Since propranolol is a well documented anti-hypertensive drug, it is likely that occlusion of the catheter or other factors which impact blood pressure, influenced blood pressures recorded at the time of treatment with propranolol.
These results demonstrate that radiotelemetry technology can be used in a zoo setting to monitor blood pressure.

LITERATURE CITED


Figure 1. Radiotelemetry measurements on a woolly monkey.
QUANTITATION OF CYTOKINE GENE EXPRESSION: A MOLECULAR APPROACH FOR EVALUATING PROTECTIVE IMMUNITY AGAINST TUBERCULOSIS

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Abstract

Tuberculosis continues to be a problem in non-domestic species. Following exposure to Mycobacterium spp., most healthy individuals mount a vigorous and effective cell-mediated immune response to curtail the infection. The protective immune response depends upon interactions between infected macrophages, activated T lymphocytes, natural killer (NK) cells, and induced cytokines which are regulatory proteins and glycoproteins that mediate interactions between cells of the immune system. A dysfunctional cell-mediated response can lead to progressive primary disease or reactivation of endogenous foci of mycobacteria.

A positive tuberculin reaction reflects delayed-type hypersensitivity to mycobacterial antigens. The test cannot distinguish between mere exposure and active disease. Isolation of the mycobacterial organism remains the definitive method for confirming infection. In some species, radiographic findings can support a diagnosis of active tuberculosis, but for other species, radiographs may not even be a realistic option. Numerous ancillary tests have been developed to aid in the diagnosis of clinical tuberculosis, particularly for hoofstock. The blood tuberculosis battery (BTB) assay, validated for deer, evaluates cell-mediated and humoral immunity in addition to inflammatory parameters. The bovine interferon-gamma (IFN-γ) assay is an ELISA designed to detect IFN-γ in whole blood incubated with bovine PPD. Because the reagents used in the ELISA to detect the presence of IFN-γ are monoclonal antibodies directed against bovine IFN-γ, there is some concern how effective this assay will be for non-bovid ungulates. Nonetheless, both the BTB and IFN-γ assays are designed to evaluate the effectiveness of an individual’s immune response to mycobacterial antigens.

In particular, production of IFN-γ, a cytokine associated with cell-mediated immune responses, is depressed in cultures of M. tuberculosis-stimulated peripheral blood mononuclear cells from humans with tuberculosis. An ELISA is also used to measure IFN-γ in humans and again there is low probability that this assay would be applicable to species other than perhaps some nonhuman primates.

Recently, investigators have evaluated the expression of IFN-γ mRNA in humans with tuberculosis and found levels to be low compared to healthy tuberculin reactors. Similar results were obtained for mRNA levels of interleukin-2, another cytokine important for the generation of cell-mediated immunity. The molecular sequences of cytokine genes are fortunately well-conserved phylogenetically making the use of consensus sequence primers to measure levels of cytokine mRNA by reverse transcription quantitative competitive polymerase chain reaction (RT-qPCR) feasible.
Consensus primers derived from mouse and human sequences have been used successfully to measure mRNA levels of IFN-γ and IL-2 in cats, dogs, pigs, cattle, and horses.\(^3\) In theory, this technique should be readily adaptable to a wide variety of non-domestic species which are exposed to mycobacteria and develop a positive tuberculin reaction. Exposed healthy individuals would be predicted to have elevated levels of mRNA for IFN-γ and IL-2 compared to animals with active replication of the organism and clinical lesions.

**LITERATURE CITED**

THEORETICAL AND TECHNICAL ASPECTS OF DIAGNOSTIC TECHNIQUES FOR MAMMALIAN TUBERCULOSIS

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Abstract

Tuberculosis (TB) infection due to organisms of Mycobacterium tuberculosis and Mycobacterium bovis causes significant disease in both humans and animals. Tuberculosis continues to persist in domestic livestock and captive cervid herds. Recent occurrences of TB at several zoos and in a privately owned elephant herd have prompted increased concern that zoo and wildlife species represent potential TB reservoirs (Mitch Essey, personal communication). The current test and depopulation method used to control tuberculosis in domestic cattle is unacceptable for endangered wildlife species.

A variety of techniques have been used to diagnose mammalian tuberculosis. Methods that directly detect bacteria from clinical specimens include acid fast and fluorescent smears, culture, and nucleic acid amplification such as polymerase chain reaction (PCR). Indirect methods include detection of antigen or antibody, and measures of cellular reactivity against mycobacterial antigen.

Direct detection of tuberculosis

Isolation of the mycobacterial organism by culture is currently the only definitive method to confirm tuberculosis infection in animals. Although acid fast smears of clinical specimens or histologic examination of tissues can detect mycobacteria, species identification requires either confirmation by culture or DNA probes. Acid fast smears have a lower limit of detection of about 1000 organisms/ml, whereas culture can detect approximately 100 organisms/ml.

Recent technological advances have seen the introduction of methods that detect mycobacteria by amplification of mycobacterial nucleic acids (DNA or RNA) followed by detection of the amplification product. Ideally these methods can detect as few as 1-10 organisms. However, in clinical trials, these tests have shown approximately a 99 % sensitivity at detecting acid fast positive, culture positive samples, but only a 60-80 % sensitivity at detecting acid fast negative, culture positive samples. They are highly specific for TB complex organisms but cannot differentiate species. Since even dead or dying organisms can be detected these methods cannot be used to monitor treatment.1,4,11

At present, two amplification methods have been FDA approved for detection of M. tuberculosis from sputum in humans: the Amplicor system (Roche Molecular Systems, Branchburg, NJ) and the
AMTD system (Gen-Probe Inc., San Diego, CA). The Amplicor system has as its target the mycobacterial 16S ribosomal RNA gene. A small target sequence within this gene is amplified (billions of copies are made) using PCR. Because of small differences in the 16S ribosomal gene, the primers are specific to organisms only of the *M. tuberculosis* complex (*M. tuberculosis, M. bovis, M. africanum, M. microti*) and only these species are detected. The AMTD system amplifies the ribosomal RNA through a method termed Transcription Mediated Amplification (TMA). Both amplification products are detected through fluorescent probes against the generated nucleic acids.1,11

**Indirect detection methods**

The standard tuberculin test is the simplest measure of cellular reactivity against mycobacterial antigens. A small aliquot of mycobacterial antigen is injected intradermally and the induration palpated or measured at 48 hr (humans) or 72 hr (humans, animals). Induration results from a local influx of lymphocytes because of cell mediated immunity. Estimates of the sensitivity of skin testing range from 68-95% and specificity is estimated to be 96-99% in cattle.12 The efficacy of the intradermal test has not been established for the vast majority of wildlife species. Results vary between species and test sites and clinicopathological correlation with test results is often lacking.8,9,10,15 In addition, tuberculin testing practices vary widely between zoos.13 Differences include the tuberculin preparation used, antigen strength, injection site and interpretation of results.

The Blood TB Test (BTB) is currently based on two assays, a lymphocyte transformation assay and an ELISA to measure antibody formation against antigens (see below). Lymphocyte transformation is measured by incubating lymphocytes with mycobacterial antigens, typically 1 µg of PPD derived from *M. bovis*, in the presence of radiolabeled thymidine (a nucleic acid precursor). T-lymphocyte populations with prior memory of an antigen will "expand," (i.e., begin to undergo cell division) and take up thymidine into newly synthesized DNA. The amount of radioactive uptake is then compared between PPD-stimulated and non-stimulated cells. The BTB test shows a sensitivity of 95% and a specificity of 92% in deer herds harboring mixed *M. bovis* and *M. avium* infections.7 Sensitivity and specificity in other species remains to be demonstrated.

ELISA measures antibody formation against specific antigens. The test is performed by adding serum to a well of a microtiter plate that contains the test antigen. Antibody against the antigen will bind to the antigen (protein, lipid, glycolipid, etc.) and remain after washing. The adherent antibody is then detected by a fluorescent tagged antibody against the animal’s antibody (for example, antibodies raised in goats against cattle IgG). ELISA shows a sensitivity 65.6% and a specificity of 56.4% in cattle.6 The test appears useful in detecting seriously infected deer but may fail to identify low grade infections.5 Positive ELISA reactions were observed in llamas 8 wk following exposure to killed *M. bovis*.16

Two technical factors limit the application of ELISA. First, unless antibodies against the animals being tested are available, detection is severely limited (there are no commercially available anti-elephant antibodies) since cross-reactivity between species is limited. Secondly, the antigen against which antibodies are measured must be chosen carefully. For example, the BTB ELISA test
measures antibodies against MPB70, a protein specific for *M. bovis*, and will not be able to detect the presence of antibodies against *M. tuberculosis*.

The gamma interferon test, accredited by the Standing Committee on Agriculture in Australia measures the release of a cytokine gamma interferon following exposure of peripheral blood mononuclear cells to mycobacterial antigens, typically PPD-bovis. A recent modification has examined the utility of detecting gamma interferon and interleukin-2 messenger RNA induced in response to PPD-bovis exposure using reverse transcription quantitative competitive (RT-qc) PCR. These cytokines are elevated in TB exposed humans who have mounted a protective immune response. Depressed levels of gamma interferon and interleukin-2 correlate with active lesions. (Suzanne Kennedy-Stoskopf, personal communication).

Last, various mycobacterial antigens have been directly measured from the blood as an indirect marker of infection. These include the *M. bovis* specific surface phenolic glycolipid (PGL) that has been shown to be able to detect experimental infection in cattle and the glycopeptide (GPL) specific to *M. avium* and *M. leprae* reported as being able to detect leprosy and *M. avium* infection in humans.

**Caveats for use in clinical situations**

Aside from the experience of skin testing, BTB test and ELISA measurements of antibody production in cattle and captive Cervidae, little data exists on the utility of these test for other species of animals. Further, none of the other methods of detection have been validated for any animal species. Until any of the tests are accepted for use in non-domestic species, careful validation ideally based on results from histology and culture at the time of necropsy is mandated.

**LITERATURE CITED**

A NEW MODIFIED LIVE VIRUS VACCINE FOR ENCEPHALOMYOCARDITIS (EMC) VIRUS PROTECTION, PRELIMINARY TRIALS AT THE AUDUBON ZOO

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Abstract

Encephalomyocarditis virus, (EMCV) has caused the deaths of many species of animals in southern zoological parks and research institutions.2,7 The Audubon Park Zoo was one of the first zoos to report an EMCV epizootic in it’s animal collection.7 Since this outbreak in the mid 1980’s, EMCV had not struck animals at the Audubon Zoo, until fall 1996. The recent outbreak has again subsided, but with current research being performed on Mengovirus an antigenically identical close relative of EMCV, attempts were again made to vaccinate the Audubon Zoo’s collection against EMC virus. Primates and other species were vaccinated with a genetically engineered Mengovirus during their annual physical exams. Serum samples taken at the time of vaccination and 21 days later were submitted for serum neutralization titers to EMCV. Preliminary results on two species of primates; black howler monkey (Alouatta caraya), talapoin monkey (Cercopithecus talapoin), tapir (Tapirus terrestris), and babirusa (Babyrousa babyrussa) showed elevated titers 21 days post vaccination for talapoin monkeys and some elevation in titers for howler monkey and tapirs who had pre-existing titers. No ill effects have been seen with vaccination.

Introduction

EMC virus is a member of the family Picornaviridae. The genus Cardiovirus contains EMCV, Mengovirus and MM all of which are antigenically indistinct.8 Transmission and maintenance of the virus is thought to be through murine rodents. The past history at the Audubon Zoo has been consistent with this theory.7,8 It is felt that the recent outbreak has again been associated with rodents, through disturbance of numerous rat nests due to construction on Zoo grounds.

Disease caused by EMCV is peracute, with anorexia and depression seen for 12-24 hr before death.1 Often death is the first visible sign.8 Gross lesions are limited to the cardiopulmonary system. Findings include hydropericardium, pale streaks throughout the myocardium, and pulmonary edema.1 Histologically lesions may show massive cardiac myocyte necrosis with edema and lymphocytic, plasmacytic and histocyte infiltrates. Pulmonary lesions of edema and congestion are secondary to acute cardiac failure.1

The viruses of the genus Cardiovirus contain a unique sequence on homopolymeric polyribocytidylate segments that may contain from 60 to 420 pyrimidine nucleotide residues in a
These poly (C) tracts and their length have been directly related to the virus’s pathogenicity.\textsuperscript{5} Genetically engineered Mengoviruses with shortened poly (C) tracts were dramatically attenuated and steadfastly maintained their artificially truncated sequences during serial tissue culture passage.\textsuperscript{6} Laboratory mice receiving the short tract strains developed life-long protective immunity against normally lethal challenge with wildtype cardioviruses.\textsuperscript{5} Vaccination with various length short poly (C) Mengoviruses and subsequent virulent challenge with wildtype EMC virus has successfully protected mice, macaques, baboons and domestic swine.\textsuperscript{4}

**Methods and Results**

Selected animals in the collection were injected during their annual physical exams with $5.0 \times 10^6$ PFU (plaque forming units) of a genetically engineered Mengovirus (vMC0). Serum samples were collected at time of vaccination and 21 days later. Serum samples were tested by two separate laboratories by serum neutralization.\textsuperscript{2,4} (Table 1) Overall results were positive with a rising titer 21 days after vaccination. No change was seen in some animals, it is not known whether the vaccine was ineffective or if more time was needed to see a measurable titer increase. An occasional animal appeared to have a preexisting titer to EMCV. A commercial version of this virus (vMC0), if available, may have efficacy for protection against EMCV caused zoo animal mortality.

**ACKNOWLEDGMENTS**

The authors would like to thank Jody Joyner, RVT, Sean MacConnell and the Audubon Park Zoo’s Mammal Department for their assistance.

**LITERATURE CITED**

Table 1. Pre-existing and 21-day post vaccination titers by and serum neutralization (SN) assays to (vMC0) vaccination in various species.

<table>
<thead>
<tr>
<th>Species, animal #</th>
<th>SN1a</th>
<th>SN2b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time 0</td>
<td>Time 21</td>
</tr>
<tr>
<td>Talapoin 1</td>
<td>Neg</td>
<td>1:80</td>
</tr>
<tr>
<td>Talapoin 2</td>
<td>Neg</td>
<td>1:160</td>
</tr>
<tr>
<td>Talapoin 3</td>
<td>Neg</td>
<td>1:160</td>
</tr>
<tr>
<td>Talapoin 4</td>
<td>Neg</td>
<td>&gt;1:320</td>
</tr>
<tr>
<td>Talapoin 5</td>
<td>Neg</td>
<td>1:80</td>
</tr>
<tr>
<td>Talapoin 6</td>
<td>Neg</td>
<td>1:40</td>
</tr>
<tr>
<td>B. Howler</td>
<td>&gt;10</td>
<td>&gt;320</td>
</tr>
<tr>
<td>Tapir 1</td>
<td>&gt;1:10</td>
<td>1:120</td>
</tr>
<tr>
<td>Tapir 2</td>
<td>&gt;1:10</td>
<td>&gt;1:320</td>
</tr>
<tr>
<td>Tapir 3</td>
<td>&gt;1:10</td>
<td>&gt;1:320</td>
</tr>
<tr>
<td>Babirusa</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>

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MANUFLEX® - AN ALTERNATIVE WAY OF TREATING BONE FRACTURES IN ZOO AND WILD ANIMALS?

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Abstract

In a survey of the quality and the clinical use of Manuflex®, an external fixator technique from Hungary (Trade-Coop Trading House for Industrial Cooperative Societies, LTD, Budapest 95 P.O. Box 20 H-1456 Hungary), this cheap and very light fixating method, composed of Kirschner-wire and a perforated aluminum bar, was used in several wild, exotic and companion animals, but especially in birds presenting with comminuted fractures.

The advantages and disadvantages of this fixation device have been judged and compared to other methods, according to the application facility, the technique, the fixation features, the healing process, the acceptance, as well as the restriction of movement for the animals. Over 4 yr, several birds, rodents, cats and smaller dogs have been treated with the external fixation, using the Manuflex®, for fractures of the humerus, radius and ulna, tibiotarsus, metatarsus, and in one case, the lower beak.

The use of the Manuflex® for fracture fixation seems to be very efficient. On one hand, the material is very light and of easy and rapid use. On the other hand, the use of aluminum and Kirschner-wires is of low cost. However during the healing period some of the animals showed complications, such as osteolysis or dislocation of bone fragments, as well as bending and instability or extraction of the fixator (Table 1). In these cases the stability and efficiency of the fixation with the Manuflex® seemed to depend on the weight and size of the animal or on the age of the fracture at the time of the surgery. Overall the Manuflex® appeared to be a recommendable alternative in the treatment of fractures of the extremity especially in birds; but, using larger aluminum bars, also in other animals.

LITERATURE CITED

Table 1. Treated bird species, location of the fractures, type of fixation and healing process.

<table>
<thead>
<tr>
<th>Species</th>
<th>Fracture</th>
<th>Fixation with Manuflex</th>
<th>Healing process</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common buzzard (Buteo buteo)</td>
<td>Humerus ri, diaphysary, oblique; Metatarsus le, splinter</td>
<td>ri: type 1-fixator le: type 2-fixator</td>
<td>both taken off after 8 wk released</td>
<td></td>
</tr>
<tr>
<td>Common buzzard (Buteo buteo)</td>
<td>Radius / Ulna le, diaphysary, oblique; Head trauma</td>
<td>type 1</td>
<td>euthanasia after 2 wk because of central blindness</td>
<td></td>
</tr>
<tr>
<td>Common buzzard (Buteo buteo)</td>
<td>Humerus le, diaphysary, oblique</td>
<td>type 1</td>
<td>taken off after 8 wk released</td>
<td></td>
</tr>
<tr>
<td>Common buzzard (Buteo buteo)</td>
<td>Humerus ri, diaphysary, oblique; Radius/Ulna le, diaphysary, splinter</td>
<td>ri: type 1 le: type 2</td>
<td>exitus 6 hr post op.</td>
<td></td>
</tr>
<tr>
<td>Common buzzard (Buteo buteo)</td>
<td>Metatarsus le, diaphysary, splinter</td>
<td>type 2</td>
<td>sequestration of a splinter, removed, filled up with bovine spongiosa, taken off after 16 wk released</td>
<td></td>
</tr>
<tr>
<td>Common buzzard (Buteo buteo)</td>
<td>Radius/Ulna le, diaphysary, splinter</td>
<td>type 1</td>
<td>taken off after 9 wk released</td>
<td></td>
</tr>
<tr>
<td>Common buzzard (Buteo buteo)</td>
<td>Radius/Ulna ri, diaphysary, splinter</td>
<td>type 1</td>
<td>refixation because of further splintering and instability, further stabilization with a intramedullary pin on the fixator, taken off after 16 wk released</td>
<td></td>
</tr>
<tr>
<td>Old World kestrel (Falco tinnunculus)</td>
<td>Tibiotarsus le, diaphysary, splinter</td>
<td>type 2</td>
<td>taken off after 10 wk volière</td>
<td></td>
</tr>
<tr>
<td>Barn owl (Tyto alba)</td>
<td>Tibiotarsus ri, proximal, oblique; Metatarsus le, diaphysary, splinter</td>
<td>ri: type 2 le: type 2</td>
<td>ri: taken off after 8 wk le: taken off after 6 wk refractured, new fixation, taken off after 8 wk</td>
<td>volière</td>
</tr>
<tr>
<td>Golden eagle (Aquila chrysaetos)</td>
<td>Tibia, malformation after fracture</td>
<td>type 2</td>
<td>not efficient, new fixation, infection of soft tissue died after 12 wk because of infection</td>
<td></td>
</tr>
<tr>
<td>Goose of Toulouse (Anser anser domesticus toulousiensis)</td>
<td>lower beak fractured and destroyed</td>
<td>type 1(beak contour)</td>
<td>taken off after 10 wk</td>
<td>Beak horn regrown badly, eats well</td>
</tr>
<tr>
<td>Rook (Corvus frugilegus)</td>
<td>Radius / Ulna le, diaphysary, oblique; Elbow luxation le</td>
<td>type 1</td>
<td>euthanasia after 4 wk, since elbow relaxation</td>
<td></td>
</tr>
<tr>
<td>Fennec fox (Fennecus zerda)</td>
<td>Femur diaphysary, splinter</td>
<td>type 1, in combination with intramedullary pin</td>
<td>taken off after</td>
<td>healed</td>
</tr>
<tr>
<td>Chinchilla (Chinchilla laniger)</td>
<td>Tibia diaphysary, splinter</td>
<td>first intramedullary pin, because of instability manuflex, type 2</td>
<td></td>
<td>euthanasia after 5 wk because of automutilation</td>
</tr>
</tbody>
</table>

Abbreviations : le-left; ri-right; hr-hours; post op-post operation.
SALMONELLOSIS (*Salmonella enterica*) IN A GROUP OF CAPTIVE BLACK RHINOCEROS (*Diceros bicornis*)

**Jeff Baier, MS, DVM* and David E. Kenny, VMD**

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Abstract

Salmonellosis caused by *Salmonella enterica subsp. arizonae* serotype 44:Z4,Z32 (formerly known as *Salmonella arizonae*) was diagnosed in three of five captive black rhinoceroses maintained at the Denver Zoological Gardens. Two animals died despite supportive treatment. Another two rhinoceroses remained asymptomatic and were negative for *Salmonella* spp. after serial fecal cultures. The source for the salmonellosis was never definitely established.

Introduction

Three of five black rhinoceroses (*Diceros bicornis*) at the Denver Zoological Gardens (DZG) contracted *Salmonella enterica subsp. arizonae* serotype 44:Z4,Z32. The signal case was a female that presented with lethargy and partial anorexia over a 1-mo period. *Salmonella enterica subsp. arizonae* serotype 44:Z4,Z32 was isolated from a nasal swab. Over the next several weeks two male rhinoceroses developed diarrhea and had positive fecal cultures for *Salmonella enterica subsp. arizonae* serotype 44:Z4,Z32.

Black rhinoceroses at the DZG are housed in the pachyderm building, which has two indoor rhinoceros exhibits, three off-exhibit holding stalls and an outside exhibit yard. The holding stalls are positioned side-by-side and sloped from west to east. The slope of the floor allows fecal material hosed from the west stall to drain through the other two stalls prior to reaching a drain during cleaning. In addition to black rhinoceroses, the building also houses hippopotamuses (*Hippopotamus amphibius*), rock hyraxes (*Procavia capensis*), Malayan tapirs (*Tapirus indicus*), and Asian elephants (*Elephas maximus*). This report will discuss the diagnosis, clinical features, antibiotic therapy, and outcome of salmonellosis in three captive black rhinoceroses.

Case Reports

**Case 1**: A 24-yr-old female black rhinoceros, weighing approximately 900 kg, developed lethargy, partial anorexia and decreased water consumption which continued intermittently for 1 mo. After epistaxis was noted in the right naris, the rhinoceros was immobilized with 2.5 mg etorphine (M99, Lemmon Co., Sellersville, PA 18960 USA) administered i.m. via pole syringe. A 1-cm-diameter ulcer was found just inside the right nostril and swabbed for bacterial culture. Blood was obtained for hemogram and serum biochemistry. Treatment consisted of 20.1 × 10^6 units of penicillin G benzathine and penicillin G procaine, 9000 International Units (IU) vitamin E (Vital E TM-300, Schering-Plough Animal Health, Kenilworth, NJ 07033 USA), and 20 mg dexamethasone (Vedco
Two days later 100 mg vitamin K1 (Veda-K1, Vedco Inc.) and 10 ml Multi B complex (Phoenix Pharmaceutical Inc., St. Joseph, MO 64506 USA) were administered i.m. Trimethoprim and sulfamethoxazole (TMS) 26880 mg (Geneva Pharmaceuticals, Inc., Broomfield, CO 80020 USA) were administered p.o. s.i.d. for 2 mo. Two weeks later, the rhinoceros became weaker, ataxic, and developed a purulent vaginal discharge. It was immobilized again to obtain blood for hemogram, serum biochemistry and culture. The vaginal discharge was also cultured. In addition, 5 L of lactated Ringer’s and 5% dextrose (LRS & 5% dex) were administered i.v. Three hundred and sixty milliliters of 0.2% nitrofurazone and 4 L of saline were infused into the uterus through a sterile catheter. Fifty-seven milliliters of trimethoprim sulfadiazine (80 mg trimethoprim and 400 mg sulfadiazine/ml [48%], Mortar & Pestle Pharmacy, Des Moines, IA 50310 USA) diluted in an equal volume of sterile water, 9750 mg amikacin (Amiglyde-V®, Fort Dodge Laboratories Inc., Fort Dodge, IA 50501 USA), 20 ml Multi B complex, 100 mg vitamin K1, 9000 IU vitamin E, 100 units oxytocin (Phoenix) and 25 mg prostaglandin F2 alpha (Lutalyse,® The Upjohn Co., Kalamazoo, MI 49001 USA) were administered i.m.

The rhinoceros’ clinical condition continued to deteriorate. Cutaneous ulcers developed on the back, neck, face, and extremities. The animal appeared to be painful and groaned when it rose. Flunixin meglumine (Banamine,® Schering-Plough Animal Health Corp.) was administered 1 g, p.o., daily for 5 days. The caudal surface of the right hock became ulcerated and exuded an odoriferous, purulent material. Four and one-half months after the initial clinical signs, the rhinoceros was found dead.

**Case 2:** A 23-yr-old male rhinoceros, weighing approximately 1140 kg, developed diarrhea. It had been housed in the stall adjacent to Case 1. This animal developed diarrhea 2 mo after Case 1. Four fecal cultures were obtained over a 1-wk period. *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32* was isolated. The flagellar (H) antigens were identical to those found in Case 1, but the somatic (O) antigen was untypeable. Treatment consisted of oral TMS (34200 mg) s.i.d. Two days later the rhinoceros became totally anorexic. Daily injections of 1.0 g ceftiofur (Naxcel,® SmithKline Beecham Corp., Philadelphia, PA 19101 USA) were administered for 17 days i.m. via pole syringe. Two weeks after onset of clinical signs, it was immobilized with 1.5 mg etorphine i.m. for hemogram, serum biochemistry and blood culture. Six liters of LRS and 3 L of LRS & 5% dex were administered i.v. Two grams of ceftiofur, 70 ml TMS 48% diluted in an equal volume of sterile water, 10 ml Multi B complex, 6000 IU vitamin E were administered i.m., and 1 g banamine was given i.v. Recovery was violent, with the rhinoceros slamming its head on the concrete floor as it struggled to rise.

Four weeks after the onset of diarrhea, the rhinoceros was found moribund. Blood was taken prior to euthanasia with 600 mg succinylcholine (Abbott Laboratories, North Chicago, IL 60064 USA) and 20 ml pentobarbital (6 g/ml) (Anpro Pharmaceutical, Arcadia, CA 91006 USA) administered i.v.

**Case 3:** A 13-mo-old male rhinoceros, weighing approximately 410 kg, and housed adjacent to Case
2, developed diarrhea. Fecal culture was positive for *Salmonella enterica* subsp. *arizonae* serotype 44:Z4,Z32, with the same flagellar and somatic antigen pattern as found in Case 2. It was given 12480 mg TMS p.o. s.i.d. for 3 wk. The stool began to become firm within 24 hr. *Salmonella enterica* subsp. *arizonae* serotype 44:Z4,Z32 has not been isolated on further fecal cultures. The animal has remained healthy since that time.

**Bacteriology**

Bacterial culture samples were transported to the Denver Zoo Hospital’s diagnostic laboratory on transport swabs (Culturette®, BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD 21030 USA). The swabs were streaked on blood agar (TSA w/5% sheep blood), MacConkey agar and hektoen plates (Remel Microbiology Products, Lenexa, KS 66215 USA) and incubated at 35°C for 24 hr. To improve recovery rates, the samples were also placed in gram-negative enrichment broth (GN Broth, Remel), incubated for 24 hr and subcultured onto the three aforementioned types of agar. Blood for culture was placed in brain heart infusion media (Becton Dickinson) and incubated at 35 °C for 1 wk. One sample was maintained under anaerobic conditions with the top screwed closed and the second under aerobic conditions with the top vented. A duplicate set of blood cultures was sent to the Colorado State University (CSU) Diagnostic Laboratory.

Bacterial identification was made utilizing the BBL Crystal Enteric/NLF ID panels (Becton Dickinson). Isolates identified as a *Salmonella* spp. were sent to CSU in Port-A-Cul® transport tubes (Becton Dickinson) for confirmation. Confirmed samples were forwarded to the National Veterinary Services Laboratories (NVSL) for additional confirmation and serotyping. NVSL utilized the Kauffman-White scheme for serotype identification.

The nasal, vaginal and blood cultures from Case 1 contained isolates of *Salmonella enterica* subsp. *arizonae* serotype 44:Z4,Z32. A culture of thoracic fluid obtained from Case 1 contained isolates of *Salmonella enterica* subsp. *arizonae* serotype 44:Z4,Z32. Fecal cultures obtained from Case 2 and Case 3 contained isolates of *Salmonella enterica* subsp. *arizonae* serotype 44:Z4,Z32. Antemortem blood culture and postmortem culture of intestinal contents obtained from Case 2 contained no isolates of *Salmonella*.

Approximately 3.5 mo after the identification of salmonellosis in Case 1, a male rock hyrax housed in the pachyderm building developed a facial abscess. Culture samples from this abscess contained isolates of *Salmonella enterica* subsp. *arizonae* serotype 44:Z4,Z32. The hyrax subsequently died 2 wk later. Fecal cultures were obtained on the remaining animals housed in the pachyderm building every 2 wk for the next 4 mo. No additional isolates of *Salmonella* were recovered.

**Gross Necropsy and Histopathology**

Case 1 had multiple areas of healing cutaneous ulcers along the backbone and on all four extremities. A 1.5-cm-diameter ulcer was present on the caudal surface of the right hock, exposing the extensor tendons. A 10 × 15 cm ulcer was noted on the caudal surface of the left elbow. Both lesions were...
thought to be due to pressure necrosis. The thoracic cavity contained a large volume of gray/black flocculent, odoriferous material. Generalized pleuritis was present and multifocal adhesions were present between the lungs and thoracic wall. The right dorsal caudal lung lobe contained a 25 × 35 cm abscess. Three gastric ulcers were present. No pericardial, perirenal or abdominal fat was present. The third digits of the left and right hind feet contained hemorrhage in the soft tissues adjacent to the distal phalanges.

Microscopically, neutrophils and histiocytes were aggregated throughout the lung tissue associated with numerous bacterial microcolonies. A distal phalanx submitted for histopathology had mild superficial bony resorption and mild epithelial hyperplasia of the dermal laminae. Moderate to marked hemosiderosis was present in the lung, liver and gastrointestinal tract.

Case 2 had multifocal areas of traumatically-induced excoriation on the face, elbows and hocks. The skin over the vertebrae was peeling. A superficial ulcer was also found on the caudal aspect of the left hock. Ulcers of the soles of both hind feet with necrosis on the lateral aspect of the second digits were also found. The coronary bands of these digits were also erythematous. As in Case 1, no abdominal, pericardial perirenal fat was noted. The mucosa of the stomach was hemorrhagic, ulcerated, and contained foul-smelling red liquid. The small and large intestines also contained blood.

Microscopically, multifocal areas of mild lymphoplasmacytic gastritis and mild catarrhal enteritis were noted. Mild hemosiderin deposition was present in lung, kidney, liver, spleen, heart, colon, and pancreas. Marked accumulations of hemosiderin deposition were noted in a visceral lymph node. The kidneys contained multifocal areas of interstitial fibrosis and tubular atrophy.

**Discussion**

Possible sources of *Salmonella* include contaminated water and nutritional supplements of animal origin, such as bone, fish or feather meal. It has been estimated that 40% of feed products of animal origin are contaminated with *Salmonella*. Infected animals are often a source of infection for other animals through fecal shedding. Other sources of *Salmonella* that have been reported and would need to be considered for this outbreak are rodents, birds, insects and reptiles. Reptiles are a common reservoir for *Salmonella* species. Tokay geckos (*Gecko gecko*) living in the building were suspected as being a possible source for infection but a direct causal relationship could not be proven. Tokay geckos had previously been released into the pachyderm building for insect control. Two geckos were subsequently captured, euthanatized and had culture samples obtained from the intestinal tracts. *Salmonella eastbourne* was isolated, but *Salmonella enterica subsp. arizonae serotype 44:Z4,Z32* was not isolated.

Case 1 was probably shedding large numbers of *Salmonella* and contaminated the adjacent stalls, exposing the two males. To prevent exposure of the other animals, the building was quarantined and movement of animals and keepers within the building restricted. Foot baths were installed and coveralls, boots, and cleaning tools were dedicated to the area. After control measures were
implemented, with the exception of the rock hyrax, no additional cases of Salmonella enterica subsp. arizonae serotype 44:Z4,Z32 occurred. Although it did not lead to identification of a causal relationship in this case, serotypic level identification of Salmonella isolates is necessary in determining the source of the infection and planning control strategies.

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LITERATURE CITED

URETERAL AND INTESTINAL OBSTRUCTION ASSOCIATED WITH ABDOMINAL FAT NECROSIS IN A HERD OF ELD’S DEER: SUSPECTED FESCUE TOXICOSIS

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Abstract

Between 1994 and 1996, two adult (6 and 7-yr-old) female Burmese brow-antlered deer, or Eld’s deer (*Cervus eldi thamin*), housed at the Conservation and Research Center (CRC) in Front Royal, VA died acutely with no premonitory clinical signs. At necropsy, both animals had large masses of firm, mineralized perirenal and sublumbar adipose tissue that partially enveloped each kidney. The masses surrounded and constricted the ureters bilaterally, causing hydroureter and hydronephrosis. A third animal, a 6-yr-old female, presented to the veterinary hospital with anorexia and depression of 2 days’ duration. Loose, tarry feces and frank blood were present on the tail and perineum. Abdominal palpation and radiographs indicated the presence of large firm masses within the abdomen, and serum chemistry showed a markedly elevated BUN (270 mg/dL) and creatinine (16.9 mg/dL). An exploratory laparotomy revealed the abdominal cavity to be filled with very large, firm white masses that adhered to most of the abdominal organs and apparently obstructed the gastrointestinal tract. The animal was euthanatized, and necropsy confirmed large masses of mineralized necrotic sublumbar and perirenal fat constricting the ureters bilaterally was well as the intestinal tract at the cecocolic junction, resulting in bilateral hydronephrosis and cecal impaction.

A retrospective study of Eld’s deer deaths at CRC from 1975 to 1996 (including those described above) revealed that, of 45 deaths of Eld’s deer housed at CRC for 2 yr or more, 11 (24.4%) had necrotic fat at necropsy. Six affected animals were female and five were male. Five of those affected had uni- or bilateral hydronephrosis associated with ureteral constriction by masses of necrotic fat, and four of the five with hydronephrosis were female. Since 1994, there have been 11 Eld’s deer deaths, and of these, seven (63.3%) had necrotic fat and four had associated hydronephrosis.

Physical examination and radiographic screening of the CRC Eld’s deer population were subsequently conducted to assess the prevalence of abnormal fat in the remaining population. Of 16 female Eld’s deer (3-15-yr-old), 15 (93.75%) had palpable firm sublumbar masses. Thirteen of the 15 masses were also visualized radiographically, and 9 appeared mineralized. Only one of seven male Eld’s deer screened (9-15-yr-old) had palpable firm abdominal masses, which were not visible radiographically. Abnormal fat was not detectable in animals of either sex under the age of 3 yr. Laparoscopic evaluation confirmed the presence of firm masses of sublumbar fat in all females...
diagnosed with abnormal fat by palpation and/or radiography. Males were not examined laparoscopically. Fat biopsies obtained laparoscopically demonstrated histologic changes similar to those observed in necropsy cases and included extensive coagulative necrosis of adipocytes with foci of saponification, fibrous tissue proliferation and occasionally focal osseous metaplasia. Inflammation was not a prominent feature in any of the cases.

The lesions in these animals are similar to those described in cattle on endophyte-infected fescue pasture.\textsuperscript{1} Fat necrosis has been reported in a wide variety of species, and it is believed to occur by different pathological mechanisms. In cattle, fat necrosis is associated with several factors: 1) obesity, 2) breed predisposition, 3) nitrogen-fertilized grasses, and 4) tall fescue (\textit{Festuca arundinacea}) forage infected with an endophyte fungus, \textit{Acremonium coenophialum}.

The CRC Eld’s deer herd has been segregated by gender and kept on pasture from March to November since 1988. Females are rotated on two pastures totaling approximately 4 acres, consisting primarily of tall fescue, orchardgrass, bluegrass, red clover, crowned vetch and alfalfa. Particularly during the cool season, tall fescue appears to be the predominant grass in the pasture, and the deer have been observed to eat tall fescue over other available grasses. During the winter months, the animals are housed indoors and fed alfalfa and a pelleted diet consisting of wheat middlings, soybean hulls, alfalfa, corn, cane molasses, beet pulp, soybean oil and vitamin/mineral supplements. The pelleted diet contains 12.5\% crude protein, 3.0\% crude fat and 21\% crude fiber.

Fescue samples from several CRC pastures, including Eld’s deer pastures, were confirmed to be highly infected with the endophyte fungus by the Auburn University Fescue Toxicity Diagnostic Center.

Similar lesions of fat necrosis, or lipomatosis, have been reported in other Asian cervid species including swamp,\textsuperscript{2} sika and sambar deer.\textsuperscript{3} In each report, the etiology of the fat necrosis was not determined, but diet was suggested as a likely cause. While age, diet and genetic predisposition may be contributing factors, the level of endophyte infection in the resident tall fescue and the increasing incidence of the problem suggest that, as in cattle, endophyte-infected tall fescue forage has played a role in the development of necrotic fat in these Eld’s deer. Moderately infected tall fescue pastures tend to become increasingly infected over time because the infected plant is, paradoxically, more insect-, drought- and disease-resistant than the noninfected plant.\textsuperscript{4-8} Therefore, it is likely that this herd has been exposed to increasing levels of infected forage over the past 9 yr.

Further studies are being conducted to determine the nature and cause of abdominal fat necrosis in Eld’s deer at CRC. In early summer 1996, the Eld’s deer pastures were treated with herbicide and reseeded with switchgrass, orchardgrass and alfalfa. Since this time, there have been no clinical developments attributed to fat necrosis in the Eld’s deer herd.
LITERATURE CITED


GIRAFFE DYSTOCIA: A RETROSPECTIVE SURVEY AND FOUR POSTERIOR PRESENTATION CASES

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Abstract

Four posterior presentations have occurred among 14 giraffe births at Albuquerque Biological Park (ABP) from 1977-1997. Thirty-four other dystocia cases were identified from a survey of North American zoological institutions for the same years. These 38 cases varied in clinical severity from mild, self-correcting problems to major life-threatening conditions, including eight fatalities among the dams. The cases were grouped into three clinical categories which reflected the complexity of the dystocia and relative risk to the dam. The first group of 15 complex cases required full restraint of the dam, obstetrical manipulation of the calf, and had significant risk for the dam. The second group of seven intermediate cases were managed by no restraint or brief chemical restraint of the dam, were resolved by simple traction without obstetrical exam, and had moderate risk for the dam. The third group of 16 uncomplicated cases were managed with no restraint or brief chute restraint of the dam, had little or no obstetrical work, and minimal risk for the dam. Use of a standing restraint chute to control the dam greatly increased its chance of survival when compared with chemical immobilization to resolve dystocia. Prolonged second stage of parturition, referred to as delayed labor, may be confused with dystocia in deliveries extending beyond 4 hr.

Introduction

Reproduction and parturition in giraffe are described in general terms in the literature.2,4 Some unusual characteristics include a long gestation of 15 mo and birthing from a standing position by the parturient dam. Births occur in all months of the year, most commonly during daylight hours, and normal fetal presentation is craniolongitudinal with dorsosacral positioning.3 Calving is normally completed within a few hours of the start of second stage labor2 and was reported in one study to vary between 20 min and 3.5 hr.4 There are few published reports of dystocia,3 yet four cases of posterior presentation occurred among 14 births at ABP between 1977 and 1997. Dystocia cases identified by a survey of North American zoological institutions and the cases from ABP, including observations of prolonged second stage labor, are presented in hopes of aiding in the evaluation of ongoing parturition and resolution of dystocia in giraffe.

Survey

Ninety-three North American zoological institutions house giraffe (all subspecies), according to the International Species Information System (ISIS) Abstracts. Seventy-one of these institutions responded to a questionnaire (76% response rate) and identified 34 additional cases of dystocia between 1977-1997. A follow-up questionnaire yielded more detailed information for each dystocia
case. The information presented here is from these cases and ABP experience. A search of ISIS data indicates 1254 giraffe were born in North America during the same years.\textsuperscript{6}

**Dystocia**

The 34 births reported as abnormal parturitions from the survey included 23 cases of fetal dystocia, three cases of maternal dystocia and eight reports which could not be classified as either a fetal or maternal abnormality. A number of factors determined how these cases were managed, including the type of dystocia, the perceived degree of risk to the dam or calf, tractability of the dam, prior reproductive history of the dam, the presence or absence of a restraint chute, and the relative risks of immobilization. ABP cases are described and classified similarly.

**Complex Cases:** These cases were managed by full restraint of the dam using chemical immobilization or a restraint chute. They required direct obstetrical exam and a corrective obstetrical procedure. Each case posed significant risk to the dam from physiological stress, injury, or prolonged restraint time. There were 14 reported cases of dystocia which fit this description. All were fetal dystocias of which 11 represented malposition or malpresentation abnormalities.

Eight of the complex cases required full chemical immobilization of the dam (seven using xylazine and etorphine\textsuperscript{1} and one with etorphine alone). The first five dams described in this series were immobilized after 6-8 hr of labor and three of the five dams died due to complications from the immobilization. The first case occurred after a fetal malposition (head flexion) was corrected and the calf pulled. During recovery the dam fell over backward, sustaining a cervical fracture, and later died. The second dam died at the conclusion of a difficult obstetrical procedure involving a limb flexion which could not be resolved by either repositioning or by fetotomy. A C-section was performed as a last resort, but the dam aspirated at the completion of the surgery. The third case in this series involved a dam that regurgitated at the conclusion of an immobilization to relieve a hip lock. The dam and calf initially survived the immobilization, but the dam later died from aspiration pneumonia and the calf was subsequently lost as well. The fourth and fifth cases were both successfully managed during immobilization, one required repelling the calf to straighten a flexed forelimb and the other to correct a minor malposition of the calf’s head. Although both calves were born alive, neither survived, but both dams returned to breeding.

The sixth case in this series, a dam with a posterior presentation, was immobilized after 24 hr of unsuccessful labor and a fetotomy was successfully performed. The seventh case involved a dam late in gestation that became briefly entrapped in electric fence wire. Thirteen days later a bloody vaginal discharge was noted along with periodic mild straining. Amniotic fluids were passed after an additional 4 days but contractions were mild and produced only more bloody fluid. The dam was immobilized 36 hr later and a fetotomy of a decomposing fetus with the head, neck and one forelimb flexed was attempted. The dam died 2.5 hr into the procedure. The final case in this series also was immobilized 36 hr after labor began and died of hyperthermia and myopathy after a dead calf was extracted. This calf had a severe malpresentation with 90 degree rotation and head flexion.\textsuperscript{3}
Four of the 14 complex cases were successfully managed by using a restraint chute to control the dam. One was a limb flexion, in which intervention was initiated after 2 hr of hard labor and both dam and calf survived. The second case was a dead calf, with the head and one limb flexed, which was removed by fetotomy after intervening 1 hr into labor, and the dam survived. The third case was another severe dystocia with head and neck flexion. Intervention occurred after 16 hr of labor and a fetotomy was successful in sparing the dam. Two of the three dams were sedated with xylazine while the third was not given any drugs. The fourth case involved a primiparous dam with a normal presentation. Two hours into labor, when there was no progress, oxytocin was given and repeated twice in the next hour. Standing sedation was initiated after 3 hr of labor, using xylazine, azaperone and detomidine, with the dam in a restraint chute. After 3 hr of unsuccessful attempts to pull the calf, the dam became recumbent in the chute and a stillborn calf was extracted. The dam stood up 2 hr later.

The final two complex cases were fatal to the dams before intervention could begin. One dam was found down in the morning with its rear legs splayed and the feet of the fetus projecting from the vulva. The head of the dead fetus was flexed and the dam died before a fetotomy could be performed. The second case presented as a 90 degree rotation of the fetus which delivered to the hips before progress ceased. The dam was unapproachable in the exhibit and fell on slick ground while attempting to swing the fetus to dislodge it. The dam’s rear legs splayed, resulting in a severe pelvic fracture, and both dam and calf died before intervention could be attempted.

**Intermediate Cases:** These cases were managed by either no restraint of the dam or by brief chemical restraint and required no direct obstetrical exam or corrective fetal manipulation. There was moderate risk to the dam from fatigue or chemical restraint. Five cases of dystocia fitting this description were reported. Three of these were posterior presentations and the other two were normal presentations.

The three posterior presentation cases included two dams that labored for 4 and 6 hr before becoming fatigued and were safely approached to pull the calves without restraint and without fetal correction. Both sets of dams and calves survived. The third case occurred in a tractable dam and was the only twinning reported in the survey. After more than 6 hr of unproductive labor a small calf (about 30 kg) was easily extracted by ropes secured to the feet. Soon thereafter a second set of hind hooves appeared and a twin of the same size was pulled. Apparently the fetuses had simultaneously wedged in the pelvic canal.

One case with anterior presentation was described as a shoulder lock. After 6 hr of labor the calf appeared dead, with an edematous head and neck protruding from the vulva. The head was lassoed and the calf was easily extracted when the dam stepped forward. The final case of normal presentation was in a dam which was recumbent from fatigue and apparent uterine inertia after 5 hr of labor. A live calf was easily pulled and survived but the dam remained recumbent and died during the night.

**Uncomplicated Cases:** These uncomplicated dystocias were managed by either no restraint of the
dam or by brief chute restraint. They involved no obstetrical exam or corrective fetal manipulation and there was minimal risk to the dam. Many were resolved by simple traction on the fetus and, in the remainder, birth was without physical intervention.

Fifteen cases of dystocia fitting this description were reported and 11 were anterior presentations. One dam was observed to have three feet and the head of a calf visible at the vulva after 3.5 hr of labor. An hour later the dam began running around the exhibit and eventually passed a 34-kg stillborn calf without assistance. Another dam, this one primiparous, gave birth after a 7-hr labor. At some point it was given an injection of oxytocin, which had an undetermined effect. Another three of the 11 cases in this series involved two different dams and were resolved by manual extraction of the calf relatively early in labor (2-4 hr) with no restraint of the dam. Early intervention was elected because of a previous stillbirth in each dam which was thought to have resulted from waiting too long. All three calves were pulled with relative ease and survived. The six remaining cases were resolved by extracting the calves with simple traction after 4-6.5 hr of labor. Three of the calves survived and three were stillborn. Two of the dams were placed in a restraint chute for the procedure and four were worked without restraint. Novel methods were used in some cases to remain at a safe distance from the dam, such as threading a rope through a section of hollow rigid tubing, leaving a loop at the end to secure the fetal extremities.

The remaining four uncomplicated cases were posterior presentations. Three of the four dams gave birth to surviving calves without intervention (one after 8 hr of labor, one after 6 hr of labor and the third had no data on the length of labor). The fourth dam was placed in a restraint chute 2 hr into labor and a live calf was easily pulled, which the mother subsequently reared.

**ABP Cases:** Four dystocias occurred between two reticulated giraffe dams. One case was in a wild-caught animal and the other three were from one of its offspring. The wild-caught dam had five consecutive normal presentation births over a 9-yr period with no problems and all were delivered within 2-4 hr of labor. The sixth birth, a complex case of posterior presentation, presented with two upside-down hoofs protruding from the vulva when the keeper arrived in the morning. No further progress occurred over the next 3.5 hr, with very hard straining during the last hour. Oxytocin (140 mg), given when the hard straining subsided, had no noticeable effect then, or when later repeated. Chemical immobilization began 6.5 hr after the labor was first seen, since there had been no progress in the delivery. The dam was confined by a crowding gate and then given xylazine (200 mg) followed by etorphine (4 mg). Obstetrical exam revealed a second degree vaginal tear and extensive contusions surrounding the urethral orifice, extending anteriorly for 18-20 cm. Three persons pulling obstetrical chains on the calf’s hind legs could not induce movement. Stronger force applied over 10 min by a modified calf puller successfully dislodged the hip lock and a 75-kg stillborn calf was delivered. The placenta separated by gentle traction over 20 min and the vaginal tear was closed using absorbable suture. The dam stood up 2 min after reversal of the etorphine. A complication occurred when the next calf was born 20 mo later. The initially healthy calf from a normal delivery died of hemolytic anemia at 7 days of age. The diagnosis was neonatal isoerythrolysis resulting from sensitization of the dam via the vaginal tear of the previous dystocia.
The other three dystocia cases were from an offspring of the previous dam. This animal had three posterior presentation deliveries out of five births in a 6.5-yr period during which it was bred back to its father. It later had three normal births and one abortion when bred to an unrelated male. The first dystocia, an uncomplicated case, had 6.5 hr of nonproductive labor with approximately 30 cm of the fetal extremities showing. Oxytocin (140 mg) was given, strong contractions were induced and the fetus progressed about 50 cm. Another oxytocin dose (100 mg) given 1 hr later induced strong contractions followed by birth of a stillborn calf 15 min later. The second dystocia was of intermediate complexity in that chemical restraint was needed. About 30 cm of fetal extremity protruded from the dam’s vulva after several hours of labor. Oxytocin (140 mg) induced about 30 cm of further progression before progress stopped. Since the dam could not be safely approached, it was then given xylazine (200 mg) followed by etorphine (4 mg), and the calf was quickly extracted by ropes placed on the hind legs just prior to the dam’s recumbency. The dam quickly regained its feet after reversal of the immobilizing drugs, but the calf died soon after the delivery. Nearly identical circumstances occurred with the third dystocia, although intervention started after 3.5 hr of labor. A lower dose of etorphine (2 mg) provided brief standing chemical restraint sufficient to pull a 75-kg live calf. Recovery was rapid after reversal of the etorphine and xylazine. This calf later required euthanasia for an unrelated problem.

Prolonged Second Stage of Parturition: Observations of a prolonged second stage of parturition (extending 4-6 hr or longer) in births not considered dystocias were reported from six other institutions and in one case at ABP. Two cases which were closely observed were seen to have two phases to the second stage of parturition. The first phase was a resting or nonproductive labor with mild to moderate contractions, usually of less than 1 min duration occurring at 10-15 min intervals or longer. This lasted 5 hr in an ABP case and nearly 7 hr in the other case. The dams remained calm, alert and exhibited no distress, even eating or chewing cud intermittently while the feet of the calf remained protruding from the vulva. Then the second phase (hard labor) began, characterized by strong braced contractions lasting 1-3 min and occurring at more frequent intervals (3-10 min). Birth occurred approximately 10 min after the head and shoulders were presented in the ABP case or once the hips were presented in the other case, which was a posterior presentation. This followed after about 60 min of hard labor in both cases. Three other live births, also not reported as dystocias, had labors of 6.5, 7.5 and 8 hr respectively with normal presentations of the fetus.

Discussion

Dystocia is defined as any abnormality in the progress of parturition, including the stages of cervical dilation, fetal expulsion and passage of fetal membranes, but it is more commonly understood to reflect a difficult labor during the phase of fetal expulsion. Although retained placenta is technically a dystocia, the survey was intended to more narrowly identify cases of abnormal second stage labor in which there was veterinary involvement. A posterior presentation birth in a ruminant is by its nature a fetal malpresentation even if the birth proceeds without assistance, thus all of these cases were included as dystocias. Observations on delayed second stage parturition were not specifically sought from the survey but were derived from the responses and correlated with APB experience.
The cases of dystocia reported here varied from minor self-correcting problems to life-threatening dystocias, including eight fatalities among the dams. The data suggest that some type of abnormality of parturition occurred in approximately 3% (38/1254) of captive giraffe births from ISIS-registered institutions in North America in the past 20 yr. About 1% of births (15/1254) were complex dystocias requiring a corrective obstetrical procedure with the dam under full restraint. Within these 15 complex cases, five of nine dams managed by chemical immobilization died, while all four dams managed in a restraint chute survived. Two other dams died prior to intervention. Calf survival from the complex dystocia cases was 2 of 15 (1 from an immobilized dam and 1 from a chute restrained dam). Compared to the complex cases, six of seven dams survived from the intermediate category, while three of eight calves lived. All 16 dams survived that had uncomplicated dystocia cases while 11 of the 16 calves survived.

The high incidence of posterior presentation at ABP probably represented individual predisposition to dystocia since three cases were from the same dam and the fourth was from its mother. Factors which may influence the occurrence of dystocia (such as inbreeding depression, nutrition, subclinical disease or management factors) could not be established from the data evaluated, although genetic predisposition was possible in the ABP cases.

A delay in the second stage of labor may be explained by the giraffe’s ability to postpone the birth process for many hours even after second stage labor has begun and the feet of the calf have appeared. This apparent adaptation to avoid predators in the wild may account for some of the labors of 4-6 hr or longer in captivity in which live births occur without assistance to the dam. Births monitored by human presence may stimulate a protective response in some dams and this was mentioned by one respondent as the cause of delay in a birth. This assessment of extended labor is based on limited observations yet best fits our experience and the data collected. One birth reported was a posterior presentation with a long resting period of 7 hr. Then 1 hr of hard labor produced a live calf without intervention. Another similar posterior presentation case delivered successfully after 6 hr. ABP experienced a labor of more than 6 hr with a 5-hr resting phase which produced a healthy calf. Differentiating a prolonged second stage labor from a true dystocia involving malpresentation or malposition will hopefully be enhanced from the information given here. Caution is warranted in assessing all cases, particularly if a restraint chute is not available while intervention is considered.

ACKNOWLEDGMENTS

The authors wish to thank all of the institutions and individuals who responded to the initial questionnaire. The complete list of respondents is regrettably too long to include. We especially acknowledge those institutions and individuals who provided data on dystocias (institutions are abbreviated and since the information is historical, individuals listed may not reflect their current place of employment): Atlanta (M. Crane), Baltimore (M. Bush), Baton Rouge (G. Pirie), St. Louis (B. Boever), Buffalo (A. Prowten), Caldwell (K. Reese), Calgary (B. Cooper), Chaffee (S. Lynch), Cheyenne Mun (D. Garrell, B. Cook), Cincinnati (K. Cameron), Detroit (D. Agnew), Ft. Wayne (K. Casserly), Gladys Porter (M. Willette-Frahm), Henry Doorly (D. Armstrong), Honolulu (B. Okimoto), Houston (J. Flanagan), Jacksonville (D. Page), Brookfield (J. Joslin), Kansas City (K. Suedmeyer), Lion Country (P. Wollenman, B. Lift), Los Angeles (G. Kuehn), Marine World (L. Gage), Pittsburgh (D. Neiffer), Reid Park (M. Flint), Riverbanks (N. Lamberski), and San Diego WAP
LITERATURE CITED

THE USE OF DIRECT CONTACT INFRARED IRRADIATION TO AID THE HEALING OF PRESSURE SORES IN ELEPHANTS (*Elephas maximus*)

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Abstract

Purpose

The purpose of this study was to determine if the treatment of chronic pressure sores on the hips of elephants with direct contact low level infrared therapy would stimulate the healing process, and if it did, to assess the quality and rate of healing.

Introduction

Decubital ulcers or pressure sores on the hips and other pressure-sensitive areas of the skin of elephants may develop when the animal lies on its side for extended periods of time. The weight of the animal may significantly decrease the circulation to various pressure spots, causing the skin and underlying connective tissue to devitalize, resulting in an open wound. If the animal continues to lay on the open lesion, the tissue is further damaged and deep ulcerations may result.

Treatment historically has been focused on solving the reason(s) the elephant may be spending more time down. Elephants reaching advanced ages or that have a foot or limb ailment may continue to lay down frequently regardless of the treatment. Rubber stall mats with extra bedding have been provided in an effort to alleviate some of the pressure to these wounds. Pressure-induced ulcerations in the skin have been kept clean, and a variety of topical antiseptic agents have been used with little success. Tissue growth factor Beta (TGFβ; Genetech, 460 Point San Bruno Blvd., South San Francisco, CA 94080 USA) has been used topically in the treatment of pressure sores with varying success.

Biostimulation of wounds of this type in humans with low level light in the infrared spectrum has been shown to accelerate the healing process by stimulating fibroblast production, quickening collagen synthesis, and enhancing the immune system to combat invading pathogens.1 A system using low level light infrared therapy (LLLT) (Equi-Light, 2100 South Dayton, Denver, CO 80231 USA) was designed and used successfully in horses. The treatment frequency, duration of therapy, and wound care were adapted to the elephant from equine protocols.

History

A 58-yr-old female Asian elephant (*Elephas maximus*), managed in a free contact system, had a
lengthy history of varying degrees of discomfort in all of its limbs. The left carpal joint became increasingly stiff starting approximately 14 yr ago. For the past 5 yr the right carpal joint has shown progressive signs of stiffness. This elephant has always tended to sleep more than the other elephants, but would lay on either side. Towards the end of 1995 evidence of lameness was exhibited in the right stifle joint. At this time the elephant would lay only on its left side.

Treatment

Two pressure sores developed in the tissue near the left ischial tuberosity. The wounds initially appeared as areas of devitalized tissue. The wounds were debrided and measured 10 × 7 cm and 0.5 × 0.5 cm at the time the treatment using the LLLT system began. Prior to each treatment the wounds were debrided, and any macerated or necrotic tissue was removed by gently scrubbing the area.

The LLLT system uses light energy created by light-producing diodes attached to a flexible pad. Each LLLT pad consists of 60 superluminous diodes that each produce 10 mw/cm² of infrared irradiation. Each pad is connected to a power source by a length of electrical wire. The pads were covered with a protective clear plastic wrap and were placed directly over the wound and attached to the surrounding skin with a wide cloth tape. The tape held the pads securely in direct contact with the wounds. The pads were arranged in such a way to allow the area being treated to be permeated with infrared radiation. After each treatment the pads were removed and a thin layer of 0.9% isotonic saline gel (Normlgel, Scott Health Care, Philadelphia, PA 19113 USA) was applied to keep the area moist. The lesions were treated initially for 30 min, with the time increased to 45 min daily for 60 days, and then every other day until the end of the experimental period. A trainer was present during the treatment times to ensure the elephant did not remove the pads. The elephant did not seem to react to the treatment.

Results

The first 4 wk proved to be a trial and learning period. The time of treatment was increased until the LLLT treatment caused the production of a clear serous fluid from the sores. Presence of this exudate had proven desirable in treating similar wounds in other species.

Not all of the devitalized tissue had been removed prior to irradiating in the first 2 wk and the wounds enlarged and deepened during this time. By the end of the third week the treatment techniques had been adjusted and the pressure sores began to fill in with healthy granulation tissue, and the wound margins began to close.

During the next 2 mo the size of the pressure sores continued to reduce in diameter. No infection or other problems were seen during that time. The healing wound margins were made up of a well vascularized connective tissue. Granulation tissue in both lesions was a deep red and the tissue was even with the epidermis. Three months after treatment began, the larger lesion measured 3.5 × 1.5 cm.
Discussion

The speed of healing was comparable to that in humans and other animals. Dressings on the wounds were unable to be maintained, and the wounds were frequently exposed to dirt and other contaminants. The lack of infection during the treatment period appeared to be significant. Irradiation of the pressure sores of this elephant seemed to promote significant granulation tissue, and allowed significantly faster resolution of these wounds when compared to similar lesions on other elephants.

LITERATURE CITED

TECHNIQUES FOR RADIOGRAPHING THE ELEPHANT FOOT AND CARPUS USING A PORTABLE EQUINE RADIOGRAPHIC UNIT

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Abstract

Diagnostic radiographs of the digits of the front and rear feet of Asian and African elephants have been obtained with a portable equine radiographic unit. Detailed films of the phalanges and metacarpal bones were obtained by using a setting of 15 mA and 80 kVP with a Fischer model FP-200 portable x-ray unit (H.G. Fischer, Inc., Franklin Park, IL; maximum output = 90 kVP and 20 Ma) and using Agfa green-sensitive rare earth all-plastic film cassettes (Agfa, 100 Challenger Road, Ridgefield Park, NJ 07660). The film/screen combination is 400 speed. Carpal radiographs have been obtained by securing a 14" × 17" rare earth film cassette to the limb of elephants trained and desensitized to allow this using a wide cloth tape. This method has proved superior to other methods of holding the cassette because of the lengthy exposure time, and the shape of the elephants’ limbs. While these films are not as detailed as those of the digits, the edges of the carpal bones can be assessed, and the joint spaces between the carpal bones can be evaluated.

It is critical to use the correct time setting. This varies with the size of the foot and may take some trials to achieve the optimal settings for each elephant. Adult elephants have distal phalangeal bones that are thin, fragile and attached to the toenail. They are best detailed using a 0.25- to 0.4-sec setting at 80 kVP and 15 mA. Diagnostic films of the proximal and middle phalanges may be obtained using the same technique with a 0.4- to 0.7-sec setting. The distal portion of the metacarpals may be radiographed by using a 0.6- to 0.9-sec setting, while the proximal edge of the metacarpals generally require an exposure time of 0.75-1.25 sec for optimum detail. These settings have been established with the elephant standing with its foot flat on the cassette, and the portable unit hand-held at an approximate 45 degree angle, with the tube head 55 cm from the surface of the foot.

Lateral films of the metacarpals and metatarsals require time settings of 3.0-4.0 sec while anterior-posterior and lateral carpal radiographs have been taken using a 4.0-sec exposure. These studies require that the x-ray unit be set at 80 kVP and 15 mA and secured to a stand with the tube head held at a distance of 45 cm from the surface of the carpus. Adequate detail may be achieved if the elephant is motionless throughout the exposure time.

Tracts caused by chronic draining wounds may be visualized and evaluated by instilling a radiopaque dye deep into the tract, plugging the opening with a cotton wad, and immediately radiographing the area.
Abstract

Analgesia in mammals can be achieved with nonsteroidal anti-inflammatory (NSAIDS) agents, opioid agonists (OA), opioid agonist-antagonists (OAA), and alpha-2 adrenergics (A2A). Other anti-inflammatory agents, such as glucocorticoids, may provide indirect analgesia.

NSAIDS have anti-inflammatory, anti-prostaglandin and analgesic effects. The anti-inflammatory and anti-prostaglandin activity results from inhibition of the enzyme cyclo-oxygenase (prostaglandin synthetase). Gastric ulceration is a potentially serious side effect of NSAID administration because the protective mucosal barrier of the stomach may be reduced by inhibition of prostaglandin E production. A few of the newer generation NSAIDS such as carprofen are more sparing of prostaglandins and are less ulcerogenic. Some NSAIDS are also nephrotoxic, especially when administered to an animal that has pre-existing renal compromise or hypotension as may occur under anesthesia. Additionally, NSAIDS have high protein binding potential which can influence the metabolism of other drugs. NSAIDS also interfere with the production of glycosaminoglycan by injured chondrocytes and may worsen joint disease with prolonged administration. At present, NSAIDS that can be given parenterally or orally are limited to flunixin meglumine, ketoprofen and ketorolac.

OAs provide excellent analgesia and have high therapeutic ceilings. The greater the dose used, the more analgesia achieved. They are especially useful for the control of moderate to severe pain but have no anti-inflammatory or anti-prostaglandin effects. OAs have shorter systemic than local duration. Examples of opioids used on animals are: morphine, codeine, fentanyl, fentanyl transdermal patches and oxymorphone. Side effects include drowsiness, respiratory depression, urinary retention, nausea and vomiting. They should not be used when head injury is present because the respiratory depression can decrease CO₂ levels which will cause cerebral vasodilation and an increase in intracranial pressure. Most opioids are available in oral as well as parenteral formulations.

OAA cause less cardiopulmonary depression but have less analgesic effect than OAs. They have agonist activity at opioid k receptors but minimal effects at opioid m receptors (sedation and respiratory depression centers). However, because they have a low therapeutic ceiling, higher dosages will not increase analgesia. Examples of OAAs are: butorphanol, pentazocine, buprenorphine, and meperidine.
A2As provide potent analgesic effects and sedation in some species, and potentiate opioid analgesia in others.\textsuperscript{2,3} They have cardiovascular side effects which may limit their use to young healthy animals. Xylazine, medetomidine, and detomidine are examples of A2As.

Babirusa (\textit{Babyrousa babyrussa}), a Southeast Asian suid, have been maintained at the Wildlife Conservation Society (Bronx Zoo) since 1985. As original breeding stock has aged, and offspring have matured, unusual numbers of animals have developed acute and chronic arthritides and degenerative joint disease (DJD). Multifactorial etiologies are probably responsible for the severity of some lesions. As with other suids, an interplay of infectious disease, nutritional factors, genetics, housing conditions, and traumatic events in individuals may result in varying degrees of acute and chronic pain.\textsuperscript{5} While underlying causes of lameness are treated or corrected, analgesia is a critical element of all therapeutic and management plans.

NSAIDS have been used most frequently in an attempt to provide relief from discomfort and to decrease joint inflammation. Dosages were determined by synthesizing information from the human and veterinary literature. As with any non-labeled use of drugs, conservative treatment plans are followed when using any agent for the first time. Fecal occult blood is routinely monitored in animals receiving any NSAID for more than 3 days. If occult blood is detected, GI protectants such as sucralfate (Carofate tablets, 1 g, Hoechst Marion Roussel), and histamine-2-receptor antagonists [cimetidine (Tagamet, 300 mg tablets, SmithKline Beecham, Philadelphia, PA 19101 USA) or ranitidine (Zantac, 300mg tablets, GlaxoWellcome, Research Triangle Park, NC 27709 USA)] are administered.

Acute onset of single limb lameness is most often treated with flunixin meglumine (Banamine Solution 50 mg/ml, Schering-Plough Animal Health, Kenilworth, NJ 07033 USA) (0.5-1.0 mg/kg p.o. s.i.d. - b.i.d.). Animals which are non-responsive after 3-5 days may be darted with flunixin (1 mg/kg s.i.d.) for 1-2 days. Oral flunixin as needed is continued when necessary. One adult male with repeated bouts of acute pain associated with chronic DJD improves dramatically with minimal flunixin doses (0.15 mg/kg p.o.) as required.

Some persistent lamenesses have responded to ketoprofen (Ketoprofen capsules 75 mg, Biocraft Laboratories, Elmwood Park, NJ 07407 USA) (0.3-0.8 mg/kg p.o. s.i.d. - b.i.d.) for 3-10 days. Aspirin has not provided adequate analgesia at 10-20 mg/kg p.o. b.i.d. Ibuprofen (Ibuprofen tablets 200mg, Interpharm Inc, Plainview, NY 11803 USA) (15 mg/kg p.o. b.i.d.) is occasionally used with variable results. Carprofen (Rimadyl 100 mg tablets, Pfizer Animal Health, Exton, PA 19341 USA) (4-6 mg/kg p.o. b.i.d.) was not effective in providing relief in one case of severe chronic DJD, and at the higher dose, fecal occult blood was present.

Transdermal fentanyl patches (Duragesic 50 mg/h, Janssen Pharmaceutica, Titusville, NJ 08560 USA) have been used 3 times on 2 animals that had severe pain due to chronic DJD. The animals refused to rise or move about their enclosures. There had been no, or poor, response to administration of NSAIDS (flunixin, ketoprofen and carprofen). Response to the fentanyl patch was dramatic, with the animals becoming fully ambulatory within 24 hr of receiving a dose of 50 mg/hr.
Fentanyl. The effect of the patch was so complete in breaking the pain cycle, that one animal which had been treated repeatedly with NSAIDS during the previous year remained asymptomatic for 5 mo. Two animals, with gradual onset of severe unilateral front limb lameness and reduction of mobility, did not improve with the use of fentanyl patches. Transdermal patches work best when oil and debris are removed from the skin with alcohol prior to application. The patch itself has an adhesive edge which is augmented by placing an elastic adhesive bandage over it and around the limb to which it is applied. Patches have been most efficacious when applied to the medial aspect of the upper forelimb.

Depending on the severity of lesions and the temperament of the individual animal, pain in babirusa can be debilitating. Failure to provide analgesia while also treating the underlying cause of the pain may result in overall failure of therapy.

ACKNOWLEDGMENTS

The contributions of Mark Stetter, DVM to the management of these cases and the extraordinary care provided by the mammal department keepers during some of the protracted clinical courses is appreciated, as is the assistance of Peter Helmer with literature searches.

LITERATURE CITED

MARINE MAMMAL INTRAVENOUS CATHETERIZATION TECHNIQUES

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Abstract

Intravenous therapy is a cornerstone of veterinary medicine and has become essential for a variety of treatments. Historically, i.v. catheters have not been routinely utilized in marine mammal medicine.1,2 The unique anatomy of marine mammals, combined with the constraints imposed by their aquatic environment, have made placement and maintenance of i.v. catheters difficult. This paper discusses several techniques for placement, and long term management, of intravenous catheters in marine mammals.

Over a 6-yr period, intravenous catheters have been utilized on nine occasions in both pinnipeds and cetaceans (two harbor seals [Phoca vitulina]), four walrus [Odobenus rosmarus]), two beluga whales [Delphinapterus leucas], one pygmy sperm whale [Kogia breviceps]), for durations up to 9 days in length. The use of a tuohy needle (Reganes Medical & Surgical Supplies, Clearwater, FL 33519 USA) in conjunction with an epidural catheter (SIMS Medical Systems, Keene, New York, 03431 USA) allows catheterization of vessels which are too deep to visualize or palpate. The tuohy needle is a 17-ga 8.9-cm metal needle which acutely curves at its most distal point (Fig. 1). The tuohy needle can be inserted perpendicular to the skin and its curved tip displaces the 20-ga nylon epidural catheter at an angle as it leaves the needle and enters the blood vessel. Although the i.v. epidural catheter technique provides rapid, reliable venous access, the 20-ga catheter is prohibitively small for adequate fluid therapy of large animals. In order to provide adequate fluid therapy, larger catheters can be placed. Traditional through-the-needle catheters can be used with marine mammals but are more difficult to place. The needle must be introduced at an oblique angle such that when the catheter is inserted through the needle and into the vein, it will not be forced to make an abrupt turn. In seals and walrus, a 10-ga 13.3-cm large animal veterinary catheter (Jorgensen Laboratories, Inc., Loveland, CO 80538 USA) is used to penetrate the vein. The metal portion is removed and then a 14-ga 61-cm catheter (Charter Med, Lakewood, NJ 08701 USA) can be introduced through the catheter sleeve. The 10-ga catheter sleeve is withdrawn and cut away leaving the longer 14-ga catheter in place for long term use. We have commonly used these larger catheters for intraoperative fluid therapy and postoperative i.v. medications for up to 9 days.

In seals and walrus, the dorsal extradural intravertebral vein is used for catheter placement. This large venous sinus is approached in the caudal lumbar region (L3 - L4) on the dorsal midline (Fig. 2). Although this location is a common venipuncture site,1,2 catheterization is difficult due to the depth of this sinus and the oblique needle angle necessary for access. The use of a tuohy needle and epidural catheter allow these obstacles to be easily overcome. These catheters are routinely used at the Wildlife Health Center for initial i.v. access during pinniped chemical restraint. Venous catheterization is commonly accomplished after intramuscular sedation and this i.v. line is then used.
for anesthetic induction. The catheter can be secured in place and used intra- and postoperatively for administration of i.v. medications.

In cetaceans, the large caudal ventral peduncle veins run on either side of ventral midline just below the laterally bulging vertebral tendon sheaths (Figs. 3 and 4). These vessels lie deep within the peduncle and can be difficult to locate and catheterize. Long term catheterization of the vessels within the fluke has had limited success. While these vessels are commonly used for venipuncture, they are not as amenable for catheterization both because of their relatively small size and because the arteries are surrounded by the periarticular venous retia. This vascular plexus has thick muscular walls which can constrict and prevent catheter placement. Due to the close association between the fluke arteries and veins, it is very difficult to specifically catheterize one versus the other. Catheterization of the ventral peduncle veins is best performed using a tuohy needle and epidural catheter. The depth of the vessel and the oblique needle angle required for through-the-needle catheters make this type of catheter difficult to use in cetaceans.

Because marine mammal catheters are not placed on an appendage, but rather on the animal’s large trunk, bandaging is impractical and securing the catheter can be difficult. It has been challenging to adequately maintain these catheters in an aquatic environment while attempting to maintain a degree of cleanliness and sterility. Due to hydrodynamic forces, catheters in cetaceans must be very tightly secured at their base and there must be a minimal length of catheter extending from the body. In some situations, a rubber flap has been sutured over the catheter to protect it from the drag of water while swimming. The use of a silicone filled rubber syringe plunger has allowed firm attachment of the catheter to the skin and created a semi-sterile barrier where the catheter exits the skin. The rubber plunger from a 20 cc syringe is removed and filled with silicone. After the catheter has been placed, a small rim of triple antibiotic ointment is applied to the catheter/skin junction. The syringe plunger is than used to cap the site by driving a needle through the silicone filled plunger and placing the catheter through the needle. The needle is than withdrawn and the catheter remains, snugly passing through the plunger. The plunger is advanced down to the skin surface where it is sutured in place. The catheter and injection cap are joined together with a rapid setting epoxy glue and sutured to the skin. A thin layer of oil based triple antibiotic ointment is placed over the external catheter and injection cap. Pinnipeds with i.v. catheters are removed from their pool and kept in a holding area with water sprinklers. Cetaceans with i.v. catheters are kept in water and briefly restrained for i.v. medication administration.

Like their terrestrial counterparts, marine mammals may require intravenous fluids or medications for a variety of reasons. Although marine mammals lack the conventional arms, legs and neck which are commonly used for catheter placement, intravenous catheters can be placed and maintained for relatively long periods in pinnipeds and cetaceans. Access to the large vessels of marine mammals requires unusually long needles and catheters. These catheters are not bandaged in place but secured with the use of rubber/silicone plungers which are sutured in place. These catheters have been used for fluid therapy, anesthetic induction, antibiotic administration, plasma transfusion, blood sampling and parenteral nutrition.
ACKNOWLEDGMENTS

The authors are indebted to Drs. Raphael, Klein, Mangold and James for their clinical assistance and Kevin Walsh, Hans Walters, Chris Baker, and the marine mammal trainers and keepers for their help. We would also like to thank Dolores Sanginito for her tremendous help with manuscript preparation.

LITERATURE CITED


Figure 1. Diagram of a tuohy needle created for human epidural catheterization. These needles have a closed terminal end and side hole (arrow), which allows the catheter to exit at an angle.

Figure 2. Schematic diagram of a seal or walrus illustrating the extradural intervertebral vein catheterization site (arrow) at the 3rd - 4th lumbar vertebrae.

Figure 3. Schematic diagram of the extradural intravertebral vein (A), cauda equina (B), and intervertebral disc (C) within a lumbar vertebral of a seal or walrus.

Figure 4. Schematic diagram of the caudal peduncle in a cetacean showing the relationship of the vertebral body (A), tendon sheath (B), and ventral peduncle vein (C).
CHARACTERIZATION OF THE ESTROUS CYCLE AND BREEDING SEASON LENGTH IN CAPTIVE AUSTRALIAN FUR SEALS (Arctocephalus pusillus doriferus) USING SALIVARY AND FECAL PROGESTERONE ANALYSIS

Cree Monaghan, BSc, BVMS,1,6 Patrick Wright, BVSc (Syd), MVSc, PhD,2 and Franz Schwarzenberger, Vet. Med.3


Abstract

Difficulties with breeding control has prompted investigation of the estrous cycle and breeding season length of Australian fur seals in captivity at Royal Melbourne Zoo, Australia. Melbourne Zoo houses seven mature female Australian fur seals and one fertile mature Australian fur seal bull. Pregnancy has been prevented by separation of the male during the wild breeding season period from November through late January. Housing difficulties with this 3-mo separation arrangement has encouraged further research into the normal estrous pattern and the length of possible breeding time. Data on wild Australian fur seals suggests that females may only ovulate once during the breeding season, at which time they are usually mated, and the time of ovulation is variable within that breeding season. Little information is available on the pattern of the estrous cycles if an ovulation passes without fertilization.

Noninvasive techniques to measure progesterone for estrous cycle characterization were sought due to the impractical nature of regular conscious serum collection. Feces and saliva were considered the two most accessible and reliable sample materials. Fecal and saliva samples were collected from three females with proven breeding history from October onwards. In addition, collections from two younger females began in February.

Seals were trained for an open mouth presentation at the beginning of the feeding session twice daily on collection days. Saliva was collected using sterile cotton tipped applicators. Saliva was spun off the swabs by a centrifuge and then frozen at -20 °C until processing time. Samples were sent to the Veterinary School, University of Melbourne, for processing; progesterone analysis was conducted using the coat-a-count serum progesterone kit. Collection of saliva occurred routinely 3-4 times/wk; however, sample quantity was dependent upon individual compliance.

Identification of an individual’s feces was achieved by installation of colored inert plastic beads into individuals food on a daily basis. Fecal samples were collected opportunistically with minor manipulation of night housing arrangements. Fecal collection difficulties occurred regularly as a result of defecation in water. Collected feces were stored at -20 °C. Fecal samples were sent to the biochemistry division of the University of Vienna and results were derived from the 20-oxo-pregnane assay.
Current saliva progesterone assay results suggest an ability to predict an estrous cycle in individuals. Fecal progesterone assay results suggest an ability to detect a corpus luteum in correlation with saliva results. Further results are pending and are expected to predict the cessation of estrous activity in individuals and consequently a time limit to yearly breeding ability. In addition, the results should allow determination of the accuracy of the two techniques for prediction of estrous cycles in the Australian fur seal and possibly for other otariid seals.
THE USE OF A LONG-ACTING NEUROLEPTIC IN THE MONGOLIAN WILD HORSE
(Equus przewalskii przewalskii) TO FACILITATE THE ESTABLISHMENT OF A
BACHELOR HERD

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Abstract

Many animal species have developed social structures which favor the formation of harem-type
social groupings. For these species, there is typically a single dominant male in the group along with
multiple mature females and their respective offspring. Species with this type of social structure
have presented some difficult problems for captive breeding programs in traditional zoos: although
the ultimate structure of these groups is typically composed of more females than males, a similar
number of both sexes are born. This gender ratio is further complicated by the fact that the majority
of traditional zoo facilities cannot provide adequate space for the subordinate males to break away
from the main group (as they would do in the wild) to escape physical harm from conflicts with the
dominant male. The result is that captive populations often have numerous males that are difficult
to maintain in a social group. The aim of this study was to assess the effectiveness of long-acting
neuroleptics as an aid in the establishment of a bachelor herd comprised of mature male Mongolian
wild horses.

The Mongolian wild horse (also known as Przewalski’s horse), which generally exhibits a single
male, multi-female social structure, is extinct in the wild and has been one of the true success stories
of organized captive breeding efforts around the world. Previous attempts to have more than one
mature (older than 5 yr) stallion in a social grouping have resulted in extreme aggression between
the males, often resulting in significant injury and on occasion, even death. As a result, the large
number of males that is necessary for the genetic and demographic aspects of the captive population
are maintained individually in zoos, thereby utilizing significant amounts of the limited space which
is available for captive breeding programs. Working with the Mongolian wild horse Species Survival
Plan (SSP), the Wilds identified eight mature (mean age 6.6 yr) males and established a fenced, 200-
acre enclosure in which these animals could be maintained together as a bachelor group.

To facilitate the formation of this group, each animal was administered a long-acting neuroleptic
(LAN), perphenazine enanthate (Trilafon® enanthate 100 mg/ml, Schering-Plough, 2000 Galloping
Road, Kenilworth, NJ 07033 USA) in combination with a mid-duration neuroleptic, haloperidol
(Haldoperidol Tablets, USP, 10 mg, Par Pharmaceutical, Inc., Spring Valley, NY 10977 USA) prior
to release into the pasture. Long-acting preparations of neuroleptic agents have been used in the
medical field for many years to aid in the treatment of human psychoses but have recently been used
extensively in wildlife species in southern Africa to aid in the translocation and the adaptation of
wild animals to new environments.

Perphenazine is a member of the phenothiazine group of neuroleptic drugs. The effects of the long-
acting formulation are generally not seen for 10-12 hr after deep, intramuscular injection but
tranquilization remains effective for approximately 7 days. The peak effect is usually reached after
a period of 72 hr, and for this reason, perphenazine enanthate is often combined with another, more
rapidly-acting neuroleptic such as haloperidol. Haloperidol is a neuroleptic drug that is a member of the butyrophenone group of compounds and may be injected or administered orally. The effects of this drug are seen within several hours of oral administration and last for approximately 10-12 hr.\textsuperscript{1}

In this study, weights of individual animals were estimated (325-375 kg) and perphenazine was given by intramuscular injection at a dose rate of 0.5 mg/kg and was administered 48 hr prior to release of the animals into the pasture. The drug was delivered by projectile syringe (Pneu-Dart, Inc. Williamsport, PA 17703 USA) and was injected into the muscles of the neck or shoulder. In addition, each animal was administered oral haloperidol at a dose rate of 0.3 mg/kg 2 hr prior to release. All animals were individually identified and marked and all entered the new pasture area within a 1-hr period. Observations of the release were performed and all animal interactions were recorded daily for a period of 10 days. Several phases of the introductory period were recorded on video tape.

Close observation revealed minimal interaction between animals upon release and there was significantly less aggression exhibited than expected. The animals formed a loosely structured group shortly after being placed together and the 7-8 day period of tranquilization was characterized by minimal aggression, excitement and anxiety without significant sedation. During this time the animals became familiar with their surroundings, the boundaries of the pasture and with each other. The effect of tranquilization on herd formation was remarkable: by the end of the observation period, when the neuroleptic drugs were assumed to be no longer effective, it was evident that a level of dominance and social order had been effectively established within the group, with no significant injuries incurred as a result of fighting. The eight Mongolian wild horses involved in this study continue to thrive in this bachelor group and after 4 mo of daily observation, the initial social order appears to have remained intact and there has been no significant aggression observed between the animals.

LITERATURE CITED

TRAINING GORILLAS (Gorilla gorilla gorilla) FOR NONINVASIVE SEMEN COLLECTION

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Abstract

Electroejaculation, a method commonly used for semen collection in exotic animals, generally results in poor quality samples from gorillas. Omaha’s Henry Doorly Zoo instituted a training program with a bachelor group of three gorillas (ages 10, 12, and 12 yr) to determine if semen quality would improve when collected in a noninvasive, nonstressful manner. This paper discusses the training process, and may be used as a guideline for other institutions initiating their own programs.

The Omaha Zoo’s training program was developed with several objectives in mind. The primary objective was to collect semen in a noninvasive, nonstressful manner. Secondary objectives included: performing a cursory physical examination, providing behavioral enrichment, preserving normal gorilla behavior, and acquiring the capability to teach personnel from other institutions how to train gorillas for semen collection. The training techniques were developed, and adapted, to meet these objectives.

Appropriate behaviors, in response to verbal prompts, were rewarded with praise and food treats (Skittles, Division of Mars, Inc., Hackettstown, NJ 07840 USA). The behaviors were progressively refined to allow a cursory physical examination and semen collection. All three gorillas were taught the behaviors listed in Table 1.

Collecting semen was the main goal of this training program. To accomplish this goal, two training techniques had to be established and consistently followed. First, gorillas were allowed to accept treats only with their lips, never their hands. Second, the trainer administered treats only with the right hand, never the left. This established a precedent which discouraged gorillas from moving their hands, and prevented the gorillas from expecting to find treats in the trainer’s left hand. Once these techniques were consistently followed, several behaviors had to be mastered before semen collection could begin. The gorillas were taught “station,” “target,” “knee,” and “hold” (Table 1). The completion of these behaviors resulted in the gorilla straddling a 7.5 × 15 cm steel sliding door that was built into the mesh caging. Once the gorilla reliably held this position, the door was opened by the trainer, and penile massage took place until the gorilla achieved an erection and ejaculated. While performing massage with the thumb and forefinger of the left hand, a small plastic bag (unopened) held in the same hand between the middle and ring fingers was used to capture the sample. The ejaculate volume, usually only a large drop, ranged from 10-140 µl.

Performing a physical examination, one of the secondary goals, was accomplished by stating the verbal prompt, and rewarding the animal when it performed the specified behavior. With the gorilla in the “hold” position, a PVC pipe was passed through the mesh. It was then used to touch the requested body part. The behavior was refined by stating the prompt and passing progressively shorter lengths of the pipe through the mesh. This encouraged the gorilla to move the body part to
the pipe. “Knee,” “foot,” and “back” behaviors resulted in palpation of that body part through the wire mesh. “Arm” allowed small volume injections and may be adapted to allow venipuncture. “Chest” permitted auscultation. “Ear” allowed the otic temperature to be taken. “Mouth” made a visual dental examination possible.

Providing behavioral enrichment while maintaining normal gorilla behaviors was another objective of this program. Gorilla participation in the training sessions was voluntary. However, they were usually eager to shift into the training area on training days. They appeared to be mentally stimulated by the training. For example, one gorilla learned to “bargain” for treats by withholding an object dropped into the cage until the trainer demonstrated a large number of treats as a reward for the object’s return.

Because semen had never been collected from gorillas in this manner, the gorillas’ behavior when not in a training session was a concern. Therefore, the training routine was varied within each session, and some sessions omitted certain behaviors, including semen collection. Semen collection was incorporated as one of many behaviors a trainer may request of the gorilla. While the effectiveness of these techniques is debatable, there was no increase in the incidence of undesirable behaviors, such as masturbation, when the gorillas were not being trained.

The final objective for this program was to have the ability to teach personnel from other institutions how to train gorillas for semen collection. Therefore, visitors were welcome to observe training sessions. The gorillas expected strangers present during training, and they generally ignored them. This program has resulted in the additional benefit of easily accommodating photographers and journalists during the training sessions.

The Omaha Zoo’s gorilla training program has been successful. Over 175 semen samples were collected from the three gorillas since the initiation of the program. Collection of the first semen sample varied from 5-14 mo after the initiation of training, with collections occurring earliest on the animal that appeared to have the best relationship with the trainer. One animal produced consistently dead spermatozoa, but the other two animals produced samples that were good quality. These samples were regularly cryopreserved, which can rarely be accomplished with electroejaculated samples. One of the cryopreserved samples was used for in vitro fertilization, and resulted in a viable offspring.

There are many benefits of developing a training program for semen collection in gorillas. Noninvasive semen collection involves no risk from anesthesia and results in good quality semen samples (N.M. Loskutoff, personal communication). Cursory physical examinations can be performed on a regular basis. Training also provides gorillas with behavioral enrichment.

As more institutions develop training programs for semen collection in gorillas and other great apes, artificial reproductive techniques have the potential to be routinely performed. The use of these techniques could lead to a more genetically stable population of captive western lowland gorillas, and may ensure the survival of the species.

LITERATURE CITED


Table 1. Trained gorilla behaviors.

<table>
<thead>
<tr>
<th>VERBAL PROMPT</th>
<th>BEHAVIORAL RESPONSE</th>
</tr>
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<tbody>
<tr>
<td>Station</td>
<td>Sit in front of the trainer</td>
</tr>
<tr>
<td>Target</td>
<td>Touch a ping pong paddle through the mesh</td>
</tr>
<tr>
<td>Hold</td>
<td>Remain in position</td>
</tr>
<tr>
<td>Okay</td>
<td>Discontinue the current behavior</td>
</tr>
<tr>
<td>Knee</td>
<td>Move knee(s) to mesh and allow it to be touched</td>
</tr>
<tr>
<td>Arm</td>
<td>Move forearm to mesh and allow it to be touched</td>
</tr>
<tr>
<td>Foot</td>
<td>Place foot on mesh</td>
</tr>
<tr>
<td>Back</td>
<td>Turn back to the trainer</td>
</tr>
<tr>
<td>Chest</td>
<td>Move chest to mesh</td>
</tr>
<tr>
<td>Ear</td>
<td>Move ear to mesh and allow it to be touched</td>
</tr>
<tr>
<td>Mouth</td>
<td>Open mouth</td>
</tr>
<tr>
<td>Give</td>
<td>Return an object dropped into the enclosure</td>
</tr>
<tr>
<td>All right, all done</td>
<td>End of training session</td>
</tr>
</tbody>
</table>
REPRODUCTIVE ASSESSMENT IN CAPTIVE BROWN BEARS

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Abstract

Background

The development of reliable methods for monitoring the reproductive cycle of bears is needed to optimize breeding management in captivity. Although there is a large database describing patterns of reproductive life history, anatomy of the female genital tract and endocrinology of bears, there is little basic information about reproductive physiology. Several species of ursids are frequently kept in zoos. Zoos and private reserves have become increasingly proactive in their management, establishing conditions necessary for successful reproduction in captivity. Increasing knowledge of reproductive behavior and animal husbandry in particular has greatly enhanced the breeding success, thus creating self-sustaining populations of some ursids in captivity, particularly brown bears (Ursus arctos arctos). The use of reproductive techniques for conservation and management programs for this species includes the introduction of effective methods of contraception. There is good evidence that bears produce progesterone during the phase of delayed implantation and we suggest that antigestagens can probably be used for prevention of implantation of the early blastocyst. The aim of the present study was to develop noninvasive procedures for monitoring the reproductive status of brown bears using transrectal ultrasonography and endocrinology to get basic data for future anticonceptive treatments during diapause.

Ultrasonography

Ultrasonographic imaging techniques have proved valuable in reproductive and veterinary studies in a wide range of exotic species. Transabdominal ultrasonography is a viable method for pregnancy detection in brown bears (Ursus arctos yesoensis).4 However, transabdominal ultrasound has not yet been successfully used for the visualization of the nongravid uterus, gonads and early pregnancies in ursids. The ability to assess the reproductive status of bears has been quite limited. Transrectal ultrasonographic imaging provides a noninvasive tool for observing reproductive events in wild carnivores. Some of the difficulties in viewing the entire female genital tract and monitoring structural changes of the uterus and ovaries were attributed to difficulties in positioning a miniaturized high frequency probe into the rectum of bears. Five female captive brown bears (Ursus arctos arctos) were examined ultrasonographically twice, once in late September and once in early December, after mating had been observed during the breeding season. All animals were immobilized with a dart gun using etorphine (1.3 ± 0.2 mg/100 kg body weight) and acepromazine (6.9 ± 0.4 mg/100 kg body weight; Large Animal Immobilon®, C-Vet Ltd., Bury St. Edmunds) with the addition of 300 IU hyaluronidase (Hylase®, Impfstoffwerk Dessau). The anesthesia was antagonized with diprenorphine (4.1 ± 0.3 mg/100 kg body weight; Reivion®, C-Vet Ltd., Bury St. Edmunds). The genital tracts of the immobilized females were examined by transrectal ultrasonography in lateral recumbency. Feces were
removed digitally using ultrasound gel (Aquasonic 100®, Parker Laboratories Inc., Orange, NJ 07050 USA) for lubrication and the rectum was then irrigated with lukewarm water. A real-time, B-mode ultrasound scanning system (CS 9100 Oculus, Picker International GmbH, Espelkamp, D-32339, Germany) equipped with a 7.5-MHz curved linear intraoperative transducer (EUP-F 334) was used. The probe was fitted in an adapter (Ultraschallkopftraeger FT2, A. Schnorrenberg Chirurgiemechanik, Woltersdorf, D-15569, Germany) and then introduced into the rectum using ultrasound gel for coupling. The urogenital tract was scanned longitudinally from caudal to cranial. The ultrasound system described allowed successful imaging of the urogenital tract of all animals investigated. All parts of the normal female reproductive tract and of the urinary system, except the ureters, could be visualized transrectally. Verification of the structures identified sonographically in situ was accomplished by performing ex situ ultrasonographic examinations of isolated urogenital tracts in two other female brown bears post mortem. In early December, three brown bears were identified as pregnant. They were characterized by local uterine enlargement (threefold), anechoic fetal fluid and endometrial proliferation at the implantation sites. The placenta appeared as a hypoechoic, discoid structure. Echogenic fetuses were easily visible within the anechoic fluid. Head, thorax, abdomen and the extremities of the fetus were clearly distinguishable at this stage. The fetal crown-rump length was 24 ± 4 mm and the fetal heart beat was detectable. With careful searching over the uterine horns, the number of fetuses (1-3 fetuses/animal) and mature corpora lutea on the ovaries could be detected.

Endocrinology

Plasma concentration of progesterone and gestagens in feces were determined simultaneously. Blood samples of two nonpregnant and two pregnant animals were taken during each ultrasound investigation. Fecal samples were collected weekly from September to November. For the determination of progesterone, 0.1 ml of plasma was extracted with 2 ml petrol ether, concentrated to dryness and the residue was reconstituted in 1 ml 40% methanol. Progesterone was measured with an enzyme immunoassay (EIA) as described and validated earlier. The antibody used was raised against progesterone-7-carboxyethylthioether-BSA and the label was progesterone-3-carboxymethyloxime-horse radish peroxidase. Fecal samples (0.5 g) were combined with 4.5 ml methanol, agitated for 30 min. and centrifuged (15 min, 1,000 g). One-half milliliter of the supernatant was diluted with 0.5 ml water. After further dilution, progesterone equivalents were quantified using the same EIA as for plasma. The plasma concentrations of progesterone were 1 to 5.5 ng/ml in all brown bears sampled in September and December, indicating functional corpora lutea. According to the limited data available, there appear to be no fundamental differences between nonpregnant and pregnant animals before and after implantation (Table 1). The preliminary results show that fecal samples obtained from 10 different female brown bears in the nonbreeding season contain low concentrations of progesterone equivalents, about 10 ng/g, whereas in fecal samples from animals with active corpora lutea, estimated concentrations of progesterone equivalents were 50-800 ng/g. A slightly increased excretion was recorded in the first two pregnant bears investigated.

Conclusions

Transrectal ultrasonography provides a tool for efficient assessment of the reproductive status in brown bears. In contrast with transcutaneous ultrasound, the advantages of the rectal method include imaging of the ovaries, including follicles and corpora lutea, and the ability to detect the nongravid uterus and early pregnancies. Noninvasive gestagen monitoring in feces may be used for pregnancy diagnosis after implantation, an important aspect particularly for breeding management.
of captive bears.

**LITERATURE CITED**


**Table 1.** Concentration of progesterone in blood plasma (ng/ml) and progesterone equivalents in feces (ng/g) of brown bears measured by enzyme immunoassay.

<table>
<thead>
<tr>
<th></th>
<th>nonbreeding season (February - April)</th>
<th>pre-implantation (September - October)</th>
<th>post-implantation (November - December)</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood plasma</td>
<td>0.1 (n = 2)</td>
<td>3.3 ± 0.9 (n = 4)</td>
<td>3.1 ± 0.1 (n = 2)</td>
</tr>
<tr>
<td>feces</td>
<td>9.6 ± 6.7 (n = 10)</td>
<td>151 ± 133 (n = 26)</td>
<td>379 ± 238 (n = 11)</td>
</tr>
</tbody>
</table>

Fecal samples in nonbreeding season originated from 10 different animals; all other samples were collected from two individuals.
ASSESSMENT OF HEALTH AND REPRODUCTIVE STATUS IN AFRICAN AND ASIAN ELEPHANTS BY TRANSRECTAL ULTRASONOGRAPHY

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Abstract

Transrectal ultrasonography was performed on 10 male and 85 female African elephants (Loxodonta africana) and on 5 male and 39 female Asian elephants (Elephas maximus) in order to develop standards for assessment of reproductive health and status.

Captive and wild African males and females as well as captive Asian males and females were examined. The entire internal urogenital tract was visualized ultrasonographically by using a 3.5-MHz, a 7.5-MHz and a 10.0-MHz transducer in combination with two probe extensions specially adapted for elephant anatomy. The findings were verified by postmortem ex situ ultrasound examinations in each species. Each part of the internal urogenital tract was sonographically detectable except for main parts of the late gravid uterus (> 13 mo p.c.) in females and the bulbo-urethral glands and the cranial portion of the ureters (in both sexes) and ductus deferentes in males. A variety of pathological alterations were found, but mostly in the captive population of African and Asian elephants. There was a very high incidence of uterine leiomyomas in the female genital tract of captive Asian elephants (35.9%). In contrast, wild and captive African elephants never develop leiomyomas but frequently have endometrial cysts (11.3% wild, 14.3% captive) and ovarian cysts (1.4% wild, 21.4% captive). This study presents results which indicate that transrectal ultrasonography may be used as an effective nonsurgical tool for reproductive and health assessment of elephants, which has implications for management, population control and assisted reproduction.

Introduction

Reproduction in African elephants (Loxodonta africana) and Asian elephants (Elephas maximus) is often less successful than in natural populations, inhibiting the establishment of self-sustaining captive populations.5, 11 Infertility due to reproductive disorders and mismanagement can have a devastating effect on captive breeding programs.5, 8 There is a dearth of knowledge regarding the reproductive anatomy and physiology of these both species.2, 3, 6, 12, 14 Sonographic imaging techniques have had a beneficial impact on reproductive and veterinary studies in a wide range of domestic13 and wild species;4, 8 however, the feasibility of this technique as a routine diagnostic procedure in elephants has not been overly successful.1 Some of the difficulties in viewing intra-abdominal testes, ovaries and monitoring fetal development were attributed to difficulties in positioning the instruments, and to the size and demeanor of these large pachyderms. Major anatomical obstacles during routine andrological, gynecological and obstetrical ultrasonography

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include locating components of the reproductive tract that in elephants lie deeply within the abdominal cavity, and the marked inclination of the axis of the proboscid pelvic inlet. Recently, the instrumentation for transrectal sonographic examinations in elephants has been greatly improved to overcome these obstacles.

Methods

Transrectal ultrasonography was performed on 10 male and 85 female African elephants and on 5 male (including 1 castrated individual) and 39 female Asian elephants to develop standards for assessment of reproductive health and status. This study included 5 wild male and 71 female African elephants from the Kruger National Park, Skukuza, South Africa, as well as captive African and Asian elephants kept in 19 different facilities with a variety of management systems. The age of the individuals ranged from 7-62 yr. The majority of the 139 elephants examined were in the reproductive age. The number of ultrasonographic examination/individual ranged from 1-62. However, most elephants had only one examination.

Ultrasonography was performed either in the nontranquilized captive African and Asian elephants in standing position (often chain- or chute-restraint) or in the immobilized wild African elephants (M99®, etorphine 10.0 to 15.0 mg, i.m., Reckett & Coleman, Hull, UK; reversed by M50-M50®, diprenorphine 30.0 mg, i.v., Reckett & Coleman, Hull, UK) which were in lateral recumbency. Both positions were satisfactory for using the transrectal approach. After manual removal of the feces and extensive irrigation in combination with the application of ultrasound gel (Aquasonic 100®, Parker Laboratories Inc., Orange, NJ 07050 USA), the transducer was inserted into the rectum and directed carefully over the reproductive tract. The internal urogenital tract was visualized ultrasonographically by using a real-time, B-mode ultrasound scanning system (CS 9100 Oculus, Picker International GmbH, Espelkamp, D-32339, Germany) equipped with either a 3.5-MHz, 7.5-MHz, or a 10.0-MHz transducer in combination with two different probe extensions adapted for elephant anatomy (Ultraschallkopftraeger II and III, A. Schnorrenberg Chirurgiemechanik, Woltersdorf, D-15569, Germany). These adapters were an essential part of our equipment that allowed good contact between the transducer and the rectal mucosa and could be extended to the cranial segment of the reproductive tract to view the kidneys, testes or ovaries. In addition to the main ultrasound monitor, we adapted a small monitor to a helmet that was worn by the examiner for orientation of the ultrasonogram during the procedure. Measurements were taken from the reproductive structures using the electronic caliper provided on the ultrasound unit. Actual scanning time was 15-25 min for each individual. The findings were verified by postmortem ex situ ultrasound examinations on the isolated urogenital organs in each species (63 African elephants and 6 Asian elephants).

Results

Each part of the internal urogenital tract was sonographically detectable except for main parts of the late gravid uterus (> 13 mo p.c.) in females and the bulbo-urethral glands and the cranial portion of the ureters (in both sexes) and ductus deferentia in males (Table 1). The use of all three ultrasound transducers in combination with the two adapters was necessary to get optimal information about the urogenital organs. The quality of the ultrasonographic images of each probe was evaluated on the basis of the following criteria: overview, detail, and surface of the organ examined (Table 1). This
knowledge concerning the appearance of healthy genital structures was necessary for clear recognition of pathological alterations. Ultrasonography was used to detect the following pathological alterations listed in Table 2.

Discussion

Assessment of the reproductive capacity of an individual is based on the health of the internal genital tract. It is therefore important to detect pathological alterations of the inner genital tract and to determine the importance or influence they may have on reproductive performance before forming breeding groups.8

Generally, exotic animals show signs of diseases or disorders very late in their progression. Sonographic examination of the urogenital organs may provide useful criteria to appraise the fitness or breeding potential of an individual. Detection of subclinical changes in these organs indicates that a clinically apparent metabolic disorder may occur under the physiological burden of breeding activity and pregnancy, which could have possible lethal consequences for mother and/or fetus.

There was a very high incidence of uterine leiomyomas in the female genital tract of captive Asian elephants (35.9%). In contrast, wild and captive African elephants never develop leiomyomas but frequently have endometrial cysts (11.3% wild, 14.3% captive) and ovarian cysts (1.4% wild, 21.4% captive). These findings correlated with a postmortem study between 1975 and 1995 of approximately 30,000 exotic mammal cases at the Institute for Zoo Biology and Wildlife Research Berlin, and the Smithsonian Institution, Washington DC, in which leiomyomas were found in the uterus, cervix, and vagina in 14 species of exotic mammals. These animals originated from the Smithsonian’s National Zoological Park (NZP) and from multiple zoos in Europe. This study indicated a very high necropsy prevalence of genital tract leiomyomas in Asian elephants as compared to other species.10

Findings regarding the general health of the animal and those specific to the genital tract should be considered in order to make appropriate decisions regarding which animals to breed.4, 8 This study presents results that indicate that transrectal ultrasonography may be used as an effective nonsurgical tool for reproductive and health assessment of elephants, which has implications for management, population control and assisted reproduction.

ACKNOWLEDGMENTS

The authors are grateful for assistance from the staffs at the following institutions: Circus Frankello; Circus Sarrasani; Circus Scholl; Dickerson Park Zoo; Fort Worth Zoo; Indianapolis Zoo; Knoxville Zoo; Kruger National Park Board; National Zoological Park; Pittsburgh Zoo; private zoo (Virginia); Ringling Bros. and Barnum & Bailey Circus; St. Louis Zoo; Tierpark Carl Hagenbeck, Hamburg; Woodland Park Zoo; Zoo Wroclaw; Zoological Garden, Berlin; Zoological Garden, Münster; Zoological Garden, Erfurt; Zoo Zürich.

Financial support was provided in part by the Institute for Zoo Biology and Wildlife Research, Berlin and the Smithsonian Institution Short Term Visitor Program. The work on the wild African elephants was supported by a grant of the Society for Technical Collaboration, Germany.

LITERATURE CITED

Table 1. Comparison of the efficiency of three transducers in the ultrasonographic imaging of elephant urogenital tracts.

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<td>overview</td>
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<td>+++</td>
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<td>cystic structures in:</td>
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<td>- urogenital tract (vestibule)</td>
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</table>

Table 2. Pathological findings detected by transrectal ultrasonography in wild and captive elephants.

* = this type of transducer did not fit in an adapter
+++ = excellent
++ = good
+ = insufficient
- = impossible
<table>
<thead>
<tr>
<th>Organ</th>
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<th>Value 3</th>
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<td>-</td>
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<td>cervix</td>
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<td>-</td>
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<td>-</td>
<td>4(39)</td>
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<tr>
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<td>11.3</td>
<td>2(14)</td>
<td>14.3</td>
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<tr>
<td>ovary</td>
<td>1(71)</td>
<td>1.4</td>
<td>3(14)</td>
<td>21.4</td>
<td>-</td>
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</tbody>
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* = in comparison to the normal age-related development
** = caused by a surgical castration
( ) = brackets contain total number of individuals investigated
SEROPREVALENCE PATTERNS OF CANINE DISTEMPER VIRUS EXPOSURE IN RACCOONS (Procyon lotor) IN WEST-CENTRAL ILLINOIS

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Abstract

The raccoon, Procyon lotor, is ubiquitous throughout the United States.7 The adaptability of raccoons to human inhabited areas presents opportunities for exposure of domestic animals and zoologic specimens to raccoon-borne infectious agents, leading, potentially, to illness, decreased productivity or loss.

Raccoons carry a number of infectious agents transmissible to domestic and exotic species.1 Although rabies has been one of the most widely recognized, canine distemper virus (CDV) can also substantially impact the health of raccoons and wild and domestic species.10

In this study, seroprevalence patterns of CDV were examined in raccoons from a state park and from a nearby farmed area in west-central Illinois. We examined the differences in seroprevalence among raccoons with different age, sex, geographic and seasonal profiles to provide more information on the epidemiology of this disease.

Raccoons were live-trapped from September 1989 through October 1993. Blood samples were collected from sedated animals. Sera were tested for neutralizing antibodies against the Onderspoort strain of CDV by the New York State Animal Diagnostic Laboratory, Ithaca, NY.2 Raccoons were classified as negative if no antibody was detected at the 1:4 dilution. Higher titers indicated that the animal had been exposed to CDV. Risk factors were screened at the univariate level and then compared, simultaneously, using logistic regression.5 Analyses were conducted using SAS11 with a P value < 0.05 considered to be statistically significant.

Of 368 raccoons tested, 85 (23.0%) were seropositive for CDV. Adult raccoons were more likely to be seropositive (39%) than juveniles (14%) or yearlings (13%). There was no significant difference in seroprevalence between males (22%) and females (25%). Raccoons captured in the farm area (19%) were less likely to be seropositive than those captured in the park (29%), however, this difference was no longer significant when adjusted for age differences. There was no significant difference in seroprevalence between trapping seasons (spring: 26%, fall: 21%).

Canine distemper virus is a common pathogen in dogs and is well known in wild canids, mustelids, procyonids, and viverrids.3 Serologic surveys in New York and Maryland detected seroprevalence for CDV neutralizing antibody titers ranging from 22-84% of raccoons.6,9 The seroprevalence in Florida raccoons was 54.5%.4 Seroprevalence detected in west-central Illinois was at the lower end of these levels.
Canine distemper virus can be devastating in raccoons, leading to high mortality and a slow return to normal population size. Previous reports have described a 4-yr distemper cycle in raccoon populations. These studies found no association between age and CDV infection during or between outbreaks. In our study, positive titers to CDV were detected in all three age groups every year with a significantly higher seroprevalence in adults than subadults. The higher seroprevalence in adults suggests that there is a constant exposure to CDV in these environments. Serologic evidence of exposure, in combination with disease related mortalities reported during the study period, suggests that CDV is enzootic in these populations. Our study found a significant decrease in seroprevalence between the 1991 and 1992 trapping years. During the summer-fall of 1992, disease related mortality was the leading cause of death in the park area. Population density in the park is estimated to be three times higher than in the farm area, however there was no significant difference in the CDV seroprevalence between the study areas (1991-1992). The disease related mortalities and decreasing seroprevalence during this time suggests that an epizootic occurred between the 1991-1992 trapping years. Adult raccoons born in 1988 or earlier were more likely to be seropositive than those adults born after 1988. The higher seroprevalence in the adults > 4-yr-old suggests that there may be a 4-yr interval between CDV epizootics in these populations. The epizootic may have resulted from exposure to a more virulent strain of CDV circulating through the populations, causing losses of both immunocompetent and naive animals, and/or the result of the number of unprotected raccoons becoming great enough that exposure could support an outbreak. Extending the time period studied would be required to confirm a 4-yr epizootic cycle in these populations of raccoons.

Raccoon populations in Illinois have increased in recent years as a result of decreased harvests. The higher density and adaptability of raccoons to human habitations and urban environments pose an infection risk for domestic and captive animal species. CDV isolated from a black leopard that died at the Rock Island Forest Preserve, Naibi Zoo, Coal City, IL, was attributed to feral raccoons. In light of the evidence for both epidemic and endemic transmission of CDV among raccoons, vaccination against CDV for domestic canids and control of potential for exposure in domestic and exotic species are strongly recommended, even if no illness has been observed in feral raccoons. Translocation of raccoons, even in areas where rabies is not epidemic, should be discouraged to prevent inadvertent transmission of CDV.

LITERATURE CITED


VACCINATION OF RACCOONS (*Procyon lotor*) AGAINST CANINE DISTEMPER: AN EXPERIMENTAL STUDY

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Abstract

Canine distemper is endemic in wild raccoons in Toronto and surrounding suburbs. Raccoons represent a constant threat to the Metro Toronto Zoo collection. We have tried to create a "barrier" of protected raccoons on the zoo site to minimize the pressure of infection on the collection but it has been our experience that vaccinated animals that are subsequently released remain susceptible and may succumb to the disease. There have been no controlled studies of humoral response of raccoons to canine distemper vaccination.

A controlled vaccination trial using a modified-live virus vaccine in 48 raccoon pups of known immune status was conducted. Antibody titers were measured for 3 mo following various vaccination schedules. Raccoons with and without titers were challenged with a virulent strain of canine distemper. They were monitored for clinical signs and humoral response for a period of 42 days. All vaccinated pups seroconverted within 2 wk of inoculation. Pups vaccinated once at 8-wk-old and pups vaccinated three times at 8, 12 and 16-wk-old had similar titers at 20-wk-old. Eight-week-old raccoons mounted titers similar to those of raccoons first inoculated at 16-wk-old.

Maternal immunity had completely waned by 16-wk-old in all pups. Pups with maternal antibodies did not mount titers as high as immunely naive pups, but all vaccinated animals survived a challenge with a virulent, raccoon-origin canine distemper virus that killed five out of six controls. Results of this study suggest that modified-live virus vaccination in raccoon pups was efficacious and yielded protection from clinical disease.
HEALTH ASSESSMENT, MEDICAL MANAGEMENT AND PRE-RELEASE CONDITIONING OF TRANSLOCATED NORTH AMERICAN RIVER OTTERS (Lontra canadensis)

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Abstract

North American river otter population numbers are improving following marked decreases resulting from the additive effects of wetland destruction, aquatic pollutants, and unregulated trapping for the fur industry in the late 19th and early 20th centuries. Although a variety of conservation efforts throughout North America have been instrumental in enhancing population numbers, the IUCN Otter Specialist Group still considers population status determinations of high priority in states and provinces where trapping for fur is currently permitted. Two of the many conservation strategies implemented in a number of states are reintroduction and translocation. The first strategy includes reintroduction of river otters from areas with dense populations to historic habitats with sparse or small populations and where introduction has previously been attempted. A second strategy involves translocation of river otters from densely populated locales to areas, within or outside the same state, from which otters have been extirpated or are found in very small numbers. There are logistical, fiscal, and biological advantages and disadvantages to each strategy. Reintroduction may ultimately be more financially costly, presents the challenge of screening for and eliminating certain infectious diseases that may be of importance to other resident mustelids, and may result in higher mortality following release.

The monetary costs of translocation, even within a state, may be relatively high also (approximately $1,000/otter) depending on the trapping methods used, the individuals responsible for trapping (state wildlife biologists vs. private trappers), the methods of transport (vehicle vs. airplane), and which health screening, treatment and preconditioning protocols are implemented to maximize survival, and ultimately reproductive success, following release. Translocation of river otters within a state does have distinct biological and logistical advantages over introduction or reintroduction. First, the probability of "foreign" diseases being introduced is low or minimal. Secondly, the stress of holding prior to transport is minimized, as well as the total transport time for individual otters.

We have developed health assessment, treatment, husbandry and preconditioning procedures over a 2-yr period as part of an ongoing collaborative river otter translocation project in the State of New York. The principles involved include the New York State Department of Environmental Conservation, the New York River Otter Project, Inc., and the College of Veterinary Medicine at Cornell University. This ongoing project has resulted in the successful release of 72 otters (92% of the individuals trapped) into three geographically separated sites in the western part of the state. Although there have been similar projects undertaken in other states, few have implemented the in depth health assessment, medical management, and infectious disease screening protocols that are now standard operating procedure for the New York River Otter Project.
THE CLINICAL AND PATHOLOGICAL FINDINGS IN FIVE BLACK-TAILED PRAIRIE DOGS \textit{(Cynomys ludovicianus)} WITH COMPLEX ODONTOMAS

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Abstract

Nodular hard palate lesions of dental origin were identified in five black-tailed prairie dogs presenting with upper respiratory distress. The five animals (three males, two females) were privately owned, originating from different breeders, and were 2.5-6 yr-old. All animals had a history of dental trauma, chewing hard materials such as metal or plastic cages, rocks or toys. The primary clinical symptoms were dyspnea, stridor, partial or complete obstruction of nasal air flow and occasional nasal discharge and open mouth breathing. Normal serum chemistry profiles and complete blood counts with differentials were found in three of the five animals tested; the other two animals had no consistent abnormalities. The nodular hard palate lesions were unilateral or bilateral and characterized pathologically as complex odontomas with obstruction of nasal airways. It is suspected that these odontogenic tumors developed as a reaction to mechanical tooth trauma.
ADRENAL ACTIVITY ASSESSED BY FECAL CORTICOIDS AND MALE REPRODUCTIVE TRAITS IN THREE SOUTH AMERICAN FELID SPECIES

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Abstract

Reproductive success in captive small-sized cats is very low or inconsistent in Latin American zoos and few of the captive males (~16%) have ever sired offspring.7 Although gonadal function is controlled by a complex interaction of biological factors, poor husbandry conditions in zoos may be partly responsible for impaired reproductive function in captive populations. In particular, inadequate nutrient intakes and an inadequate environment (physical and/or social conditions) can compromise reproduction through behavioral and/or physiological mechanisms.1,4,7 Most zoos manage small-sized cats identically, without considering possible physiological and psychological differences among species. It is quite possible that optimal conditions for one species are not appropriate for another. Thus, in this study we compared reproductive activity and fecal corticoid levels (as an indicator of stress) among male ocelots (Leopardus pardalis), tigrinas (Leopardus tigrina) and margays (Leopardus wiedii). Specifically, we: 1) evaluated testicular volume and seminal characteristics; 2) assessed the stress response to captive conditions and experimental manipulations; and 3) calculated correlations between fecal corticoid levels and sperm production and quality.

Three males of each species were housed individually and fed a meat-based diet with vitamin and mineral supplementation. Each male was submitted to monthly reproductive evaluations over a 14-mo period. After 12-24 hr fasting, anesthesia was induced in ocelots with tiletamine-zolazepam (10 mg/kg, i.m., Telazol, Fort Dodge Laboratories Inc., Fort Dodge, IA) and in tigrinas and margays with a combination of ketamine HCl (20mg/kg, i.m., Ketaset, Fort Dodge Laboratories Inc., Fort Dodge, IA) and xylazine (1 mg/kg, i.m., Rompun, Mobay Corp., Shawnee, KS). Semen collection and analysis followed standardized protocols.3 The reproductive parameters analyzed were testicular volume, ejaculate volume, total number of sperm cells/ejaculate, sperm motility and status, and sperm morphology. Fecal samples were also collected once/wk during the experimental period for analysis of corticoid metabolite concentrations. Steroids were extracted from feces as described previously,7 and corticoids quantified using a previously validated double-antibody 125I-corticosterone assay (ICN Biomedicals Inc., Costa Mesa, CA).6 Assay sensitivity, based on 80% of maximum binding, was 12.5 ng/ml. Intra- and inter-assay coefficients of variation were < 10%. Hormonal data are expressed “per g of wet fecal weight.” Peak and baseline concentrations were calculated as previously described.5 Differences among species in endocrine and reproductive data were determined by a one-way analysis of variance (ANOVA) followed by Duncan’s New Multiple Range tests.

Mean values (± SEM) for body weight, testicular volume, ejaculate characteristics, and fecal
corticoid levels for each species are presented in Table 1. Spermatogenic activity, as determined by both quantitative and qualitative assessments, was higher in the ocelot, whereas margay males exhibited the lowest sperm production and highest rates of primary spermatogenic defects, mainly abnormal acrosomes and sperm head malformations. Tigrinas had intermediate values for almost all of the characteristics analyzed, with the exception of total number of sperm cells. Surprisingly tigrina males produced numbers of sperm cells/ejaculate similar to that for ocelot males. When analyzed on a “per unit of testicular volume” basis, tigrinas produced approximately five and sixfold more sperm cells than margays and ocelots, respectively. In comparison to previous assessments for these same three species, an improvement in reproductive characteristics was noted, particularly those related to quantitative semen measurements. These findings may be the result of feeding an improved diet enriched with vitamins and minerals, which began 5 mo prior to initiation of this study. Frequency of sperm collection, however, may also play a role, since significant (p<0.05) correlations were observed between number of previous sperm collections and 1) testicular volume (r = 0.71, r = 0.47, r = 0.52); 2) ejaculate volume (r = 0.45, r = 0.59, r = 0.51); and 3) total number of sperm cells/ejaculate (r = 0.36, r = 0.34, r = 0.47) in tigrinas, margays and ocelots, respectively.

Fecal corticoid levels differed significantly among species. Overall mean, baseline and peak corticoid levels were lower (p<0.05) in ocelots than in the other two species. All three species responded to different “stressors” (pre-anesthetic fasting, anesthesia, electroejaculation and “usual” captive stressors, such as routine caretaking procedures and climatic changes) by excreting higher levels of corticoids. However, tigrina and margay males seemed to be more sensitive than ocelot males. A significant (p<0.05) seasonal effect was observed only in margays, with higher levels observed during winter (June-August) in comparison to summer (December-February). A negative correlation between corticoid levels and ambient temperature (r = 0.52; p<0.05) was also found in margays. Corticoid excretion in ocelots and tigrinas tended to increase with ambient temperature increments, although a significant correlation was observed only in the ocelot (r = 0.46; p<0.05). For all data combined, correlation coefficients between corticoid levels and 1) body weight (r = -0.43), 2) testicular volume (r = -0.40), 3) total sperm cells / ejaculate (r = -0.20), and 4) % of normal sperm cells (r = -0.32) were all significant (p<0.05).

In conclusion, although maintained under similar environmental and husbandry conditions in captivity, different small-sized cat species appear to respond differently to various acute stress factors. There also were species differences in basal adrenal activity as determined by fecal corticoid analysis. Thus, fecal corticoid measurements may provide us a means of monitoring stress responses associated with the captive environment. Finally, although this study was not specifically designed to establish cause-effect relationships, there was a modest relationship between higher fecal corticoid concentration and impaired spermatogenic function in small felids kept in captivity.

ACKNOWLEDGMENTS

The authors thank the Curitiba Zoo and Itaipu Binacional for providing animals and assistance with sampling. We are also grateful to Laura H. Graham for the radioimmunoassay technical assistance. This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), the New Opportunities in Animal Health Sciences (NOAHS) Center and Friends of National Zoo (PONZ), British Airways, and The Philip Reed Foundation.

LITERATURE CITED


Table 1. Body weight, reproductive characteristics and fecal corticoid concentrations in male ocelots (L. pardalis, n = 3), tigrinas (L. tigrina, n = 3) and margays (L. wiedii, n = 3). Data are \( \bar{x} \pm SEM \).

<table>
<thead>
<tr>
<th></th>
<th>Ocelot</th>
<th>Tigrina</th>
<th>Margay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>14.3 ± 2.6(^a)</td>
<td>3.0 ± 0.3(^b)</td>
<td>3.4 ± 0.3(^b)</td>
</tr>
<tr>
<td></td>
<td>(41)</td>
<td>(41)</td>
<td>(41)</td>
</tr>
<tr>
<td>Testicular volume (cm(^3))</td>
<td>32.0 ± 8.5(^a)</td>
<td>3.9 ± 1.1(^b)</td>
<td>6.2 ± 1.5(^c)</td>
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<tr>
<td></td>
<td>(41)</td>
<td>(41)</td>
<td>(41)</td>
</tr>
<tr>
<td>Ejaculate volume (ml)</td>
<td>1.4 ± 0.6(^a)</td>
<td>0.3 ± 0.1(^b)</td>
<td>0.5 ± 0.2(^c)</td>
</tr>
<tr>
<td></td>
<td>(39)</td>
<td>(36)</td>
<td>(35)</td>
</tr>
<tr>
<td>Total sperm (× 10(^6))</td>
<td>137.9 ± 113.7(^a)</td>
<td>103.7 ± 91.2(^a)</td>
<td>32.0 ± 23.2(^b)</td>
</tr>
<tr>
<td></td>
<td>(37)</td>
<td>(36)</td>
<td>(35)</td>
</tr>
<tr>
<td>Motility Index **</td>
<td>77.5 ± 8.4(^a)</td>
<td>72.7 ± 13.9(^b)</td>
<td>70.5 ± 7.7(^b)</td>
</tr>
<tr>
<td></td>
<td>(41)</td>
<td>(40)</td>
<td>(37)</td>
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<tr>
<td>Normal sperm (%)</td>
<td>82.4 ± 7.7(^a)</td>
<td>53.1 ± 24.5(^b)</td>
<td>57.4 ± 16.4(^b)</td>
</tr>
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<td></td>
<td>(42)</td>
<td>(38)</td>
<td>(35)</td>
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<tr>
<td>-Primary defects (%)</td>
<td>4.9 ± 2.89(^a)</td>
<td>9.28 ± 12.30(^b)</td>
<td>15.79 ± 8.92(^c)</td>
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<tr>
<td></td>
<td>(42)</td>
<td>(38)</td>
<td>(35)</td>
</tr>
<tr>
<td>-Secondary defects (%)</td>
<td>12.92 ± 7.5(^a)</td>
<td>37.6 ± 19.8(^b)</td>
<td>26.8 ± 11.8(^c)</td>
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<tr>
<td></td>
<td>(42)</td>
<td>(38)</td>
<td>(35)</td>
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<tr>
<td>Fecal corticoids (ng/g wet feces)</td>
<td>424.7 ± 431.0(^a)</td>
<td>978.4 ± 1405.6(^b)</td>
<td>1215.2 ± 1266.7(^c)</td>
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<tr>
<td>-Overall mean</td>
<td></td>
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<tr>
<td>-Baseline concentration</td>
<td>253.1 ± 137.3(^a)</td>
<td>419.5 ± 195.2(^b)</td>
<td>421.9 ± 234.4(^b)</td>
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<td>(122)</td>
<td>(127)</td>
<td>(106)</td>
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<tr>
<td>-Peak concentration</td>
<td>1006.5 ± 563.4(^a)</td>
<td>2087.5 ± 2000.8(^b)</td>
<td>2181.9 ± 1339.4(^b)</td>
</tr>
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<td></td>
<td>(36)</td>
<td>(64)</td>
<td>(87)</td>
</tr>
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</table>

\(^a,b,c\) different superscripts differ significantly (\(P < 0.05\)).

\(^*\) (n) = number of samples considered in the analysis.

** Sperm Motility Index = \( \frac{\text{% motility} + (20 \times \text{sperm progressive motility})}{2} \)
AN OUTBREAK OF FELINE INFECTIOUS PERITONITIS IN CAPTIVE SERVALS (Felis serval): CLINICAL, PATHOLOGICAL, AND IMMUNOHISTOCHEMICAL FINDINGS

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Abstract

Feline infectious peritonitis was diagnosed in two captive servals (Felis serval) according to clinical, pathological, and immunohistochemical findings. They died 9 days apart, although the second serval, with effusive disease, was euthanatized during the acute phase of infection owing to a poor prognosis. Clinical signs consisted of abnormal behavior (1/2), anorexia, depression, abdominal distension, and leukocytosis (1/2). The two other servals in the colony remained asymptomatic. The main pathologic findings were severe fibrinopurulent to pyogranulomatous meningitis and ventriculitis in the serval with abnormal behavior and ascites, fibrinonecrotizing to pyogranulomatous peritonitis, and fibrinous perisplenitis in the serval with effusive disease. Feline Infectious Peritonitis virus antigen (spike protein) was detected in the affected tissues of both servals by immunohistochemistry using monoclonal antibodies.

Introduction

Feline infectious peritonitis (FIP) has been documented both in domestic and exotic cats.2 In captive exotic felines, FIP had been sporadically documented1 until a severe outbreak of FIP and other coronavirus-associated diseases caused death in 18 out of 35 cheetahs (Acinonyx jubatus) in a wildlife preserve in the early 1980s.1,3 Recently, FIP has been recognized as the cause of death in five of eight captive adult European wildcats (Felis silvestris) during a 10-yr period.6 To our knowledge, this is the second report on FIP in exotic felines diagnosed by immunohistochemistry.

Case Report

A group of four servals (Felis serval) was housed in a wire-fenced, outdoor enclosure at the Barcelona Zoo (Spain); stray cats did not have direct access to the enclosure. Indoor sleeping quarters were also available to the animals. In early 1993, a 2-yr-old male serval (No. 1) stopped entering the indoor part of the enclosure three days prior to death, what was considered an abnormal behavior. Five days later, an 8-yr-old serval (No. 2) began to show anorexia, depression, and a moderate abdominal distension. Hematology revealed leukocytosis and moderate anemia. This animal had been implanted with melengestrol acetate for contraception purposes. A differential diagnosis of pyometra and FIP was made. Exploratory laparotomy was performed and effusive FIP-like lesions were seen. Due to a poor prognosis, serval No. 2 was euthanatized 9 days after the death of its cagemate. The two other servals remained asymptomatic; one of these animals had a pre-epizootic, negative feline coronavirus IFI antibody titer (1:25).

At necropsy, no gross lesions were seen in serval No. 1. Serval No. 2 had approximately 100 ml of...
a yellow-opaque fluid in the abdominal cavity, and multiple, firm, 1-3 mm white-yellowish nodules scattered throughout the abdominal serosal surfaces. There was a marked fibrinous perisplenitis. Microscopically, serval No.1 had a fibrinopurulent ventriculitis in the brain, with associated mononuclear perivascular cuffing; severe pyogranulomatous leptomenigitis; and focal pyogranulomatous nephritis. Serval No. 2 had fibrinonecrotizing to pyogranulomatous lesions in the mesentery and serosa of the liver and gall bladder, as well as fibrinous perisplenitis with focal pyogranulomatous-necrotizing splenitis.

Paraffin-embedded tissue sections from both servals were screened for the presence of FIP virus antigen by the avidin biotin peroxidase complex method using monoclonal antibodies to the spike protein of FIP virus. Positive staining was found in scattered macrophages within the aforementioned lesions of both servals.

Discussion

On the basis of specific immunohistochemical studies, FIP was diagnosed in two servals. Clinical signs were similar to those commonly found in cats with FIP, but a presumptive diagnosis of FIP could only be obtained in serval No. 1 by histopathology, and in serval No. 2 at the exploratory laparotomy. In the domestic cat, clinical signs and results of hematology, biochemistry, analysis of effusions, and coronavirus serology seems to have a poor sensitivity and specificity for the antemortem diagnosis of FIP. Previous to this report, immunohistochemical diagnosis of FIP had been reported in European wildcats (Felis silvestris). The degree of susceptibility of different feline species to feline coronaviruses is poorly understood. The explosive nature of the cited outbreak of FIP in cheetahs could be related to the extreme genetic monomorphism observed in this species, especially at the major histocompatibility complex. Servals (Felis serval), along with other felines, are included in a group of species with a moderate to high genetic diversity.

LITERATURE CITED

DISSEMINATED TOXOPLASMOSIS IN SUSCEPTIBLE ZOO SPECIES - A SPORADIC DISEASE?

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Abstract

Disseminated toxoplasmosis is a primary, highly fatal disease in some zoo species, mainly New World monkeys, prosimians, and Australian marsupials. High risk situations are the practice of feeding raw meat to susceptible species and the presence of stray cats within the zoo grounds. During the period 1992-1995, disseminated toxoplasmosis was a considerable problem at the Barcelona Zoo, with epizootic episodes affecting golden lion tamarins (Leontopithecus rosalia) and slender-tailed meerkats (Suricata suricatta). A high case-fatality ratio (apparently 100%) was observed. Unexpected death with no premonitory signs was a common outcome, so a rapid diagnosis at necropsy is often required to initiate an appropriate therapy.

Introduction

Toxoplasma gondii is a ubiquitous intestinal coccidian parasite both of domestic and wild cats; congenital and neonatal toxoplasmosis can be sporadically seen in zoo cats. The list of intermediate host species is wide, but their susceptibility to T. gondii varies considerably. Some of the most susceptible species can be commonly found in zoos throughout the world.

The purpose of this communication is to discuss some conflictive aspects about the occurrence of disseminated toxoplasmosis (DT) in susceptible zoo species, based mainly upon the literature and the authors’ experience originated from epizootic DT episodes in golden lion tamarins (Leontopithecus rosalia) and slender-tailed meerkats (Suricata suricatta) at the Barcelona Zoo (Spain) during the period 1992-1995. Spontaneous DT has been rarely reported in callitrichids, and meerkats were recently added to the list of susceptible zoo species.

Epizootiology

During the last years, DT at the Barcelona Zoo has frequently been a highly fatal epizootic condition in small breeding groups, although isolated cases also occurred. When we compared the number of cases of DT and avian tuberculosis (ATB), a disease with a high incidence at the Barcelona Zoo during the period 1990-1994, it was fairly evident that DT can become a serious problem.

High-risk situations for DT in zoos seem to be mainly the practice of feeding raw meat to susceptible species and the presence of stray cats within the zoo. It is easy to avoid the first high-risk situation; however, many reports of epizootic DT in zoo species have been thought to be the result of feeding raw meat. It may be an inappropriate food item for some zoo species because it can be a source not only of T. gondii, but also of other pathogens, such as Streptococcus zooepidemicus. Moreover,
feeding raw meat to zoo felines contributes to the presence of *T. gondii* in zoos.\(^7\)

If stray cats are a problem, then prevention is not always easy. For example, in our case, trapping cats was an unpopular procedure and there was always an ongoing release of cats within the park where the zoo is located. Therefore, it becomes a problem difficult to eradicate for the veterinarian. Stray cats had easy access to the enclosure of a group of meerkats with DT.\(^12\) Most reports on epizootic DT in herbivorous marsupials provided evidence of stray cat involvement in the transmission of *T. gondii*.\(^6,13,14\)

In the absence of these high-risk situations, other sources of infection (e.g., ingestion of mice or birds, transports hosts of oocysts)\(^7\) will most likely account, in our opinion, for enzootic DT. These other sources of infection seem to be sporadic or not major pathways for DT to occur, and, anyway, they are mostly the reflection of the presence of stray cats within a zoo. Ingestion of feral mice could be the source of infection in three golden lion tamarins (*Leontopithecus rosalia*) that died of DT at the Barcelona Zoo (Juan-Sallés et al., submitted).

Latency and reactivation of *T. gondii* in highly susceptible zoo species do not seem to be common. However, evidence has accumulated about the ability of even highly susceptible species to survive the acute phase of toxoplasmosis;\(^2,6,8\) therefore, latency and reactivation of *T. gondii* could account for some DT cases. For example, reactivation of *T. gondii* has been reported in an experimentally-infected owl monkey (*Aotus lemurinus*),\(^8\) and apparently in a Barbary macaque (*Macaca sylvana*) with previous, experimental inoculation of simian immunodeficiency virus.\(^16\) Although we could not confirm it, a litter of newborn meerkats could die of DT between two DT episodes;\(^12\) in such case, transplacentary or transmammary transmission from a latently infected dam would have been a likely source of infection. The possibility of *T. gondii* latency in highly susceptible zoo species makes this parasite an undesirable candidate to be introduced in wild populations of endangered species that are being reintroduced in their natural habitats, such as the golden lion tamarin.

The susceptibility to *T. gondii* varies between different species, perhaps depending on their ecology in the wild and the characteristics of their natural habitat:

1. The absence of felines in the habitats where lemurs and Australian marsupials evolved seems to be responsible for their high susceptibility to *T. gondii*.\(^2,7\)
2. The arboreal habits, and therefore the lack of contact with cat-contaminated food, of most New World primates has been incriminated as a factor contributing to their high susceptibility to *T. gondii*.
3. The feeding ecology of some susceptible species in the wild (the absence or sporadic presence of meat in their diet) seems to be a major contributing factor for their susceptibility to *T. gondii*. For example, the feeding ecology together with the arboreal habits of callitrichids would account for their extreme susceptibility. Surprisingly, there are very few reports on DT in callitrichids.\(^15\) Our experience both with zoo and pet callitrichids shows that DT can become a serious problem if high-risk situations occur.
4. The temperature and humidity influence on the survival of *T. gondii* oocysts; some species living in dry, desert regions, such as the gondi (*Ctenodactylus gondi*), have proved to be highly susceptible to *T. gondii*.\(^4\)

The feeding ecology of the slender-tailed meerkat as well as the characteristics of its habitat are perhaps good reasons for its apparently high susceptibility to *T. gondii*.\(^12\) Other mammals belonging to the Order Carnivora seem to have a better balanced parasite-host relationship; for example, DT
in bears and mustelines is mainly reported in neonatal or young specimens, whereas sporadic toxoplasmosis cases in foxes, raccoons, and red pandas are often associated with canine distemper.12

Pathology

DT can be tentatively diagnosed at necropsy if smears or touch imprints of organs with lesions are taken.2,12,13 This simple procedure is of great value to rapidly initiate a *T. gondii*-specific therapy before DT kills most animals in a group. Major gross findings that can be seen in DT cases are:

1) Lymphadenopathy and/or necrotizing are common findings at least in New World primates, prosimians, and meerkats (Juan-Sallés et al., submitted).4,5,12
2) Pulmonary edema and/or reddened, non-collapsed lungs (sometimes with pale foci), suggestive of interstitial pneumonia (Juan-Sallés et al., submitted).1,2,9,12,14
3) Hemorrhages, pale streaks or foci, and/or mineralization can be frequently seen in marsupials.1,13 Occasionally, severe myocarditis may be present.12
4) Gastrointestinal ulceration and hemorrhagic enteritis have been described in marsupials and callitrichids (Juan-Sallés et al., submitted),1 but they do not seem to be very common. However, intestinal lesions can become prominent microscopic findings (Juan-Sallés et al., submitted).1,5,14
5) Splenomegaly, multifocal necrotizing hepatitis, hydrothorax, ascites, and hydropericardium are sometimes present.1,5,9,12,13

Microscopically, DT affects most tissues; kidneys are rarely damaged. Lesions are often necrotizing with varying degrees of mixed inflammatory response, but some tissues have characteristic lesions. For example, acute to a subacute interstitial pneumonia and/or pulmonary edema are frequent in meerkats, marsupials, and New World monkeys (Juan-Sallés et al., submitted).1,2,12,14 The occurrences of syncytial cells in the lungs of zoo animals with DT and interstitial pneumonia is not described, but we did find them in meerkats and callitrichids (Juan-Sallés et al., submitted).12

*T. gondii*-like organisms become widely distributed through the tissues of animals with DT and can be readily seen in H&E sections of most DT cases. However, especially in peracute and acute cases, tachyzoites can be overlooked; this is also true for some severe lesions containing few *T. gondii* organisms.

DT is often consistent with a primary infection with *T. gondii*, whereas localized toxoplasmosis, fairly uncommon in susceptible zoo species, is more consistent with the reactivation of a latent *T. gondii* infection, at least in the mouse and man.3 The distribution of lesions in localized toxoplasmosis may reflect the common distribution of *T. gondii* tissue cysts in the muscular tissues and brain of latently-infected hosts.

Therapy

DT is rarely suspected antemortem since short clinical courses with nonspecific signs or unexpected deaths are common in highly susceptible species. Therefore, getting a rapid diagnosis at necropsy during epizootic episodes is the key to initiate an appropriate therapy. Harper and co-workers used five drug regimens in experimentally-infected squirrel monkeys (*Saimiri sciureus*); although the treatment began 48 hr after intragastric inoculation, two animals became parasitemic and recovered.9 Sulfamethoxazole alone or combinations of sulfonamides with trimethoprim or pyrimethamine were the most effective drugs in preventing clinical disease and death.9 The clinical course of DT in
meerkats seemed to lengthen when animals were treated with clindamycin, but it did not prevent death.\textsuperscript{12} Trimethoprim-sulfadiazine therapy was successful in clinical cases of suspected toxoplasmosis in Australian macropods, as well as in a binturong.\textsuperscript{10,13}

**Prevention**

Depopulating stray cats and discontinuing the practice of feeding raw meat to susceptible zoo species and large cats are major pathways to prevent DT from devastating valuable breeding groups of zoo species. Control of rodents and birds, often difficult, would be a minor pathway since they have to be preyed on to become infectious and, therefore, they would only account for sporadic DT cases.

**LITERATURE CITED**

YEAR-ROUND URINARY OVARIAN STEROID MONITORING IN A CLINICALLY HEALTHY CAPTIVE OWL-FACED GUENON (*Cercopithecus hamlyni*)

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Abstract

Owl-faced or Hamlyn’s guenons (*Cercopithecus hamlyni*) are native to eastern Zaire and Rwanda. Limited information is available regarding the natural history of this species in the wild. Field observations of other cercopithecine species provide some evidence for reproductive seasonality, although breeding may occur year-round in some wild populations.1 As of December 1996, the international zoologic population of owl-faced guenons included 55 (14.37.4) animals in 14 International Species Information System (ISIS) institutions.2 Despite their relative abundance in captivity, little published information exists describing their captive management, and there are no reports describing the reproductive biology of this species.

In May 1994, the Philadelphia Zoo received two male owl-faced guenons (one adult, one sub-adult) from an ISIS institution. In June 1994, the Zoo received an adult female from a second ISIS institution. For 14 days following the female’s arrival, the males remained physically isolated, but have visual, auditory and olfactory contact. Immediately following physical introduction and for a period of approximately 6 hr thereafter, both males repeatedly copulated with the female. The three animals were housed together for 3 mo, after which the subadult was separated from the group. During the 7 mo subsequent to initial introduction no copulatory behaviors were observed and the female exhibited no physical signs of ovarian activity (e.g., changes in perineal skin color or profile; overt menses). Physical examinations were performed on each of the animals in June and October 1994. The adult male was not ejaculated, nor was invasive reproductive examination performed upon the female. Physical, hematologic and serum chemical findings were unremarkable.

A longitudinal examination of urinary ovarian steroid metabolites was undertaken to 1) determine if the female was exhibiting ovarian cyclicity, and 2) characterize the basic features of the female reproductive-endocrine cycle. Freshly voided urine samples (1-5 ml) were collected daily over a 12-mo interval and stored frozen until analysis. Hormone determinations were performed at the Conservation and Research Center’s Endocrine Research Laboratory, Front Royal, Virginia. Urinary estrogen conjugates (EC) and pregnanediol-3-glucuronide (PdG) were analyzed as described previously;3 both assays were validated for use in owl-faced guenons. All urine samples were indexed by creatinine (Cr) measurement and hormone values were expressed as mass units/mg Cr excreted. The female became pregnant during the study, but experienced a first trimester abortion.

Longitudinal measurement of steroid metabolite excretion revealed that this female exhibited spontaneous year-round ovarian cycles with no evidence of reproductive seasonality. The overall mean nonconceptive ovarian cycle duration was 25.4 ± 2.5 days (n = 12 cycles; range 20 - 29 days), whereas the mean luteal and follicular phase durations were 15.3 ± 1.1 days and 10.6 ± 3.2 days, respectively. During nonconceptive cycles, EC concentrations increased ~7 fold over baseline follicular phase levels.
(3.96 ± 0.85 ng/mg Cr) to midcycle pre-ovulatory peaks (26.36 ± 5.27 ng/mg Cr) and declined rapidly thereafter to basal concentrations (Fig. 1). Urinary PdG concentrations remained low during the follicular phase (22.72 ± 9.51 ng/mg Cr), but rose rapidly around the time of the pre-ovulatory EC peak (Fig. 2). Peak luteal PdG concentrations (192.93 ± 88.11 ng/mg Cr) were achieved 5-10 days following midcycle EC peak and exceeded basal concentrations by ~9 fold (Fig. 3). During early pregnancy, both EC and PdG concentrations increased (EC, 16 fold; PdG, 100 fold) over follicular phase concentrations; a rapid decline in both metabolites reflected an abortion during the first trimester of pregnancy.

The general endocrine profiles of urinary EC and PdG provide an accurate representation of ovarian function, including the diagnosis of pregnancy and abortion, in the owl-faced guenon. Caution must be exercised in applying the data presented herein to other owl-faced guenons. Urine assays performed on other captive owl-faced guenons would substantially supplement this data set.

LITERATURE CITED


Figure 1. Urinary estrogen conjugates in a clinically healthy female owl-faced guenon. The EC profile on approximate Julian days 110 to 135 was concurrent with pregnancy an spontaneous abortion.
Figure 2. Urinary pregnanediol-3-glucuronide in a clinically healthy female owl-faced guenon. The EC profile on approximate Julian days 110 to 135 was concurrent with pregnancy and spontaneous abortion.

Figure 3. Mean ovarian cycle profile (over 1 yr) of a clinically healthy female owl-faced guenon. Data points recorded during pregnancy and abortion in this animal were excluded for mean ovarian cycle determination.
DISEASES OF LEMURS IN CAPTIVITY

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Abstract

Diseases of lemurs in captivity were identified by review of medical records, literature searches, and experience of Species Survival Plan (SSP) advisors. Diarrhea was one of the most common presenting complaints, and may be associated with parasitic (usually protozoal) or bacterial enteritis. Trichobezoars and dental disease also occur. Pneumonia is uncommon unless stress is a factor; cases with Klebsiella pneumoniae infection are often rapidly fatal. Two proliferative periosteal diseases have been reported, one associated with progressive renal disease and death. Integumentary system problems are limited, and most often are related to trauma. Renal disease is common in aged lemurs. Other urinary tract disease is uncommon. Diseases of the nervous system and cardiovascular system are less common, although seizures, cerebrovascular accidents, and aneurysms have occurred. Cases of toxoplasmosis (usually fatal) have been reported, most frequently in ground-dwelling species, such as ringtailed lemurs.

Introduction

The Malagasy prosimians include five extant families. However, this presentation will focus on lemurs because of their predominance in zoological collections and therefore, the predominance of medical information available. While prosimians are certainly susceptible to a wide variety of disease syndromes, discussion will be limited to conditions with unique significance to prosimians.

Methods

The material in this presentation has been collected from several sources. The initial data collection was generated by a detailed review of medical records of 287 black lemurs13 and an extensive multispecies literature review, which includes medical reviews for lemurs in captivity1,5,11,13 and in the wild.14 This was supplemented with case material presented to lemur SSP advisors, prosimian taxon advisory group (TAG) veterinary advisors, and field research veterinary advisors.

Gastrointestinal System

Loose or otherwise abnormal stools were the most common cause for medical intervention in two surveys of diseases of lemurs.5,11 Bacteria isolated from fecal cultures from lemurs with evidence of gastrointestinal disease include Yersinia enterocolitica,4 Campylobacter fetus jejuni, Salmonella typhimurium, E. coli,5 and Klebsiella pneumoniae.1 With cases of bacterial enteritis that are associated with profuse or persistent diarrhea, attention must be given to prevention of dehydration, electrolyte imbalances, and secondary systemic bacterial infections.

Hemochromatosis (hepatic iron storage disease) has historically been considered a common problem in lemurs in captivity. Hemochromatosis is the development of pathological changes in the liver as a result of excess iron storage. Incidences varied with species, with Eulemur macaco most commonly
affected, followed by *Varecia* and *Lemur catta*. The condition is suspected to be the result of the combination of excess dietary iron, excess ascorbic acid, and lack of tannins in the diet. Recognition of the mechanism of this problem has resulted in a reduced incidence. A common sequela to iron storage disease was the initiation of neoplastic transformation of the damaged cells, which resulted in a high incidence of hepatic neoplasia.\textsuperscript{1,11,20}

Prevalence of gastrointestinal parasitism varies. Parasitism may be a cause of diarrhea in captive lemurs; however, a survey of medical records on black lemurs indicated 61\% of fecal examinations positive for parasites were asymptomatic (no diarrhea). Protozoal infestations are more frequently associated with clinical diarrhea than nematode infestations; in the same survey 53/63 (84\%) of parasitic diarrheas were protozoal.\textsuperscript{13} Commonly identified organisms include *Entamoeba*, *Trichomonas*, *Giardia*, and *Balantidium*. Protozoal infestations respond to treatment with metronidazole (25 mg/kg b.i.d. for 7 days) or paromomycin (12.5 mg/kg b.i.d. for 5 days); potentiated sulfas and tetracyclines have also been used.

Common nematode parasites include organisms in the genera *Strongylus*, *Strongyloides*, *Gongylonema*, and *Physaloptera*, as well as ascarids. Gastrointestinal nematodes respond to a variety of anthelmintics (ivermectin 0.2 mg/kg p.o. or s.c. once, mebendazole 10-20 mg/kg p.o. for 3 days, thiabendazole 50 mg/kg p.o. once, and pyrantel 5-10 mg/kg p.o. for 3 days). *Physaloptera* may present a challenge for diagnosis and treatment. The eggs of this gastric nematode are shed intermittently and may be difficult to find in fecal flotations. In addition, it appears to be resistant to standard anthelmintic regimes. Treatment with ivermectin 0.2 mg/kg p.o. s.i.d. for 7 days, or levamisole 2.5 mg/kg p.o. s.i.d. for 14 days, have been successful.

Dental disease may occur in captive lemurs. Plaque and tartar accumulation is common, and tooth root abscesses are reported. Trichobezoars may occur, especially ruffed lemurs (*Varecia*). Surgical intervention may be required to alleviate gastric obstruction in severe cases. Regular administration (weekly or biweekly) of laxatives or lubricants (such as oral cat laxatives) will prevent the occurrence.

**Respiratory System**

Bacterial pneumonia in lemurs is not common under good management conditions; however, in stressful conditions, such as acclimating to a new environment, it can occur. Cases have been reported in newly captive animals that have been exposed to environmental changes. Clinical signs are as expected, and include fever, inappetence, and labored breathing. Incidences of *Klebsiella pneumoniae* pneumonia are often peracute and fulminant, resulting in rapid fatalities.

The incidence of tuberculosis in prosimians appears to be low, with eight cases in three species reported.\textsuperscript{7,10,16,19} Two cases involved ringtailed lemurs living in a North American zoo, the others were recent imports (mongoose lemurs) or in captivity in Madagascar. Lesions were described as typical tuberculosis granulomas in lungs, liver, spleen, kidney, and lymph nodes, with acid fast bacilli.\textsuperscript{7,10,16,19} Culture results from two cases produced “typical human tubercle bacilli”\textsuperscript{16} and *M. tuberculosis*.\textsuperscript{10} Of the three infected mongoose lemurs tested by intradermal tuberculin injection, one was positive, one equivocal and one negative. Among black lemurs in North American zoos, 222 intradermal tuberculin tests in 112 individuals were reported, with no positive results. The current Prosimian Taxon Advisory Group testing recommendation is to use 0.1 ml modified old tuberculin intradermally.

Pleural effusion has been reported several times in ringtailed lemurs. Effusions have been related to systemic fungal disease,\textsuperscript{6} but they have also apparently been spontaneous. Spontaneous cases have been
managed conservatively with repeated thoracocentesis, and have resolved. Cytological examination and culture of aspirates have identified the fluid as a sterile transudate.

**Musculoskeletal System**

Two significant skeletal diseases have been reported in lemurs. A familial bone disease involving periarticular new bone formation coincident with progressive renal failure has been described in black lemurs. This disease, described as periarticular hyperostosis (PH), is characterized by nonpainful enlargement of knee and ankle joints initially, with concomitant progressive renal disease. Proliferation of bone and degeneration of kidneys continue throughout the course of illness. Typically, progress to death due to end stage renal disease or euthanasia in 6-12 mo. The etiology of PH in lemurs is not known.

A second syndrome was described in ruffed lemurs (*Varecia variegata*), and is characterized by irregular periosteal bone proliferation along the diaphyses of long bones (primarily tibia, fibula, radius, and ulna). This syndrome has been theorized to be a nonspecific inflammatory response.

Age-related degenerative arthritis is uncommon in prosimians. A variety of analgesic drugs have been used with apparent success. Traumatic skeletal injury also occurs infrequently, and seems to respond well to standard techniques of fracture repair. Either external stabilization by casting or internal fixation with pins works well. Skeletal trauma to small appendages (digits, distal tail) is often managed by amputation, with few complications.

**Integumentary System**

A common cause for medical intervention for the integumentary system, as well as for all systems combined, is traumatic injuries to the skin (lacerations and abrasions). Lacerations are usually superficial fight wounds, but may be caused by enclosure features as well, and may occasionally be deep and involve other tissues. Most heal well with little intervention; however, suturing and prophylactic antibiotics may be appropriate. Bite wounds inflicted by lemurs do not seem to be prone to produce an abscess.

**Urogenital System**

Renal disease is common in aged lemurs. Glomerulonephritis, glomerulosclerosis, and chronic interstitial nephritis are diagnosed at postmortem examination of aged individuals. Urethral obstructions also have occurred but are not common. Urethral obstructions due to coagulum plugs may occur after electroejaculation. In these cases, coagulum material produced by electroejaculation became lodged in the urethra at the ischial arch, and required surgical intervention for removal.

Reproductive complications are not common. Dystocia is rare, and is usually associated with multiple births (note - multiple births are normal in *Varecia*). A vaginal prolapse in a black lemur was associated with multiparity (11 offspring in seven pregnancies); correction was attempted unsuccessfully with several surgical interventions, and the animal was eventually euthanatized. Vaginitis and vaginal discharge have been associated with a variety of bacteria (*Proteus, Staphylococcus, Streptococcus, E. coli, Citrobacter*) and have responded to systemic antibiotics or antiseptic flushes.

**Nervous System**
Seizures may occur in relation to several generalized or system illnesses. Epilepsy (recurrent seizures of unknown etiology) may occur rarely, and in cases where the incidence of seizures is high, anticonvulsant therapy may be warranted. Oral phenobarbital has been effective in a black lemur.

Both viral and bacterial encephalitis has been diagnosed. A case of nonsuppurative meningoencephalitis in a ruffed lemur was caused by a herpesvirus. The clinical signs associated with the disease; included intermittent rear limb lameness, progressing to seizures, coma, and death. Bacterial meningitis and encephalitis caused by Klebsiella pneumoniae have been diagnosed in a ruffed lemur and a black lemur.

**Cardiovascular System**

Dissection and rupture of aortic aneurysms has been one of the most common causes of mortality among captive lemurs in Madagascar. Aneurysms occurred as the result of migration of the parasite Spirocerca lupi. Dissecting aneurysms and cardiac tamponade have occurred in crowned and black lemurs; in the black lemur, the aneurysm was suspected to be secondary to hypertension based on signs of cardiac hypertrophy. Atherosclerosis, myocardial scarring, thickening of aortic valve leaves, “heart failure,” and myocardial infarction have been recorded in pathology surveys.

**Systemic Diseases and Miscellaneous Conditions**

Lemurs are quite sensitive to fatal disease associated with Toxoplasma gondii infection. Malaria parasites have been identified in blood samples of Eulemur species in Madagascar. Several species of Plasmodium have been described; these organisms are not pathogenic in the lemur hosts, and do not infect humans. Toxicoses are not common. Nightshade toxicosis and aflatoxicosis have been reported.

**LITERATURE CITED**


MEDICAL MANAGEMENT OF MEGACHIROPTERANS

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Abstract

The Megachiroptera includes a single family of fruit- and nectar-feeding bats, the Pteropodidae. Several species are endangered and many are threatened with extinction. The major disease concerns for captive management of megachiropterans are traumatic, nutritional and viral. Several important zoonotic viral diseases have recently been associated with megachiropterans.

Introduction

The following emphasizes important aspects of the medical management of megachiropterans; a more comprehensive review is forthcoming.6

The mammalian Order Chiroptera is subdivided into Microchiroptera and Megachiroptera. The Megachiroptera includes a single family of fruit- and nectar-feeding bats, the Pteropodidae, with 42 genera and 166 species.9 This family is confined to the subtropical and tropical regions of the Old World, east to Australia and the Caroline and Cook islands.11 The term “flying fox” is generally applied to the large bats of the genera Pteropus and Acerodon while many of the other species are labelled “fruit bats.”

The major disease concerns for captive management of megachiropterans are traumatic, nutritional and viral. When provided with appropriate nutrition and environmental requirements, these mammals are remarkably problem-free.

Rules and Regulations

In 1994 there were 12 megachiropteran species in North American zoological institutions. There are seven flying fox species listed on Appendix 1 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES): Truk (Pteropus insularis); Marianas (P. marniannus); Ponape (P. molossinus); Mortlock (P. phaeocephalus); large Palau (P. pilosus); Samoan (P. samoensis); and insular (P. tonganus).7 All other megachiropterans belonging to the genera Pteropus and Acerodon are listed as Appendix 2.7

In the United States all Pteropus spp. are classified as injurious wildlife and, therefore, require a permit from the US Fish and Wildlife Service for their possession. This permit requires they be housed and transported in double-wired enclosures.

All bats require a permit from the Centers for Disease Control and Prevention (CDC) for importation and movement within the United States.

Anesthesia
In island flying foxes (400-600 g) ketamine alone (30 to 37.5 mg/kg i.m.) produced short-term chemical restraint, but poor muscle relaxation and struggling during recovery. A xylazine (2 mg/kg i.m.)/ketamine (10 mg/kg i.m.) combination produced short-term immobilization (30 min) with good muscle relaxation and quiet recovery.

Inhalation anesthesia is recommended for both short- and long-term restraint. Isoflurane (5% by mask decreased to 2.5% when relaxed) provides rapid (1-2 min) induction and recovery, as well as easily adjusted anesthetic depth. Megachiropterans > 350 g are intubated with a ≥ 2 mm internal diameter endotracheal tube. Good anesthetic relaxation and dorsal recumbency facilitate intubation. For visualization of the glottis, gauze is placed around the upper and lower jaws to open the mouth and the tongue is displaced forward. A laryngoscope with a small straight pediatric blade is used for illumination. Topical anesthetic applied to the glottal opening decreases reflex coughing. Glycopyrrolate (0.01 mg/kg i.m.) administered prior to induction will reduce the sometimes profuse pharyngeal secretions. Heart rate and rhythm, and peripheral blood flow are monitored with a Doppler flow probe secured over either the tibial artery behind the knee or the pedal artery on the palmar surface of the feet. Temperature is monitored using a probe placed in either the rectum or esophagus; the latter is preferred because it more accurately reflects core body temperature. To prevent hypothermia the wings are folded to the body, the animal placed on a circulating water blanket and where possible wrapped in either a blanket or bubble-wrap. Avoid electric or chemical heating pads which have caused severe burns. For recovery, bats are wrapped in a drape and left in a quiet cage to prevent struggling and wing-flapping; the animals are usually sufficiently recovered when they crawl out of the wrap.

Venipuncture

Venipuncture is facilitated by anesthesia. In megachiropterans ≥ 150 g large blood volumes are collected from the median artery or vein on the lateral aspect of the humerus. Small blood samples are collected into microhematocrit tubes from the cephalic vein, which extends along the leading edge of the patagium, and from pedal veins. The author prefers heparinized blood samples for biochemical analysis; this allows the blood to be centrifuged immediately after collection and the plasma removed from the blood cells and frozen until analysis. If the plasma remains in contact with the red cells for even a few hours there will be marked spurious elevations in potassium and decreases in sodium, chloride and glucose concentrations.

Preventive Medicine

All bats are examined at least once/yr. There are no currently recommended vaccinations. Parasite control is directed at detection and treatment, and appropriate and frequent sanitation of housing. For transport, the CDC requires that bats be free of rabies and Histoplasma capsulatum. Bats from captive collections should be quarantined a minimum of 90 days. During quarantine a minimum of three fecal flotations should be performed to detect nematode ova. Additionally, pooled rectal fecal samples are cultured for H. capsulatum.

Parasitic Diseases

The nematode Toxocara pteropodis is found in south-east Asian and Australian flying foxes. Adult parasites live in the upper gastrointestinal tract of suckling pups. Eggs passed in the feces of the pups are ingested by adult flying foxes during grooming and feeding. Fertile eggs are ovoid to spheroid, 80-110 μm in diameter and the outer layer is pitted. The ova are bulkier than those of related ascaridoids
because of a thicker external coat which, while not providing mechanical strength, is thought to protect against dessication. The eggs hatch and the larva pass through the portal system to the liver where they encyst. In adult male bats the larvae do not develop any further. In the adult female bats the larvae are activated at the end of parturition and during lactation to migrate to the mammary gland where they pass in the milk to the suckling pup to complete the cycle. The adult worms are shed from the pup when it ceases to suckle and begins to eat solid food. There are usually only a few adult worms/pup (< 5) and they rarely cause morbidity or mortality. A captive island flying fox pup died from an intestinal volvulus associated with 20+ worms.\(^3\) Despite these examples, it is questionable whether it is necessary to treat *T. pteropodis* in captivity. *T. pteropodis* was once thought to be a possible cause of human hepatitis but this has subsequently been disproved.

**Viral Diseases**

Until recently there was very little information on megachiropteran viruses. However, recent events in Australia and Africa have precipitated an increased interest because of their suspected role in harboring several zoonotic viruses.

**Lyssaviruses (Rabies).** Bat lyssaviruses have been reviewed by Constantine.\(^1\) Within the family Rhabdoviridae the genus *Lyssavirus* contains five serotypes: classic rabies virus (serotype 1), Lagos bat virus (serotype 2), Mokola virus (serotype 3), Duvenhage virus (serotype 4) and European bat virus (serotype 5).\(^2\) All can cause rabies or rabies-like diseases in infected animals.\(^2\) In 1996 in Australia a 5-mo-old female black flying fox (*Pteropus alecto*) was found under a tree unable to fly.\(^2\) Histological examination of brain tissue revealed a severe nonsuppurative encephalitis. A second case, in 1995, was identified after retrospective examination of archived paraffin-embedded tissues.\(^2\) The affected animal, a juvenile female of the same species, was reported to be more aggressive than usual and was euthanatized. Although the encephalitis was very mild, many eosinophilic, cytoplasmic inclusion bodies were present in various parts of the brain. An indirect immunoperoxidase test for rabies performed on paraffin-imbedded tissues using an antirabies monoclonal antibody showed positive reactions over wide areas of the brains of both bats. Additionally, similar reactions were observed in neuronal cytoplasmic in gastrointestinal nerve plexuses. Electron microscopy examination of ultrathin sections of hippocampus from the 1996 bat showed aggregates of viral nucleocapsids within the cytoplasm of cell bodies. Virus was isolated from a weanling mouse injected with tissue homogenate. Sequence comparisons were done by using the nucleocapsid proteins of known lyssaviruses and the virus now designated pteropid lyssavirus. Phylogenetic analysis of both the nucleotide and amino acid sequences showed that the virus is closely related to the European bat virus as well as the classic street rabies strains. The virus has subsequently been identified by immunohistochemical techniques in five bats in three different virus isolations.\(^2\) Some of these bats were from another species, the little red (*P. subscapulatus*) and from locations as far apart as 1,700 km along the Australian east coast. Subsequent to the discovery of the virus in flying foxes the virus has been isolated from a Queensland woman in which the virus produced neurological disease, coma and death. The woman was a rehabilitation worker who was scratched by a sick fruit bat. Studies at CDC indicate that human, veterinary and subunit vaccines protect against the lyssavirus and sera of rabies-vaccinated people neutralizes the virus, as does hyperimmune reference sera.

**Bat Paramyxovirus (Equine Morbillivirus).** Two outbreaks of a previously unknown disease occurred in humans and horses in 1994 in Queensland, Australia.\(^15\) The outbreaks occurred within 1 mo of each other at two locations approximately 1,000 km apart. In one incident 14 of 21 infected horses died or were euthanatized because of severe acute respiratory disease. One of two humans with less
well defined clinical signs also died. In the second incident, one person and two horses died. A paramyxovirus isolated from four of the horses and one human was designated equine morbillivirus. Serological sampling (neutralizing antibody) demonstrated antibody in all four Australian flying fox species; spectacled \((P. conspicillatus)\), black \((P. alecto)\), little red \((P. scapulatus)\) and grey-headed \((P. poliocephalus)\) with a prevalence rate of about 9%. A virus indistinguishable from equine morbillivirus and defined as bat paramyxovirus was subsequently isolated. This virus has not been associated with disease in either flying foxes or in persons who have had extensive exposure to bats. Research is ongoing into the biology of this virus and its mode of transmission. The author is working with Dr. Peter Young from Australia to perform a serological survey of the flying fox collection at the Lubee Foundation. The author subsequently plans to coordinate compilation of samples from other collections in North America for testing.

**Filoviruses (Ebola).** Filoviruses are best known for their propensity to cause fatal hemorrhagic disease of humans with person-to-person spread. This lethality suggests primates are incidental victims of infection and not true reservoir hosts. To determine the possible source a wide range of vertebrates, invertebrates and even plants were injected with Ebola Zaire isolated from a human patient. This study was based on the principle that animals able to survive with circulating virus for prolonged periods without becoming ill were suspected reservoirs. The virus replicated in both microchiropteran and megachiropteran bats. Virus antigen was detected in the lung tissue of one bat and on day 21 from the feces of a megachiropteran \((Epomorphus wahlburgi)\).

**Kasokero (Yogue).** A virus related to the unclassified virus Yogue was implicated as the cause of a mild to severe illness in four laboratory workers in Uganda. The virus was originally isolated by mouse inoculation from the blood of Egyptian fruit-bats \((Rousettus aegyptiacus)\) collected from Kasokero cave in Uganda. Serological studies concluded the isolates from both bats and laboratory workers were strains of the same virus.

**Nutritional**

Nutrition is a major problem area in the captive management of bats. This is a reflection of bat diversity, limited knowledge of nutrient requirements, the small size of most bats and adaptation for flight.

**Hypovitaminosis E.** Hypovitaminosis E has been associated with a dilated cardiomyopathy in flying foxes. The animals had been in captivity approximately 2-3 yr prior to the development of clinical signs. Significant findings included unmeasurable plasma vitamin E levels in affected bats and low to unmeasurable levels in many bats without dilated cardiomyopathy \((0.10 \pm 0.18 \mu g/ml)\). Tissue levels of \(\alpha\)-tocopherol were much lower in the affected than the unaffected bats. Additionally, \(\gamma\)-tocopherol levels were undetectable in affected bats. The diet was calculated to contain 56 IU/kg (dry basis) of vitamin E. Increasing dietary levels to \(\geq 240 \text{ mg/kg} \) appeared to resolve the problem. An important natural source of vitamin E is green leaves. Although large flying foxes have traditionally been thought to feed exclusively on fruits and/or flowers, it has recently been shown that some species chew leaves and ingest the fiber-free juices. These leaves may be an important source of essential nutrients lacking in a purely frugivorous diet. Captive flying foxes have been observed crawling to the ground to consume or chew grasses.

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Abstract

Visceral larval migrans (VLM) caused by nematode parasites in the genus *Baylisascaris* has been described in a wide variety of mammalian and avian species. Experimental infection of nonhuman primates with *B. procyonis*, the raccoon ascarid, has been well described and increasingly natural infections of captive primates are being reported. Previously, the only report of *Baylisascaris* sp. infection in callitrichids was of *B. columnaris*, the skunk ascarid, in several species of marmoset (*Callithrix* sp). In this report we describe cases of VLM due to *Baylisascaris* sp. in golden-headed lion tamarins (*Leontopithecus chrysomelas*) from two zoos. Observations regarding exhibit design and tamarin behavior possibly predisposing to infection in this species are also discussed.

Two histologically confirmed cases occurred at the Salisbury Zoo in Salisbury, MD. These tamarins were housed in a screen-topped, outdoor exhibit with abundant overhanging foliage which was shared with a two-toed sloth and several iguanas. In March of 1996, an 8-yr-old male golden-headed lion tamarin was examined for a recent onset of ataxia and a mild head tilt. Over the next 5 mo partial improvement was observed with intermittent courses of antibiotics and prednisone given orally. In August of 1996, the monkey’s condition worsened and it could no longer feed itself; euthanasia was performed. At about the time this animal was euthanatized, it’s cagemate, a 7-yr-old female began showing similar neurologic signs. Treatment with antibiotics and prednisone resulted in only slight improvement. Euthanasia was performed in September 1996. Histologic examination of both monkeys revealed focally extensive areas of granulomatous and eosinophilic encephalitis which was most severe in the cerebellum and occipital lobes of the cerebrum. In both animals, foci of inflammation contained cross sections of nematode larvae ranging from 30-67 μm in diameter with a single pair of prominent lateral alae and paired triangular excretory columns that were smaller than the central intestine. These findings were considered to be consistent with *Baylisascaris* sp. Lesions consistent with parasite migration tracts were also observed in the spinal cord of the male and the optic nerve and retina of one eye in the female tamarin. Review of clinical and necropsy records revealed two other tamarins over the preceding 3 yr with similar neurologic signs, one of which had a granulomatous and eosinophilic encephalitis consistent with larval migrans; no larvae however were demonstrated histologically. Raccoons had been a persistent problem within the park despite an aggressive trapping program and several raccoon latrines were observed adjacent to the tamarin exhibit. Fecal flotation of raccoon feces obtained from one of these latrines contained large numbers of ascarid ova. The two-toed sloth has remained asymptomatic.

Additional cases involve a family group of 3 golden-headed lion tamarins from the Los Angeles Zoo housed in an outdoor exhibit with a chain-link top and overgrowing foliage which allowed debris to fall
into the exhibit. This exhibit also contained white-fronted marmosets separated from the tamarins by a wall. Between September 1992 and September 1993 all 3 tamarins in this group began to display neurologic signs characterized by a head tilt, intention tremors, weakness and ataxia. Hematologic and serum biochemical analyses as well as routine radiography were all within normal limits. Animals were treated with varying regimes which included prednisone and antibiotics and had a variable clinical course characterized by periods of improvement and resolution followed by return of clinical signs. In October of 1994, magnetic resonance imaging was performed on two animals, including the most severely affected monkey, with negative results. All three tamarins had negative serologic tests for toxoplasmosis and lymphocytic choriomeningitis virus. The most severely affected animal had negative results for blood lead. In July 1994, all 3 tamarins were treated weekly for 4 wk with oral ivermectin (0.03 ml of 1% ivermectin solution/monkey) because of suspected VLM. In August 1994, the tamarins were removed from the exhibit because of suspected repeated ascarid infection associated with soil contamination. In November of 1994, a 2.5-yr-old male from this group was euthanatized because of persistent, severe clinical signs. A complete necropsy was performed and histologic examination of the brain demonstrated a granulomatous meningoencephalitis which was most severe in the cerebellum, basal ganglia and occipital lobes. Cross sections of a larval nematode parasite consistent with Baylisascaris sp. were demonstrated in inflammatory foci. The remaining 2 tamarins eventually recovered sufficient neurologic function to breed and raise offspring. Skunks and raccoons had been persistent pests within the zoo. Fecal flotation of skunk feces obtained within the park have been positive for ascarid eggs. The white-fronted marmosets within the enclosure have remained asymptomatic.

These are the first reports of Baylisascaris sp. larval migrans in golden-headed lion tamarins. Cases from the Salisbury Zoo were most likely due to Baylisascaris procyonis based on recurring problems in controlling wild raccoon populations and on the discovery of infected raccoon latrines adjacent to the exhibit. At the Los Angeles Zoo, cases could have been due to either B. procyonis or B. columnaris because of recurring problems with skunks as well as raccoons. Clinical signs common to both groups of tamarins included a head tilt and ataxia. As is evident from these clinical histories, long clinical courses extending over many months may be observed in affected tamarins. While Baylisascaris sp. infection was not confirmed in the two surviving tamarins from the Los Angeles zoo, VLM is the most likely explanation for the clinical signs observed in these animals. Based on this observation it appears that some golden-headed lion tamarins may be able to survive infection with Baylisascaris sp.

In both zoos tamarins were housed in outdoor exhibits that provided overhead access by wildlife. Open outdoor exhibits, such as these with dense overhanging foliage may create situations that are attractive to raccoons and subsequently predispose an exhibit to contamination with Baylisascaris sp. eggs. In particular, raccoons are known to create latrines in wooded areas at the bases of trees, tree forks and raised horizontal surfaces. These cases emphasize the need to aggressively control raccoon and skunk populations in zoological parks and to design exhibits which have a minimal chance of becoming contaminated by feces containing eggs of Baylisascaris sp.

Of particular interest is that in both zoos tamarins were housed adjacent to or with other primate species that did not demonstrate clinical signs of infection with Baylisascaris sp. Keepers from both zoos reported subjectively that the tamarins would spend greater amounts of time on the ground foraging compared to the other primate species. Such a difference in behavior indicates that the tamarins might have contact with a greater number of ascarid eggs with an increased likelihood of VLM. Alternatively, golden-headed lion tamarins may be naturally more susceptible to disease caused by Baylisascaris sp. migration.
ACKNOWLEDGMENTS

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LITERATURE CITED

LEPTOSPIROSIS: AN UNDER-REPORTED DISEASE IN ZOO ANIMALS?

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Abstract

Leptospirosis, the disease caused by the spirochete Leptospira interrogans, is a zoonosis with worldwide distribution. L. interrogans is known to infect and cause disease in a broad range of mammalian species. A great deal of work and research has been done on this agent in domestic animals. The development of a vaccine against the five most common serovars (L. pomona, L. hardjo, L. icterohaemorrhagiae / copenhageni, L. grippotyphosa, L. canicola) has greatly decreased the incidence of this disease problem in vaccinated populations. As a result, although aware of it, many veterinarians may have little practice experience with this pathogen.

Classically, after being shed by a carrier host into the environment, organisms gain entry into the susceptible host via mucocutaneous or percutaneous routes. The spirochetes multiply, and are spread hematogenously and localize in the liver, kidney and uterus. In the acute form of the disease, particularly with certain serovars (e.g., L. canicola) which can produce a hemolysin, hemolytic anemia and icterus are prominent gross manifestations of the infection. Acute hepatic injury from bacterial localization within hepatocytes can cause elevation of liver enzymes and hyperbilirubinemia. Following the hepatic phase of the disease, organisms localize in the kidney leading to focal to diffuse tubulonephritis. In addition to hepatic and renal involvement, organisms may also localize to the lungs, heart and brain with subsequent inflammation in these tissues. Chronically infected tissues are frequently infiltrated by lymphocytes, plasma cells, and fibrous connective tissue. Subclinical infections of adults can result in abortions or neonatal losses and have a significant impact on reproduction programs.

Diagnosis of leptospirosis is ideally achieved through the collection of paired serum samples and the demonstration of a rise in titer against a specific serovar. Examination of urine by phase contrast microscopy and visualization of the spirochetes or, evaluation of formalin fixed urine by electron microscopy are other methods of diagnosis. Fluorescent antibody (FA) testing of fresh urine or fresh or frozen tissue collected at the time of necropsy will confirm the presence of leptospiral antigen. Cultivation of leptospires is problematic due to the fragile and fastidious nature of the organism. Of all diagnostic methods, it is the one with the lowest yield.

As is true of many other diseases, the diagnosis of leptospirosis in zoo animals presents a serious challenge. While serologic testing is always the preferred method of diagnosis, reluctance to immobilize zoo species for repeated blood sampling at fixed time intervals often makes it impossible to obtain paired samples to demonstrate a rise in titer. In addition, zoo animals may die with no antecedent clinical work up or blood samples temporally related to the time of death. Vaccination of animals in the absence of prior baseline serology can further complicate interpretation of serologic results. In the absence of serologic data, diagnosis often rests on histopathologic evaluation of tissues. In cases where animals are in an autolysed condition, the success of culture attempts is decreased and histopathologic
interpretation of changes in hepatocytes and renal tubular epithelium is more difficult. Silver stains may be used on fixed tissue sections to detect leptospires but this technique too is fraught with complications. Immunohistochemical identification of leptospires exists but is not yet widely available. Financial limitations may impede the ability of an institution to routinely submit fresh tissues for FA as a screening method. Physical lack of freezers may make it impossible to preserve tissue samples at -70 °C for FA testing if indicated by histopathology. Most important of all, in addition to the previously described handicaps, leptospiral infections in non-domestic species may not “fit” classic descriptions of the disease. Atypical presentations of the disease may lead both the clinician and pathologist to overlook this diagnosis and underestimate the impact this disease may be having on a zoological collection.

At the Wildlife Conservation Society, the loss of a red panda (*Ailurus fulgens*) resulted in a heightened awareness of this disease threat and also raised two very important issues. First, although it was impossible to do serologic testing on the panda which died acutely with massive pulmonary hemorrhage and renal tubular necrosis and hepatocellular dissociation, silver positive organisms were demonstrated on histologic sections and FA was also positive on liver and kidney. Serology performed on the exposed cagemate was initially reported as negative by the diagnostic laboratory. Since it was possible the serovar infecting the red panda was not necessarily the same as the five serovars seen and tested for in domestic species, serologic testing for an additional 12 serovars was performed. A rise in titer against *L. autumnalis*, a serovar most commonly associated with rodents, was demonstrated. Given their location within a large urban park, the red pandas could readily have been exposed to leptospirosis via rodent vectors, in spite of rigorous attempts at rodent (mice/rats) control within the confines of the zoo.

This case raised concerns as to how many cases might previously have gone undetected. Up until that time, all leptospira serologic testing had been limited to the five common serovars. As a result of this case, all 17 serovars are routinely tested for now. However, the limitations of even the extended panel must be recognized, given the fact that over 200 pathogenic serovars have been described and can potentially be transmitted by any mammal.

The detection of an “unusual” serovar raised a second issue. The red panda had not been vaccinated against leptospirosis due to the risk of using commercially available multivalent canine vaccines and inducing canine distemper. However, even if a leptospire bacterin had been used, it might not have provided any protection against the specific serovar causing disease in this individual as *L. autumnalis* was not included in any commercial bacterin. This prompted discussions as to the logic of vaccination programs currently used in zoological collections.

The Department of Pathology began a retrospective study and reviewed 10 yr of pathology records for known or suspected cases of leptospirosis. Tissues from incoming “sudden death cases” were submitted for FA, and culture, and silver stains were routinely performed on tissue sections. Twenty cases, involving 16 species of exotic mammals (Table 1), housed both in indoor and outdoor exhibits, were confirmed by FA testing of fresh tissues collected at necropsy or those available in the frozen archive library.

Several cases (red panda, Senegal bushbaby, degu, sugar glider) presented with acute hemolytic crisis very similar to the syndrome reported in domestic animals. Of greater interest, however, were those cases which exhibited no detectable gross lesions at necropsy as was the case with most of the hoofstock neonates. “Poor-doer” calves are common necropsy submissions in zoological collections. Failure to
nurse, hypothermia and maternal neglect are often contributory factors leading to death. In the absence of specific gross findings, unless the prosector has a high index of suspicion for leptospirosis, it is possible that appropriate diagnostic tests may not be performed. As the leptospiral status of the dam is often unknown, failure to pursue the diagnosis in neonates may result in under-reporting of the disease as a whole. This can have serious implications for breeding herds and captive propagation programs.

The loss of four sun squirrels (Heliosciurus gambianus) challenged another “classic” concept of leptospirosis. Rodents have generally been reported as carrier species and are considered one of the main sources of infection for other mammals. And yet, all sun squirrels died with massive peracute leptospiremia and tissue necrosis. This particular exotic rodent species, at least, proved to be extremely susceptible to the disease.

These preliminary studies on leptospirosis in zoo animals have raised more questions than answers and challenged many basic assumptions about the disease. Certainly all institutions should already have stringent pest control programs in place. Protecting food and water stations from contamination is standard procedure in zoos. However, from a realistic point of view, no matter how intensive a zoos’ rodent/feral mammal control program might be, it is unlikely that introduction of this disease will be prevented. The problem is probably far more widespread than is currently suspected. For, even if zoos can prevent accidental exposure of collection animals by indigenous wildlife, there is still the risk of acquiring infection from subclinical carriers already within the herd. More aggressive screening of all incoming quarantined animals may help detect carriers prior to release. A combination of urinalysis, vaginal swabs and paired serologic tests is the best hope of diagnosing this disease. Concerns over the risks of handling or immobilizations necessary to perform these procedures need to be weighed against the potential risk of losing animals to the disease.

The importance of serologic testing cannot be over-emphasized. Without it, while the other tests will confirm the presence of leptospirosis in general, only culture or a rise in titer will help pinpoint the actual serovar involved. This information will be necessary before vaccination programs can be evaluated. Given the number of pathogenic serovars which might potentially be introduced to a zoo collection via feral mammals or in-house carriers, do currently available vaccines actually provide protection against disease? If so, for how long? There is evidence that exotic species do not respond to vaccination in a uniform manner (Dr. Bolin, personal communication). That being the case, it is obvious there will be a need for standardized clinical vaccine trails in key species before this question can be answered. Until then, it would be best not to be lulled into a false sense of security by an animals’ vaccination status. The identification and treatment of this disease problem within zoological collections will not be as straightforward as it might be in domestic species. But, given the importance of the individuals found in zoos and the impact fetal death or decreased reproductive performance may have on the future of certain species, additional studies are warranted.

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LITERATURE CITED

Table 1. Species in which Leptospira infection was confirmed between 1986-1996.

Animal
Red panda (Ailurus fulgens)
Roosevelt elk fetus (Cervus elaphus roosevelti)
Flying squirrel (Glaucomys sabrinus)
Pere David’s deer fetus (Elaphurus davidianus)
Indian rhinoceros fetus (Rhinocerus unicornis)
Barasingha fetus (Cervus duvauceli)
Sambar deer neonate (Cervus unicolor)
Sun squirrel (Heliosciurus gambianus) (n = 4)
Senegal bushbaby (Galago senegalensis)
Degu (Octodon degus)
Pygmy goat (Capra hircus)
Okapi (Okapia johnstoni)
Sugar glider (Petaurus breviceps)
Slenderhorn gazelle (Gazella leptoceros)
Wild mouse (Mus musculus)
Eld’s deer (Cervus eldi thamin)
Wild muskrat (Ondatra zibethicus)
EVIDENCE OF Brucella sp. INFECTION IN PACIFIC HARBOR SEALS (Phoca vitulina richardsii) AND CALIFORNIA SEA LIONS (Zalophus californianus) FROM PUGET SOUND, WASHINGTON, AND POSSIBLE MODE OF TRANSMISSION WITHIN Parafilaroides sp. LUNGWORMS IN PACIFIC HARBOR SEALS

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Abstract

The Washington Department of Fish and Wildlife and National Marine Mammal Laboratory have been conducting annual blood testing on Pacific harbor seals on Gertrude Island and California sea lions at Shilshole Bay in Puget Sound, WA.⁸ Forty-eight of 200 harbor seals (12%) and 4 of 84 California sea lions (5%) had antibodies to Brucella abortus by the Brucella card test, Brucella buffered plate hemagglutination test, Rivanol test and complement fixation procedure.¹ Three of the seropositive stranded harbor seals.⁵ This isolate is similar to a strain isolated from a seal in the United Kingdom.⁹ Necropsy of most affected seals revealed emaciation and lungworm infection. Histopathology revealed severe verminous pneumonia with intraleisonal Parafilaroides sp. lungworms.⁴⁶ In Giemsa-stained sections, large numbers of minute bacterial coccobacilli were detected along the inner membrane of the uterus and within the gut lumen of some of the Parafilaroides sp. adult worms.⁶ Immunohistochemistry using a Brucella abortus antibody revealed large quantities of antigen within the gut lumen and/or uterus of several of the Parafilaroides sp., corresponding to areas where bacteria were detected within the worms.⁶ Labeled Brucella antigen was also detected within the cytoplasm of leukocytes in the surrounding pulmonary parenchyma.⁶ Bacteria within the uterus and gut lumen of the worms had ultrastructural morphology typical of Brucella sp.² The significance of Brucella sp. infection to the lungworm and to the seal is unknown. Because seals frequent habitat shared by terrestrial mammals, and because seals are occasionally used for human consumption, there may be some risk of transmission to terrestrial wildlife, domestic livestock and humans.

LITERATURE CITED


GASTRIC SPIRAL BACTERIA AND INTRAMUSCULAR SARCOCYSTS IN FREE-RANGING AFRICAN LIONS (Panthera leo) IN NAMIBIA

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Abstract

Ten lions (six juvenile males, one adult male, and three adult females) from Etosha National Park, Namibia were presented for necropsy. Gross lesions included gunshot trauma and enteric cestodiasis in all individuals, and pulmonary nematodiasis in 3 individuals. The parasitism was not considered clinically significant.

Histologic evaluation of Steiner stained gastric tissue revealed two of four adults and one of six juveniles had moderate numbers of gastric spiral bacteria. The gastric spiral bacteria were located extracellularly in fundic and pyloric glands, and also intracellularly within parietal cells in the fundic region. The organisms were 4-8 µm long by light microscopy, and 0.53-0.73 µm wide with a periodicity of 0.50-0.67 µm by transmission electron microscopy (TEM). The bacteria had blunted ends with multiple flagella. No periplasmic fibrils were observed. The histologic and ultrastructural characteristics of the bacteria were considered most consistent with Helicobacter-like organisms (HLO). Gastric inflammation in the infected individuals consisted of mild to moderate, diffuse, lamina propria, lymphoplasmacytic and eosinophilic infiltrate with moderate to marked lymphofollicular hyperplasia in both the fundic and pyloric regions. However gastric inflammation in uninfected animals did not differ significantly from that observed in the infected individuals. Possibly the bacteria are commensals or an opportunistic pathogen, which has been previously suggested for HLO infection in other species.4 HLO have been previously observed in domestic cats (Felis domesticus) and various captive felids including cheetahs (Acinonyx jubatus), and one cougar (Felis concolor), but histologic gastritis and clinical gastric disease are only variably observed in these cases.3,5,7,8 Continued study, particularly controlled pathogenesis studies, are needed to adequately ascertain the role of these bacteria in gastric pathology. To the authors’ knowledge, this is the first report of gastric spiral bacteria in a free-ranging animal.

Histologic examination of skeletal muscle revealed all adult lions had rare to moderate numbers of sarcocysts within individual myocytes measuring 40 × 70 µm to 50 × 260 µm. Sarcocysts were not observed in juvenile lions, however all juveniles had enteric Sarcocystis sp. oocysts within small intestinal lamina propria macrophages. Enteric Sarcocystis sp infection was not observed in adults. TEM was performed on affected skeletal muscle. All observed cysts were mature, and were contained within myocytes. The cyst wall consisted of a 44 to 66 nm, granular, electron dense parasitophorous membrane with subjacent, 0.9-2.0 µm thick, granular and fibrillar ground substance which also extended into the cyst interior as thin septa. The parasitophorous membrane was folded into irregularly spaced, 0.8-1.3 µm tall villi centrally containing small amounts of ground substance. The membrane was continuous in the villar projections but divided into discrete aggregations of the electron dense material between villi. Bradyzoites within the interior of the cyst were approximately 3 µm × 12 µm. The
sarcocysts were determined to be consistent with *Sarcocystis felis* based on the characteristic ultrastructural appearance of the cyst wall.\(^1\) The life cycle and definitive host for *Sarcocystis felis* has not, to date, been ascertained, however it likely employs a scavenger species as the definitive host.\(^1,6\) Intramuscular *Sarcocystis felis* sarcocysts have been previously reported in a single lion in eastern Africa.\(^2\)

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**LITERATURE CITED**

WHEN BAD THINGS HAPPEN TO GOOD ANIMALS: CHRISTMAS EVE 1995 AT THE
PHILADELPHIA ZOO

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Abstract

In the late evening/early morning hours of 23-24 December 1995, there was a fire in the ceiling of a
cinder-block holding/exhibit building housing 1.5 western lowland gorillas (Gorilla gorilla gorilla), 1.2
Bornean orangutans (Pongo pygmaeus pygmaeus), 3.1 white-handed gibbons (Hylobates lar), 2.4 ring-
tailed lemurs (Lemur catta), 1.1 ruffed lemurs (Varecia variegata variegata) and 1.1 mongoose lemurs
(Eulemur mongoz mongoz). Each of the 23 nonhuman primates in that exhibit died of smoke inhalation,
before the Philadelphia fire department arrived at the scene. None of the animals was actually burned.
No humans were physically injured. Necropsies were performed on each of the animals, smoke
inhalation was confirmed as the cause of death, and specimens were saved and sent to numerous
researchers. Smoke inhalation poisoning is directly related to blood carbon monoxide levels (%COHb).
The Zoo engaged a fire toxicology expert to generate a model to estimate the risk to mammalian species
of smoke inhalation. Other events surrounding the fire, including the media and the public’s grieving
process, insurance and other legal matters occupied a great deal of time and attention from Zoo staff
members. As we speak, construction is set to begin on a $21 million new primate facility.

Introduction

Fire is a primal element. It is capable of wreaking devastating destruction in incredibly short time
periods. Zoos have moral, legal and ethical obligations to safeguard their animal collections, their staff
and visitors, (including fire prevention and fire safety training), along with the “mundane” health and
safety needs of the animals, staff and public. Since most captive wild animals cannot be evacuated from
a burning building in the same manner as able-bodied human beings, a unique set of parameters must
be considered during the design phase for animal exhibits/buildings.

The Fire

The Zoological Society of Philadelphia does not operate with night keepers. The Zoo has a 24-hr
security force; during night hours this is a two-person team which patrols the entire facility. In
December 1995 there was snow totalling nearly 30 cm on the ground and the air temp was below 0°C.
At approximately 22:00 on 23 December 1995, two guards thought they smelled smoke as they were
conducting their patrol in the vicinity of the Zoo’s World of Primates exhibit. This facility housed 23
nonhuman primates: 1.5 western lowland gorillas (Gorilla gorilla gorilla), 1.2 Bornean orangutans
(Pongo pygmaeus pygmaeus), 3.1 white-handed gibbons (Hylobates lar), 2.4 ring-tailed lemurs (Lemur
catta), 1.1 ruffed lemurs (Varecia variegata variegata) and 1.1 mongoose lemurs (Eulemur mongoz
mongoz).
Major Amtrack/Conrail rail lines lie just outside the Zoo facility, approximately 200 m from the World of Primates. The guards assumed the smoke was coming (as it some times had in the past) from a small fire in the rail right-of-way. The guards ignored the smoke and did not return to the area until almost 3 hr later, when they saw flames coming from the roof of the World of Primates facility. The alarm was received by the City of Philadelphia’s Fire Communications Center at 00:44 24 December 1995. Companies responded and had visible fire in the ceiling area of the north section of the World of Primates exhibit. At the same time, Zoo staff, including veterinarians, technician, curators, keepers, registrar, maintenance, public relations, guest relations, the Zoo President and the Sr. VP for Business Affairs began arriving.

The “fire building” was a series of three one-story masonry buildings (total area 23 x 70 m) constructed in 1986. The cinder block buildings were utilized as the indoor sleeping and holding area for the animals and there were public observation areas that were covered but not enclosed. The fire was confined to the enclosed ceiling areas between the gorilla and orangutan buildings. However, smoke permeated through the entire facility via HVAC ductwork and thick black greasy soot was heavily deposited on all surfaces inside the animal enclosures. A separate animal holding facility (Discovery Center) was connected to the World Of Primates by underground ductwork. All 10 primates (bicolor and golden-headed lion tamarins) and one acouchi in this facility survived, although two pregnant female tamarins later miscarried. No humans were physically injured during the fire and its aftermath.

The fire site was cordoned off to the press as the Administration Building (at the other side of the Zoo) became the focus for the media, the site of press conferences, etc. It was several hours before the Fire Marshall’s Office examined the fire scene and allowed the staff to remove the animals’ bodies from the World of Primates. This operation was conducted while all the press were occupied at the Administration Building. By 07:00 24 December, all of the bodies were identified, tagged, weighed and in the pathology walk-in cooler awaiting necropsy.

**The Staff**

Although Christmas Eve is one of 5 days in the year that the Philadelphia Zoo is closed to the public, the staff, volunteers and members of the Board of Directors began arriving at the Zoo as soon as they heard about the fire and the animals’ deaths. This was apparently a visceral reaction to the news and a need on their part to do something to be helpful. The keepers assigned to work that day went about their duties; several keepers (even though it was a holiday) stayed all day and were extremely helpful during necropsies by recording gross findings and assisting with measurements, collection of specimens, and keeping the prospectors supplied with instruments, etc. Many Zoo volunteers and Board Members were able to help staff the Zoo’s main switch board and field incoming calls. The guest services manager volunteered to ensure that food would be available to staff dealing with the carcasses and staff in the Administration Building. Managing staff was an important and delicate part of what happened during this long, long day. Ensuring that people ate and took rest breaks was a very important and necessary function.

Immediately following the fire and for several weeks thereafter, professional counselors were available for staff and volunteers who requested assistance. In the months following the fire, intensive fire safety inspections were conducted, over $500,000 in new fire detection and safety systems were installed throughout the Zoo, and training classes in safety and fire safety were conducted for all staff.

**The Necropsies**
The Pathology Department has a set of three ring binders in the Necropsy Suite which contain basic necropsy procedures, basic and special anatomy drawings, and individual sections on SSP species’ special necropsy instructions (one binder for Birds, one for Herps and three for Mammals). While the carcasses were being prepared, one keeper volunteered to photocopy 23 copies of all the necessary forms. We were able to set up five necropsy stations and run concurrent gross necropsies (Zoo veterinarians and veterinary pathologists conducted the necropsies), and six carcasses were transported to the Wildlife Conservation Center’s Pathology Department. By 19:00 24 December, a total of 13 necropsies had been completed. By 26 December, all 23 animals had received complete necropsies. Considerable volunteer assistance, from numerous staff and colleagues from other institutions, made this possible in a relatively short time.

Photographs and (amateur) videotape were shot of the interiors of the buildings and the sites where the animals were found. Not one of the animals was burned. The animals were found in typical resting/sleeping locations in peaceful repose positions. There was no evidence of any struggle in any of the animal areas. Several of the lemurs had apparently evacuated their colons perimortem. Each of the animals’ skin/fur was coated with thick greasy black soot, which extended into the mouth and nose, pharynx, trachea, bronchi and into the lungs. Each of the 23 animals was basically healthy. One adult gorilla was pregnant, the adult orangutan had an unsuspected air sacculitis, and some of the older animals had various degrees of arthritis. Several of the animals had bright pink subcutaneous tissues. Gross findings in each case were compatible with death by smoke (and carbon monoxide) and soot inhalation.

Confirmation of a diagnosis of death by smoke and soot inhalation requires toxicology results indicating elevated carboxyhemoglobin (%COHb) levels in the blood. Analysis of at least 2 ml of whole blood from the affected Zoo animals on a co-oximeter resulted in %COHb levels significantly above those considered lethal in humans (G. Purnell, personal communication). Clotted blood will not work in a co-oximeter; however, there is a micro-diffusion technique that can be used to qualitatively measure COHb. This method requires only 0.5-1.0 ml and can be performed on a postmortem clot (G. Purnell, personal communication).

Soot samples from the animals’ skin and fur and from various sites within the building were collected and analyzed for PCB’s, as we were concerned about potential risks to staff and clean-up workers. All samples tested negative for PCB’s (S. Packham, personal communication).

Since the fire occurred on a holiday, some of the researchers on the SSP special request lists were unavailable to confirm that they wanted tissues. We received numerous phone calls on Christmas Eve itself, while we were conducting the necropsies, from researchers requesting parts. I believe that most of this attention was directly related to the animal taxa involved. The great apes were of interest as sidelights to researchers working on human prostate disease, human bone metabolism, etc. and these inquiries were all opportunistic. I took information and later relayed it to SSP coordinators. In addition, I explained to the researchers (none of whom knew much about zoos) about SSPs and gave them names of people to contact for future specimen requests. At this point, we did not know the composition of the soot, and feared it could have toxic components, so I denied all requests for skin, hair and full body mounts (several museums called with requests for whole bodies).

The Lawyers, the Insurance, the Public and the Media

A catastrophic event like this fire will soon involve police/fire/insurance investigators, teams of lawyers
for all potentially involved parties, and the press (both print and broadcast). It is very important to establish and maintain a chain of custody for all evidence. The principle of a chain of custody is to establish that evidence found in situ was witnessed at the time of discovery and has not been altered in any significant way (e.g., no tampering, substitution, mishandling, mislabelling or contamination). This includes identifying each piece of evidence (a routine component of a complete necropsy) and controlling access to the specimens.

To assist the Zoo in handling of various aspects of the fire, we retained the services of a nationally known fire toxicologist, Dr. Steven C. Packham. He flew in and consulted with us, our lawyers and our insurance company. He took soot samples from various sites within the building and from the animals. He coordinated running samples with the City of Philadelphia’s Medical Examiner’s Office (PMEO). He also generated a model which can help estimate the risk to various mammal species of smoke inhalation. His model indicates that the animals in the World of Primates would have died in fewer than 15 min from smoke inhalation. These results were presented at the 1997 American Zoo and Aquarium Association (AZA) meeting.

The Philadelphia Zoo is closed to the public on Christmas Eve, Christmas Day, New Year’s Eve, New Year’s Day and Thanksgiving. After the fire, we chose to remain closed to the public until 2 January 1996. The visiting public had formed attachments to the great apes at the Zoo, and were probably as emotionally devastated by the fire as the staff. We received hundreds of faxes from around the world, thousands of letters from children and adults, over $1M in contributions from individuals, schools and corporations to a Zoo Renewal Fund, donations of numerous artworks and sculptures. We created a “Memorial Gallery” which opened to the public on 2 Jan 1996. This Gallery contained examples of the faxes, letters and drawings, photographs of the animals, brief histories of each animal and conservation information on the species involved in the fire. The keepers from the World of Primates were integral participants in the creation of the Memorial Gallery. The Gallery was open until March 1996 and filled needs of both the public and staff to remember and grieve.

We received tremendous support from the AZA, including the support of PR/media specialists. The days immediately following the fire saw incredibly intense media focus on all aspects of the fire. Ill-considered comments made early on were amplified. For example, a mini-controversy over disposition of the bodies was fueled by People for the Ethical Treatment of Animals (PETA) taking a position for burial in a pet cemetery as opposed to any other method of disposition.

Dispositions

I firmly believe that we have an obligation to learn as much as possible from each of the animals in our care. Part of that obligation includes supporting recognized researchers who make requests through the SSP coordinators and such facilities as the U.S. Fish & Wildlife Service (USF&WS) Forensics Laboratory. We sent specimens to as many researchers as we were able. We sent the skeletons of 1.1 of each species (and the young orangutan) to the USF&WS Forensics Lab, and the other skeletons were sent to a zooanthropology museum.

We arranged to have life molds and casts made from the hands and feet of 1.1 adult gorilla, orangutan and gibbon. Molds and casts are being made of the entire skeletons of selected individuals of each species. We see this as a means of ensuring the continuing contribution of these animals to the education of humans worldwide. The Zoo’s portion of the proceeds from these sales goes to a restricted account for Primate Conservation, managed by the Philadelphia Zoo.
The soft tissues were cremated and returned to the Zoo for inclusion in a planned memorial in the new primate exhibit, located on the site of the old exhibit, which was completely demolished in March 1997. There has been an accelerated design phase for the new exhibit, and construction is set to begin in fall 1997, with the grand opening set for 1999.

Discussion

Fires can have four components: flame, heat, smoke and gases. Heat sufficient to cause harm to lungs is often sufficient to cause fatal obstructive glottal edema in humans. Combustion of plastics introduces other gases, including chlorine, phosgene and hydrochloric acid (HCl). A latent period of 1-6 hrs may elapse before pulmonary signs of exposure to HCl manifest. Concentrations of greater than 100 ppm of HCl may result in pulmonary edema and laryngospasm. Heat-retaining concrete releases HCl for as long as 60 min after fire “knock down”. Although most authors use smoke inhalation and carbon monoxide intoxication interchangeably, there is evidence that immediate respiratory distress may be seen due to the simple mechanical irritation of smoke particles. Carbon particles may also enhance toxic gas uptake by adsorption. We analyzed soot samples for PCB’s. Soot can also be analyzed for chlorine, hydrogen cyanide and dioxins.

Carbon monoxide (CO) is the most common and most serious gaseous hazard during a fire. In concentrations of 0.5-1% it may be lethal to humans in 5 min. CO poisoning causes a multitude of effects due to tissue hypoxia and possibly cellular poisoning, primarily affecting the central nervous system and the myocardium. It is possible to use the %COHb data with known respiratory intoxication rates to generate a “clock” to time the growth of the fire up to the point of death (S. Packham, personal communication). Carbon monoxide exposure during pregnancy is teratogenic and has been reported to cause stillbirth and congenital defects. It was interesting that, of the surviving animals in Discovery House, the only two pregnant animals did not have successful births. Both of these mothers later conceived and bore healthy, live offspring.

Cherry red skin and mucus membranes are NOT commonly found. Analysis of blood samples for %COHb is proof of carbon monoxide poisoning. Blood samples collected during necropsy (even hours after death) and properly handled will yield valid results. However, blood samples collected hours after a necropsy may have artificially low %COHb (G. Purnell, personal communication).

We attempt to learn as much as possible from each of our captive wild animals. In this case, we tried to ensure that, even after death, these animals can continue serving as eloquent ambassadors for their species.

ACKNOWLEDGMENTS

I had a tremendous amount of help from a large number of people during a time that was difficult for everyone. I am indebted to Drs. K. Hinshaw, D. Ialeggio, K. Wright, M. Goldschmidt, D. Dambach, D. McAloose, A. Osborne, M. Lin and to S. Skeba, K. Kranz, Dr. S. Packham, Dr. T. Grand, G. Purnell, P. Farina and a terrific group of keepers.

LITERATURE CITED

A NEWLY RECOGNIZED FATAL BACULOVIRUS INFECTION IN FRESHWATER CRAYFISH

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Abstract

A newly recognized, emerging baculovirus infection has caused high mortalities in several populations of penaeid shrimp in the United States and several Asian countries, and most recently in a population of feeder crayfish at the National Zoological Park (NZP). The National Zoo’s Department of Invertebrates (DOI) receives weekly shipments of feeder crayfish which are fed live to cephalopods (mainly common cuttlefish exhibited in the collection). In early December 1995 the DOI reported a high mortality rate in their feeder crayfish for at least 1 mo prior to submitting the dead crayfish to the Zoo’s Department of Pathology. At the onset of this epidemic, two species of crayfish (Orconectes punctimanus and Procambarus sp.) were supplied by two different local distributors. Between December 1995 and mid-March 1996, approximately equal numbers of the crayfish species were received and combined in large plastic holding tanks in the DOI until fed out. At the peak of the epidemic, greater than 90% of Orconectes either arrived dead or died within the first 24 hr after arrival. There were no mortalities of Procambarus until mid March 1996, at which time 11 out of ~100 died within 4 days after arrival. At the end of March, Orconectes sp. were no longer purchased due to the high losses. Shortly thereafter, Procambarus mortalities increased to 20-30% through the summer of 1996.

Crayfish were submitted for postmortem examination at seven time points between December 1995 and May 1996. Gross lesions were limited to exoskeleton discoloration and mottling, especially prominent on the cephalothorax, with occasional extension down the chelipeds. Histologically, within the basal epithelial cell layer of the integument, there were large, deeply basophilic intranuclear inclusion bodies, often with attendant necrosis and separation of the overlying chitinous layer of the integument. Additionally, there were similar intranuclear inclusions within skeletal muscle and epithelial cells of the gill, stomach, intestine, hepatopancreas, compound eye and antennal gland, with multifocal necrosis. Transmission electron microscopic examination of the cuticular epithelium and antennal gland revealed large rod-shaped to elliptical intranuclear viral particles that measured ~100nm -350nm, with morphological characteristics of the Baculoviridae family.

No further baculovirus-associated mortalities occurred in DOI until early February 1997, when approximately 20% of a shipment of crayfish (Orconectes punctimanus) died within 24 hr after arrival to the DOI. Mortalities attributed to the virus have continued to occur at a low rate as of April 1997.

In 1992, a baculovirus was determined to be the causative agent of a fatal shrimp disease which resulted in high mortalities and economic loss to the shrimp culture industry of Taiwan and other Asian countries.2,4,7 Until then, baculoviruses had been isolated from and caused disease only in arthropods, mainly insects. In 1995, the cultured shrimp industry in the southern United States experienced...
outbreaks of a baculovirus disease which resulted in high mortalities.\textsuperscript{5,6,9} The source was believed to be from imported Asian shrimp. Clinical signs in the acutely affected shrimp included lethargy and anorexia, and loose cuticles with white target-like spots on the exoskeleton measuring 0.5-2.0 mm in diameter. These spots, composed of abnormal deposits of calcium salts, were most prominent on the inside surface of the carapace and led to the name White Spot Disease or White Spot Syndrome. The etiologic agent was called White Spot Syndrome Virus (WSSV). WSSV is extremely virulent and the natural host range includes several species of penaeid shrimp (\textit{P. japonicus}, \textit{P. monodon}, others).\textsuperscript{2-4,7,8} Similar to the crayfish at the National Zoo, some populations of shrimp may have cumulative mortalities up to 100\% within 3-10 days of onset of clinical signs.\textsuperscript{5} Experimental susceptibility to WSSV has been demonstrated in many cultured and wild shrimp, crabs, lobster and the red swamp crayfish, \textit{Procambarus clarkii}.\textsuperscript{1,11}

Using in-situ hybridization probes developed from the Thailand WSSV isolate and an isolate from the Yellow Sea region of China by co-author DVL\textsuperscript{5,8} at the University of Arizona Aquaculture Pathology Laboratory, a strong positive signal was demonstrated in the intranuclear inclusions of affected NZP crayfish. Additionally, infectivity trials utilizing disease-free shrimp that were injected with, or fed our WSSV probe-positive crayfish yielded a syndrome identical to WSSV reported in penaeid shrimp. These results strongly indicate the virus present in crayfish from the National Zoo is an Asian-derived WSSV.

The source of the baculovirus has so far been undetermined. At this time, because of multiple vendor involvement, it has not been clear when or if any of these crayfish originated from wild populations. Significant natural crayfish mortalities have not been reported to our knowledge, but the possibility remains that crayfish are becoming infected at their point of origin (either natural habitats or crayfish farms) in the Southeast United States. Other possible sources of infection include the introduction of baculovirus-contaminated or infected food fed to the crayfish, or cross contamination during handling by vendors or staff at the Zoo. Since the early mortalities occurred right after delivery of \textit{Orconectes} to the National Zoo, it is likely they arrived infected. Contamination of holding tanks may have contributed to perpetuation of the virus following the earlier outbreaks.

The significance of this newly-recognized, fatal baculovirus in decapods is threefold. First, the American Fisheries Society Endangered Species Committee has determined 112 out of 338 native crayfish species are either endangered or threatened.\textsuperscript{10} The introduction of nonindigenous crayfish species alters the local habitat, and now represents a potential source of baculovirus transmission to wild, endangered crayfish. Second, baculovirus from infected crayfish from any source may be transmitted to other wild decapods. Third, economic losses to the farmed crayfish and shrimp industries are likely. Therefore, it is of utmost importance to eliminate exposure of farmed and wild populations of decapods to baculovirus-infected arthropods.

\textbf{ACKNOWLEDGMENTS}

The authors thank the following individuals for their contributions to our study: Dr. Mary Allen, Andrew Keech, Christopher James and Earl Pinkney. This research was supported in part by a Friends of the National Zoo Senior Fellowship, FONZ 96-3545A.

\textbf{LITERATURE CITED}


A RETROSPECTIVE ANALYSIS OF NECROPSY INFORMATION FROM SPEKE’S GAZELLE (Gazella spekei) AT ST. LOUIS ZOOLOGICAL PARK

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Abstract

Speke’s gazelle (Gazella spekei) originate from the dry open bushland of eastern Ethiopia and Somalia. They have formed part of the St. Louis Zoological Park’s animal collection since the mid-1960's. The gazelles were born in the collection or were derived from the same breeding line, with the exception of three animals from Qatar. Over 120 Speke’s gazelle have been necropsied at the zoo and tissues en bloc have been collected and stored from the majority of cases since 1970. Analysis of the necropsy records revealed that the cases fell into six age categories: 1) aborted fetuses and stillborn calves, 2) neonates up to 2 wk-old, 3) adolescents from 2 wk and to 1-yr-old, 4) young adults from 1-2-yr-old, 5) adults from 2-4-yr-old, and 6) aged adults greater than 4-yr-old, proportionally the largest group.

Within defined age groups, cause of death followed a distinct pattern. With abortion in early gestation the dam was not always identified, while late abortion occasionally followed restraint for treatment of an unrelated medical or surgical problem. In neonates, poor mothering and congenital limb deformities were the main problems. Limb deformities and injuries were seen also in young adults. In the former cases, despite apparently favorable surgical outcomes, often the gazelles declined weeks to months later and at gross examination of the carcass aspiration pneumonia was suspected. Approximately one third of the deaths in young adults occurred in heifers peripartum and were associated with prolonged labor with malpresentation or pregnancy toxemia. The widest range and least specific lesions were seen in adults two to 4-yr-old. Aged animals frequently died in renal failure and often showed signs of chronic arthritis. Review of histological material has shown that amyloidosis, not restricted to the renal system, is a frequent finding. Ongoing studies aimed at identifying the extent and form of the deposits, which are thought to be most consistent with the form of amyloid seen following prolonged inflammation and synthesis of acute phase reactants.
THE USE OF FRACTIONAL EXCRETION FOR EARLY DIAGNOSIS OF RENAL DAMAGE IN CHEETAHS (Acinonyx jubatus)

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Abstract

Renal failure is a major cause of morbidity and mortality in the captive cheetah population. Specifically, there are high prevalences of glomerulosclerosis (82%) and renal amyloidosis (38%) with lesions seen in cheetahs as young as 1-yr-old. Because renal disease limits the viability of the captive cheetah population, early diagnosis and therapy may be beneficial to prolong and improve the quality of life for affected captive cheetahs.

Serum concentrations of blood urea nitrogen (BUN) and creatinine are insensitive indicators of renal damage. With renal damage, increased flow of fluid and filtered protein in remaining tubes results in solute loss. The net effect is an increased clearance of electrolytes relative to creatinine. Fractional excretion and urine protein:creatinine ratios have been evaluated for diagnosis of renal damage in domestic cats. The urine protein:creatinine ratio and fractional excretion of sodium, chloride, potassium, calcium, and phosphorous have been shown to increase in domestic cats with chronic renal disease. The purpose of this study was to determine if fractional excretion tests could reliably diagnose renal damage earlier than elevated serum BUN and creatinine in cheetahs.

Paired urine and serum samples were obtained from 13 healthy cheetahs (7.6) and six cheetahs with renal disease (2.4) during annual physical exams. A minimum of two sequential samples were obtained from each animal. Cheetahs were maintained at White Oak Conservation Center and fed a commercial ground horse meat-based diet (Nebraska Brand Canine Diet; Central Nebraska Packing Co., North Platte, NE 69101 USA) supplemented weekly with ox tails. Water was available ad libitum. Cheetahs were anesthetized and urine was obtained by sterile catheterization. Urine and serum were stored frozen (-70°C) until analysis. Urine and serum chemistries were measured at a commercial laboratory.

Fractional excretion (FE) of electrolytes was calculated using the following formula:

\[
\text{Fe}_{\text{electrolyte}} = \frac{[\text{Serum}_{\text{creatinine}}][\text{Urine}_{\text{electrolyte}}]}{[\text{Urine}_{\text{creatinine}}][\text{Serum}_{\text{electrolyte}}]}
\]

The ratio of urine protein to urine creatinine was also calculated.

Cheetahs that never developed persistently elevated BUN/creatinine over the 4 yr of the study were classified as normal. Abnormal cheetahs had at least one sample point in which BUN/creatinine were normal but developed persistently elevated BUN/creatinine during the study period. A comparison of the accuracy of diagnostic tests generally requires an independent gold standard. Because there is no reasonable independent gold standard available for diagnosing renal disease in cheetahs, the result of outcome serves as the gold standard.
There was a significant difference ($P < 0.05$) between values from normal and abnormal cheetahs for all tests. Normal ranges were calculated using mean and 2 SD and are shown in Table 1. The maximum test efficiency (accuracy) was calculated for each test at the time of diagnosis of renal disease and 1 yr prior to diagnosis. All of the tests evaluated had high levels of accuracy for early diagnosis of renal disease. Sensitivity, specificity, and predictive values (data not shown) were also greater than 80% for most tests. Relative to the other tests, the low accuracy of the urine protein:creatinine ratio was probably the result of placing all abnormal cheetahs in the same group regardless of disease etiology. All of the abnormal cheetahs with glomerulosclerosis confirmed by histopathology had increased urine protein:creatinine ratios.

The use of fractional excretion tests has been limited in domestic cats because values are affected by diet and time of day in addition to renal status. Because most cheetahs in captivity receive a similar diet and are fasted prior to annual physical exam, this dietary limitation of fractional excretion tests is minimized. In this study, fractional excretion tests were able to diagnose renal damage earlier than serum BUN and creatinine elevation. Further research is needed to evaluate the potential of fractional excretion tests to differentiate between glomerulosclerosis and renal amyloidosis.

ACKNOWLEDGMENTS

The authors wish to thank Cyd Shields Teare, Kelly Hernandez, and Lisa Kolbach at White Oak Conservation Center for their assistance with sample collection and analysis. We would also like to thank Dr. Linda Munson for histological examination of kidneys.

LITERATURE CITED

Table 1. Normal range and accuracy of fractional excretion tests and urine protein:creatinine ratio for diagnosing renal damage at the time of diagnosis by increased BUN/creatinine and 1 yr prior to abnormal BUN/creatinine.

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal Range</th>
<th>Accuracy %</th>
<th>Accuracy % 1 yr prior</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE Calcium %</td>
<td>0 - 0.13</td>
<td>89</td>
<td>94</td>
</tr>
<tr>
<td>FE Chloride %</td>
<td>0 - 0.20</td>
<td>95</td>
<td>88</td>
</tr>
<tr>
<td>FE Potassium %</td>
<td>0 - 11.70</td>
<td>95</td>
<td>75</td>
</tr>
<tr>
<td>FE Phosphorous %</td>
<td>0 - 16</td>
<td>89</td>
<td>82</td>
</tr>
<tr>
<td>FE Sodium %</td>
<td>0 - 0.07</td>
<td>89</td>
<td>76</td>
</tr>
<tr>
<td>Urine Protein:Creatinine</td>
<td>0 - 0.01</td>
<td>78</td>
<td>81</td>
</tr>
</tbody>
</table>
HEMATOLOGY AND SERUM BIOCHEMISTRY PARAMETERS IN LIVE-TRAPPED RIVER OTTERS (*Lutra canadensis*)

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Abstract

Successful translocation depends on the relocation of healthy wildlife. Medical evaluation, including clinical pathology screening, of animals scheduled for translocation should be conducted.5 Clinical pathology baselines are helpful in evaluating the health status, nutritional state, and the presence of infectious and noninfectious diseases. This study established baseline hematology and biochemistry parameters of eastern North Carolina (USA) North American river otters (*Lutra canadensis*) live-trapped during the winter months of December-February.

One hundred fifty-five clinically healthy, live-trapped river otters from eastern North Carolina (USA) were sampled over a 4-yr period between the dates of December 8 through February 28. Otters were live-trapped, using various sized standard and modified leg-hold traps, within a 10 county range in central eastern North Carolina for the North Carolina Wildlife Resources Commission (NCWRC) Otter Restoration/Translocation Project (Raleigh, NC USA). Otters were weighed, then anesthetized using regimes that had been previously published for river otters6,7 or sea otters.8 Complete blood counts were performed using automated cell counters and 50 sera were submitted for biochemical analysis in 1992 and 1996. Statistical analysis used nonparametric methods because of the non-normal distribution of the data.

Adult male and female clinical pathology values were summarized together because the statistical differences found were not clinically significant. Microfilaria, most closely resembling *Dirofilaria lutrae*,4 could be seen by scanning under low magnification (100x) and were found in 18.1% of all blood smears examined during the 4 yr of the study. Serum CPK decreased in concentration as the number of days held prior to work-up increased. An evaluation of hematology and biochemistry parameters considering degree of injury showed a slight but significant increase in the median leukocyte count (12.9 x10^3/µl) and absolute numbers of neutrophils (9793.5 µl) as degree of injury increased (Table 1).

The river otter was found to have a higher concentration of smaller erythrocytes than other species of otters and has a lower blood urea nitrogen, amylase, and creatinine than sea otters (*Enhydra lutris*).9 There were no significant differences between adult males and females for the study years. It appears that *D. lutra* is commonly found in river otters in the eastern United States1-4 including North Carolina. The baseline clinical pathology values established in this study of clinically healthy live-trapped wild river otters varied slightly from other otter species and significant differences were found when comparing age, gender, and trapping injury.
ACKNOWLEDGMENTS

This study was funded in part by the North Carolina Zoological Park; the North Carolina Furbearers Association; the College of Veterinary Medicine and the Environmental Medicine Consortium, North Carolina State University.

LITERATURE CITED


### Table 1. Hematology and biochemistry values for adult live-trapped river otters (*Lutra canadensis*).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>N</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>× 10^3/µl</td>
<td>132</td>
<td>11.3</td>
<td>4.7 - 33.2</td>
</tr>
<tr>
<td>RBC</td>
<td>× 10^6/µl</td>
<td>132</td>
<td>10.99</td>
<td>6.10 - 14.50</td>
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<tr>
<td>Hgb</td>
<td>g/dL</td>
<td>132</td>
<td>15.1</td>
<td>10.4 - 19.0</td>
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<tr>
<td>Hct</td>
<td>%</td>
<td>132</td>
<td>47.6</td>
<td>32.2 - 60.8</td>
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<tr>
<td>MCV</td>
<td>fl</td>
<td>132</td>
<td>43.3</td>
<td>38.3 - 49.0</td>
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<tr>
<td>MCH</td>
<td>pg</td>
<td>132</td>
<td>13.7</td>
<td>11.3 - 15.8</td>
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<tr>
<td>MCHC</td>
<td>g/dL</td>
<td>132</td>
<td>31.4</td>
<td>27.8 - 39.2</td>
</tr>
<tr>
<td>Plt</td>
<td>× 10^3/µl</td>
<td>132</td>
<td>565</td>
<td>298 - 931</td>
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<tr>
<td>Neutrophils</td>
<td>µl</td>
<td>132</td>
<td>8878.5</td>
<td>3003.0 - 28220.0</td>
</tr>
<tr>
<td>Bands</td>
<td>µl</td>
<td>132</td>
<td>94.0</td>
<td>0.0 - 486.0</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>µl</td>
<td>132</td>
<td>1254.0</td>
<td>123.0 - 4950.0</td>
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<tr>
<td>Monocytes</td>
<td>µl</td>
<td>132</td>
<td>452.3</td>
<td>52.0 - 2380.0</td>
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<tr>
<td>Eosinophils</td>
<td>µl</td>
<td>132</td>
<td>312.0</td>
<td>0.0 - 1833.0</td>
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<tr>
<td>Basophils</td>
<td>µl</td>
<td>132</td>
<td>88.0</td>
<td>0.0 - 219.0</td>
</tr>
<tr>
<td>Ca</td>
<td>mg/dL</td>
<td>50</td>
<td>8.4</td>
<td>6.8 - 10.0</td>
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<tr>
<td>Phos</td>
<td>mg/dL</td>
<td>50</td>
<td>5.8</td>
<td>3.2 - 8.3</td>
</tr>
<tr>
<td>Na</td>
<td>mEq/L</td>
<td>50</td>
<td>152</td>
<td>136 - 158</td>
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<tr>
<td>K</td>
<td>mEq/L</td>
<td>50</td>
<td>4.4</td>
<td>3.5 - 5.3</td>
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<tr>
<td>Cl</td>
<td>mEq/L</td>
<td>50</td>
<td>113</td>
<td>94 - 121</td>
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<tr>
<td>TCO₂</td>
<td>mEq/L</td>
<td>21</td>
<td>24</td>
<td>19 - 28</td>
</tr>
<tr>
<td>ALT</td>
<td>IU/L</td>
<td>50</td>
<td>194</td>
<td>46 - 990</td>
</tr>
<tr>
<td>AST</td>
<td>IU/L</td>
<td>50</td>
<td>85</td>
<td>34 - 1260</td>
</tr>
<tr>
<td>Alk Phos</td>
<td>IU/L</td>
<td>50</td>
<td>85</td>
<td>29 - 282</td>
</tr>
<tr>
<td>T. Bili</td>
<td>mg/dL</td>
<td>50</td>
<td>0.2</td>
<td>0.1 - 0.5</td>
</tr>
<tr>
<td>Chol</td>
<td>mg/dL</td>
<td>29</td>
<td>152</td>
<td>63 - 279</td>
</tr>
<tr>
<td>Trig</td>
<td>mg/dL</td>
<td>29</td>
<td>31</td>
<td>9 - 72</td>
</tr>
<tr>
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**GRANULOMATOUS AORTITIS AND CARDIOPULMONARY ARTERITIS IN FAIRY BLUEBIRDS (*Irena puella*) WITH MYCOBACTERIOSIS**
GRANULOMATOUS AORTITIS AND CARDIOPULMONARY ARTERITIS IN FAIRY BLUEBIRDS (Irena puella) WITH MYCOBACTERIOSIS

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Abstract

Two adult male fairy bluebirds (Irena puella) from two different zoological collections had histologic inflammatory lesions in the aorta, pulmonary trunk, and coronary arteries. Lesions in the aorta and pulmonary trunk were transmural, while coronary artery lesions were limited to the media and adventitia. The intima in vessels with transmural lesions was characterized by variably thick subendothelial plaques consisting of collagen and/or spindloid cells and macrophages. Aggregates of epithelioid macrophages with abundant granular basophilic cytoplasm were present occasionally in the subintima and, more typically, in the media and adventitia. Medial epithelioid macrophage infiltrates were often arranged in rows, parallel to the medial collagen and elastin fibers and there were occasional foci of medial mineralization. Inflammation was most marked in the adventitia of all vessels, which contained poorly organized infiltrates of lymphocytes, plasma cells, macrophages, epithelioid macrophages, and heterophils. Occasional multinucleated giant cells were also present. Ziehl-Neelsen stains identified large numbers of acid-fast bacilli in the cytoplasm of the epithelioid macrophages. Both birds had other lesions of mycobacteriosis. In one bird these were limited to the presence of acid fast organisms within histiocytic nodules adjacent to secondary and tertiary parabronchi in the lung. The other bird had a very small number of similar pulmonary nodules and disseminated granulomatous inflammation in the liver, both containing acid fast organisms.

Granulomatous arteritis related to mycobacterial infection has not before, to our knowledge, been reported in animals. The distribution and nature of the lesions in these cases is similar to a syndrome in humans called Takayasu’s arteritis. Takayasu’s arteritis may involve the aorta, its branches, and the pulmonary arteries and is characterized by granulomatous inflammation of the media and adventitia. The etiology of the disease remains undetermined. Epidemiologic data, including an elevated incidence of active tuberculosis or positive skin reactivity to purified protein derivative (PPD) in affected patients compared to the general population, suggests a link with mycobacterial infection. Patients with Takayasu’s arteritis also have levels of serum antibodies to Mycobacterium tuberculosis extract, its 65 kDa heat shock protein, and a purified tuberculosis-specific 38 kDa protein, that are significantly higher than those in control sera and are comparable to, or higher than, sera from patients with pulmonary tuberculosis.1,2 It has been suggested that Takayasu’s arteritis may be an immune mediated disorder triggered by mycobacterial antigens with immunologic similarity to native antigens in the arterial wall. The arteritis observed in the birds differs from the human syndrome in that macrophages with intracytoplasmic Mycobacteria are present within the avian lesions but are not observed in Takayasu’s arteritis.

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CONSERVING AND USING THE GENETICS OF THE WILD CATTLE OF SOUTHEAST ASIA

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Abstract

The wild cattle species of southeast Asia are experiencing a rapid decline in numbers due to many factors including fragmentation of tropical forests, dense and expanding human populations, poaching and disease (resulting from overlapping ranges with domestic animals). At a meeting of the Asian Wild Cattle Specialists Group in 1995, 33% of the 21 Asian wild cattle species surveyed were considered critical, 24% endangered, 14% vulnerable and 5% low-risk. A partnership of scientists representing the National Zoo’s Conservation and Research Center, Omaha’s Henry Doorly Zoo and Disney’s Animal Kingdom of the United States and Khao Kheow Open Zoo, Chulalongkorn University and the Department of Livestock Development of Thailand have established a collaborative effort to address conservation, education, research and animal agriculture issues regarding these taxa. These species represent a valuable, genetically diverse resource that is well adapted to tropical conditions and, in some cases, resistant to disease. One study (Azmid and Hilmi, 1988, unpublished) indicated that interbreeding between Malaysian gaur and domesticated cattle can produce hybrid progeny that exhibit improved growth rates and feed efficiency compared to domesticated counterparts. Furthermore, the development of a hybrid domestic breed better adapted to tropical forest habitation may reduce the incentive to destroy these areas for conventional farming. Gaur and banteng have been chosen as the charter species for this project. A recent survey has identified approximately 915 gaur and 470 banteng in protected areas in Thailand, and no individuals outside the protected areas. This is at least a 60% reduction in gaur and an 80% reduction in banteng populations in Thailand over the last 20 yr. Preliminary studies in semen cryopreservation have been conducted in both species, and continued development of assisted reproductive techniques in gaur is ongoing at the Henry Doorly Zoo.

Funded by the U.S. Agency for International Development (USAID), the goals of this project are to 1) develop and compare reproductive parameters among the wild cattle species of southeast Asia; 2) establish techniques in germplasm cryopreservation and initiate the maintenance of a genome resource bank for wild cattle in Thailand; 3) test the feasibility of assisted reproductive techniques for increasing wild cattle population size; 4) provide training to Thai veterinarians and scientists in anesthesia, gamete biology and endocrinology; and 5) utilize wild cattle genetics as a means of improving the economic efficiency of Asian domestic cattle. As a result of this project, several Thai scientists have already received training at U.S. institutions, and a new Experimental Research Center has been constructed at the Khao Kheow Open Zoo in Chonburi, Thailand. The new center will house gaur and banteng, and is equipped with laboratory space and a working chute for manipulating animals for research, veterinary care and gamete collection. Results obtained through this collaboration will serve as a foundation for
future in situ conservation efforts in other wild cattle species threatened with extinction.

LITERATURE CITED


IMMOBILIZATION OF FREE-RANGING GIRAFFE (Giraffa camelopardalis) USING MEDETOMIDINE AND KETAMINE

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Abstract

A non-opiate drug combination of a potent alpha2 agonist and a dissociative anesthetic was evaluated for capture of 10 free-ranging giraffes (Giraffa camelopardalis) in the Kruger National Park, South Africa. A medetomidine and ketamine combination was given at a ratio of 1:20 via a dart containing 7500 IU of hyaluronidase. The study animals included 5 males and 5 females with estimated weights of 190 to 850 kg.

The dosages for medetomidine ranged from 41 to 116 µg/kg with the corresponding 0.8 to 2.3 mg/kg of ketamine (1 to 20 ratio). The time from darting to the onset of signs varied from 1 to 5.5 min (average 2.33 min) while the time from darting to recumbency varied from 2.75 to 13 min (average 7 min). Both the time of onset and time to recumbency were related to the dosage. Eight of 10 giraffes became recumbent. Two adult females receiving 48 and 61 µg/kg medetomidine and 1 and 1.2 mg/kg ketamine did not become recumbent, while 2 others receiving 41 and 50 µg/kg medetomidine and 0.8 and 1 mg/kg ketamine did. All giraffes receiving 78 to 116 µg/kg medetomidine and 1.6 to 2.3 mg/kg ketamine became recumbent in less than 9 min.

There was minimal excitement during the induction phase as the giraffe tended to stand and went down in a controlled manor. Once recumbent the giraffes were monitored every 5 min during a 30 min period for heart rate, respiration rate, rectal temperature, indirect blood pressure, and blood gases. Hyperventilation was present in all giraffes during the procedure with an average of 74 breaths/min (range of 24 to 126). There was a snoring respiration that was not corrected by repositioning the head and neck. Heart rates remained above 30 beats/min in all animals with an average rate of 44/min. The blood pressure was well maintained with an average systolic pressure of 190 mm Hg and a diastolic of 110 mm Hg. There was no elevation in body temperature during the procedure. The blood gas evaluation showed an initial slight acidosis which corrected itself by the end of the recumbency.

The giraffes were given atipamezole i.m. at dosages of 114 to 222 µg/kg (average 189 µg/kg) to antagonize the medetomidine, which produced a smooth and rapid recovery in an average of 6 min and 40 sec (range 2 to 14 min). Four of these giraffes were observed 24 hr later and they appeared normal.

There were major difference noted when comparing anesthetic protocol which use opiates (etorphine or carfentanil).1-3 Giraffes given a medetomidine and ketamine combination: 1) are aware of ground personnel and would avoid or strike out at them so it was not possible to use a rope to contain and cast them, 2) did not exhibit a forced pacing behavior, and went down in a controlled manner rather than crashing down or going over backwards, 3) did not show respiratory depression, but instead hyperventilated, and 4) are sedated and not anesthetized since they respond to minor manipulation with increased heart rates, therefore supplemental anesthetic agents would be required prior to any
painful procedure and to obtain complete muscle relaxation.

A medetomidine (78 to 116 µg/kg) and ketamine (1.6 to 2.3 mg/kg) combination proved to be a safe and effective combination for capture of free-ranging giraffes. This combination should greatly improve the safety and reliability of anesthesia in captive giraffes. The use of concurrent anesthetic are indicated in potentially painful manipulations.

LITERATURE CITED

ANALYSIS OF URINE FROM FREE-RANGING MOUNTAIN GORILLAS (Gorilla gorilla beringei) FOR NORMAL PHYSIOLOGICAL VALUES

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Abstract

Urinalysis can reveal much information about the health of an animal, in particular, regarding the status of carbohydrate metabolism, kidney and liver function, acid-base balance, and urinary tract infection. Urine was collected from habituated gorillas in the Parc National des Volcans, Rwanda to establish reference intervals for various physiological values. The urine was collected midstream as the animal urinated or from pools formed in the foliage. Sixty-six samples were collected between August, 1996 and January 1997. Thirty-four midstream samples, including two collected during anesthesia, and nine samples from the foliage were of a quality sufficient to be included in the study. This represented samples from 16 males, eight females, and one of unknown sex, of which five individuals were juveniles. Multiple samples were collected from nine gorillas. The color and turbidity were evaluated by visual observation. The pH, specific gravity and presence of protein, glucose, ketones, bilirubin, urobilinogen, blood and nitrate were evaluated using commercially available dipsticks (Combur 10 Test, Boehringer Mannheim, Mannheim, Germany and Ames Multistix 10 SG, Miles, Inc., Elkhart, IN 46515 USA). The specific gravity was also determined using a hand-held refractometer calibrated against distilled water. A portion of the samples with sufficient quantity was centrifuged for 5 min at 2000 rpm. The supernatant was removed, and an aliquot of the deposit was examined microscopically for the presence of cells, casts, crystalline and other deposits. The color ranged from pale yellow to yellow (79% of samples), to dark yellow or brown (21%). The majority (88.3%) of samples were clear, the rest had varying degrees of cloudiness. The mean ± SD specific gravity as measured by the refractometer was 1.013 ± 0.003 (n = 32) and the mean ± SD pH was 8.45 ± 0.42 (n = 43). Eighteen out of the 43 samples (41.9 %) had a trace of protein. Seven (16.3 %) had a trace of leukocytes and eight (18.6%) were positive for nitrite, four (9.3%) of which were positive for both tests. Three (7%) were considered to be low positive for bilirubin. All other samples were negative for the above tests. All samples were negative for glucose, blood and ketones, and all samples were considered to be within normal limits for urobilinogen. Thirty-three of the 37 samples (89.1%) examined microscopically contained some deposits in the urine. Twenty-nine (78.4%) samples contained varying amounts of crystalline material mostly suspected to be phosphate crystals, and 13 (35.1 %) had varying amounts of amorphous deposits. Rare casts were seen in 12 samples (32.4%) and three (8.1%) had very rare epithelial cells. From this study it appears that it is feasible to collect urine from wild mountain gorillas for diagnostic purposes. The establishment of normal physiological values will hopefully improve the noninvasive diagnosis of disease in mountain gorillas and allow for prompt and effective treatment of individual gorillas with minimum disturbance to the population.
EVALUATION OF ISOFLURANE AND PROPOFOL ANESTHESIA FOR INTRA-ABDOMINAL TRANSMITTER PLACEMENT IN NESTING FEMALE CANVASBACK DUCKS

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Abstract

Intra-abdominal radio transmitters are used preferentially over externally-mounted transmitters because they appear to have less impact on behavior, health, survival and reproduction in ducks. Inhalant anesthetics (isoflurane and methoxyflurane) are often used to anesthetize birds during surgery but require expensive equipment such as a vaporizer and oxygen delivery system. Propofol is a new, rapidly-metabolized intravenous anesthetic which provides smooth induction and recovery, excellent muscle relaxation and short duration of anesthesia. Female canvasback ducks (Aythya valisineria) were assigned randomly to one of three treatments: propofol (n = 39), isoflurane (n = 39) and control (flushed not captured; n = 40). Rapid recovery characteristic of isoflurane anesthesia precludes placement of ducks into their natural environment prior to complete recovery. However, the use of propofol allows recovery to take place on the nest. Overall nest abandonment differed among treatment groups (χ^2 = 6.115, 2 df, P = 0.047), with lower than expected abandonment in control and propofol groups. No significant difference in abandonment was detected between propofol and control groups (χ^2 = 1.22, 1 df, P = 0.27), confirming that the relationship was driven by the higher than expected abandonment in the isoflurane group. A comparison with results from Arnold et al., where birds were captured and nasal-marked, revealed that surgery resulted elevated abandonment (χ^2 = 11.75, 2 df, P = 0.003). Abandonment rate in the propofol group did not differ (χ^2 = 1.12, 1 df, P = 0.29) from rates reported by Arnold et al., suggesting that the former relationship was again driven by the isoflurane group. Stress at time of recovery may be responsible largely for nest abandonment. Propofol requires minimal equipment, reduces anesthetic cost and decreases the risk of investigator induced abandonment making it an ideal anesthetic for field studies of waterfowl.

Introduction

Intra-abdominal radio transmitters are used preferentially over externally-mounted transmitters because they appear to have less impact on behavior, health, survival and reproduction in ducks. Inhalant anesthetics (isoflurane and methoxyflurane) are often used to anesthetize birds during surgery but require expensive equipment such as a vaporizer and oxygen delivery system. A simpler anesthetic technique, using methoxyflurane placed on a gauze, has also been employed in reducing stress and disruption of normal behavior after handling and external transmitter attachment. Methoxyflurane decreases nest abandonment after transmitter placement but inability to control depth of anesthesia may result in complications and mortality. Violent recoveries associated commonly with methoxyflurane anesthesia make this technique unsuitable for over-water nesting species, such as canvasback ducks. Methoxyflurane also poses a significant human health risk. Recovery from isoflurane-anesthesia is too rapid to allow placement of ducks into their natural environment prior to complete recovery. This may cause unacceptably high abandonment when attempting to place birds on their nests following surgery, although this has not been tested. Propofol is a new, rapidly-metabolized intravenous anesthetic which
provides smooth induction and recovery, excellent muscle relaxation and short duration of anesthesia. Preliminary work in the laboratory suggested that recovery from propofol would allow placement of lightly anesthetized birds on their nests following surgery.

**Materials and Methods**

In most brood survival studies, females are equipped with transmitters during mid-to late incubation to reduce nest abandonment rates which in turn lowers (a) human-induced reproductive failure, (b) lost information and (c) equipment costs. Therefore, female canvasbacks were captured at 15 to 18 days of a 25 to 26 day normal incubation period. Female canvasbacks were assigned randomly to one of three treatments: propofol, isoflurane and control (flushed from nest but not captured). Sterile dummy silicone implants weighing 15 to 18 g were surgically implanted while birds were anesthetized with either propofol (Group 1, n = 39) or isoflurane (Group 2, n = 39). Birds in the control group (Group 3, n = 40) were considered to be normal with minimal intervention since they were flushed only from the nest.

Propofol (group 1) was delivered through an 24-gauge i.v. catheter placed in the medial tarsal vein. Ducks were induced with 10 mg/kg of propofol given slowly over 1 min. Depth of anesthesia was assessed and additional boluses (1-2 mg) were given until the bird could be intubated with a non-cuffed endotracheal tube. Anesthesia was maintained with additional boluses (1-3 mg) given as needed and birds were ventilated throughout the procedure with a 0.5 L pediatric self-inflating resuscitation apparatus.

Isoflurane (group 2) was delivered through a non-rebreathing system by an Isotec 3 vaporizer (Ohmeda, BOC Health Care, West Yorkshire, England). Anesthesia was induced with isoflurane starting at 1 % and stepped up to 5 % with an oxygen flow of 2 L/min. Following induction, birds were intubated with a non-cuffed endotracheal tube. Anesthesia was maintained at 1.5 to 3.5 % with oxygen flow rate of 1 L/min. Birds that became apneic were ventilated with a 0.5 L rebreathing bag attached to the circuit.

Anesthetic depth was assessed by monitoring (a) heart rate using an esophageal stethoscope, (b) nictitating membrane movements, (c) swallowing or coughing, (d) response to stimuli and (e) movement. During anesthesia and surgery, heart rate and respiratory rate were monitored. Anesthetic depth was adjusted to maintain the bird at a constant level of anesthesia. Surgery was performed as described by Olsen et al. After discontinuation of anesthesia, heart rate and respiratory rate were monitored in both groups until normal breathing resumed. Oxygen flow rate was maintained at 1 L/min for birds receiving isoflurane and ventilation was provided as necessary for both groups. The endotracheal tube was removed when the bird began to lift its head to cough or swallow. Respiration was monitored for a few minutes following extubation to ensure that ventilation was maintained.

At the nest, group 1 birds were given an additional bolus of propofol. Respiratory rate was monitored and depth of anesthesia was sufficient to allow removal of the catheter. Bleeding was stopped by applying Blood Stop Powder (Dominion Veterinary Laboratories LTD, Winnipeg, Manitoba, Canada) and manual pressure. Group 1 birds were allowed to recover on the nest while group 2 birds were released on the nest after recovery.

Monitoring for nest abandonment in all groups was accomplished by recording nest temperature every 4.6 min for 6 days after treatment. Temperature was recorded on a HOBO XT Temperature Logger (Onset Instruments Corp., Pocasset, MA USA) attached to a thermistor implanted into a dummy egg.
The dummy egg was anchored to a metal rod measuring at least 15 cm and placed in the center of the clutch. Nests were visited 6 days after surgery to retrieve the HOBO Temp and to determine fate of the nest (abandoned, active or destroyed). Nests were considered abandoned if the temperature pattern indicated that a bird did not return to the nest following surgery (group 2) or flushing (group 3). In group 1, nests were considered abandoned if the bird left the nest within a 6-hr recovery period and did not return.

Logistic regression and chi-square were employed to determine whether nest abandonment varied with year, treatment and the interaction of these two variables. Results were considered significant when $P < 0.05$.

**Results**

All birds survived surgery. One mortality occurred prior to surgery using propofol during a period when ventilation and monitoring of the canvasback duck was inadvertently stopped. This bird is not included in the analysis. One canvasback duck in the control group was found dead on the nest and was likely killed by a mink (*Mustela vison*).

In canvasback ducks, nest abandonment occurred in all treatment groups in both years (Table 1). No difference in nest abandonment between years was found (logistic regression, $P = 0.55$), nor was there a year by treatment interaction ($P = 0.83$). When these terms were dropped from the model, a weak treatment effect was detected (logistic regression, $P = 0.06$), owing to the greater abandonment ($P = 0.02$) in the isoflurane group. These analyses were appropriate because the data fit the logistic models adequately (model goodness of fit, $Ps > 0.8$). Categorical analyses confirmed these findings; there was a significant difference among treatments in nest abandonment ($X^2 = 6.115$, 2 df, $P = 0.047$), with propofol and control groups having lower than expected abandonment. Since abandonment in propofol and control groups did not differ ($X^2 = 1.22$, 1 df, $P = 0.27$), this indicates that the former relationship between groups was driven by the higher than expected abandonment in the isoflurane group.

**Discussion**

Human disruption of nesting waterfowl may lead to lowered production and survival of ducks, decreased sample size and loss of transmitters. Time and stress associated with handling, capture, transport, surgery and release can interfere with normal reproductive behavior and lead to nest abandonment. This study suggests that anesthesia at time of release reduces nest abandonment after surgery. Waterfowl anesthetized with isoflurane are completely aware at time of release whereas birds anesthetized with propofol are allowed to recover in their natural environment without human disturbance. A study by Arnold et al. reported a 9.9% nest abandonment rate (39 abandoned, 353 did not abandon) in female canvasback ducks following capture and marking. A comparison of nest abandonment in the two surgery groups in this study with results of Arnold et al. revealed that surgery produced higher abandonment rates ($X^2 = 11.75$, 2 df, $P = 0.003$). However, when results for the propofol group were compared with findings of Arnold et al., no significant difference in nest abandonment ($X^2 = 1.12$, 1 df, $P = 0.29$) was found, suggesting that the former relationship was driven primarily by the isoflurane group. Stress at time of recovery in the isoflurane group may be responsible largely for the higher nest abandonment rate.

Although nest abandonment in the propofol group (15.4%) wasn’t significantly different from results of Arnold et al. (9.9%), it is unwise to conclude that surgery and anesthesia had no effect. Further
investigation with larger numbers of females in treatment groups is required to resolve this issue. Since propofol provides little intra- and post-operative analgesia, supplemental analgesia is required for surgical procedures. In this study, bupivacaine was administered prior to surgery to provide both intra- and post-operative pain control. The effectiveness of bupivacaine to provide analgesia is undetermined in avian species and further investigation is required.

ACKNOWLEDGMENTS

Funding for this project was provided by Delta Waterfowl and Wetlands Research Station, Ducks Unlimited Institute for Wetland and Waterfowl Research and Western College of Veterinary Medicine Wildlife Health Fund (University of Saskatchewan). Support in kind was provided by the Canadian Wildlife Service and Malinkrodt Groups Inc. I would like to thank Robert Brua, Robert Clark, Marnie Cooper, Nigel Caulkett, Shannon Lind and Margaret Yole for their assistance and advise on this project. I am also grateful to the numerous field assistants who located the nests and provided technical support during this study.

LITERATURE CITED

Table 1. Nest abandonment of female canvasback ducks in late incubation following random assignment to 3 treatment groups. Number of females (% abandoned) and group sample sizes (n) for each year and years combined (Total) are shown.

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<td>39</td>
<td>11 (28.2%)</td>
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DISEASE SURVEILLANCE IN FREE-RANGING HUMBOLDT PENGUINS (*Spheniscus humboldti*) IN CHILE

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Abstract

The Humboldt penguin (*Spheniscus humboldti*) is native to the Peruvian and Chilean coast, and is one of the world’s most endangered penguin species. It is listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and currently it is one of two species of penguins in North American zoos and aquaria to be managed under an Association of Zoos and Aquaria (AZA) Species Survival Plan. Although the population in the wild has decreased dramatically over the past several decades, and the species has been in captivity for many years, review of the literature revealed that very little has been published about the natural biology and behavior of the Humboldt penguin.

In 1994, the Milwaukee County Zoo initiated a 5-yr study of the natural ecology of the Humboldt penguin residing on Ex-isolote de los Pajaros Ninos near Algarrobo, Chile. The team of field researchers originally included members of the veterinary, curatorial and keeper staff of the Milwaukee County Zoo, a Chilean graduate student, and a field assistant. Contact with a well known Chilean ornithologist had been established during an earlier study of the colony and continues; he obtains the necessary permits, and functions both as a liaison between the field team and the Chilean government, and as a guide. Although several of the original co-investigators have left the zoo they remain active members of the project and field team (they are supported by their institutions, the Riverbanks Zoo and Denver Zoo, through paid leave and small monetary contributions). Researchers from the United States are able to spend approximately 8-10 wk annually in the field, while the graduate student and field assistant monitor the research site approximately every 2 wk during the rest of the year.

The study is multifaceted, investigating nest site tenacity, pair-bonding, incubation time, weight loss of eggs during incubation, longevity, morphometrics for sex determination, causes of mortality, chick growth rates, and prevalence of disease as determined by serologic assays.

Establishing the prevalence of disease in an apparently healthy, free-ranging population is valuable for several reasons. First, if the population were to experience a significant increase in mortality in the future, comparisons between the serologic prevalence of diseases in the ill population and those established previously may help elucidate the pathogenic agent, and source of disease. Second, comparison of the prevalence of disease in wild versus captive populations may indicate those diseases that occur primarily in captivity and lead to improved husbandry. Finally, knowledge of the diseases affecting a free-ranging population is useful if it becomes necessary to import wild birds as new genetic stock for captive propagation.
During the first 2 yr of the study, approximately 750 penguins were individually marked, and blood samples were collected. Plasma was removed, frozen, and transported to the United States for assay. To date, 200 plasma samples have been assayed for titers to paramyxovirus-1 (PMV-1 or Newcastle’s disease), PMV-2, and PMV-3 (hemagglutination inhibition assays), and avian influenza (agar gel immunodiffusion). Ninety-nine samples have been assayed for Aspergillus titers (ELISA), and 99 for titers to Chlamydia (complement fixation). Preliminary results indicate that the percentage of samples with positive titers to PMV-1, Aspergillus, and avian influenza is low to none. Approximately 20% of the samples had positive titers to Chlamydia, nearly 50% were positive for PMV-2, and roughly 10% of the samples had titers to PMV-3.

The use of caution cannot be overstated when interpreting serologic results and using them to make assumptions about the overall health of the population. Except for the Aspergillus assay, none of these assays has been validated for Humboldt penguins. Production of humoral antibodies in response to the PMV-1 virus varies widely among taxonomic groups and individuals; the same may hold true for PMV-3. It is interesting to note that one reference states that antibodies to PMV-3 haven’t been documented in feral birds. The direct complement fixation (CF) test for chlamydia detects only immunoglobulin G activity, and therefore is useful to detect past infection only. In addition, this test method may not be accurate in many bird species. Elementary body agglutination is more worthwhile for detecting current infection. However, CF may be useful epidemiologically to follow the status of a population. Serial sampling of individual penguins will be necessary to document changes over time and is in our research plan. Investigations into the Chlamydia titers using elementary body agglutination, as well as prevalence of diseases caused by Plasmodium spp. (avian malaria), Salmonella pullorum, and Mycoplasma spp. will ensue as funding allows.

ACKNOWLEDGMENTS

We would like to thank the Zoological Society of Milwaukee for their contribution to this project, especially funds for stipends and for the serologic assays. We would also like to thank the Windway Foundation, of Sheboygan Wisconsin, for underwriting our airfare and other expenses. We wish to extend our gratitude to the Consejo de Monumentos Nacionales and the Corporacion Nacional al Forestal of Chile for their permission to do this study, and to the Cofradía Nautica del Pacifico at Algarrobo for allowing us to access to the island. Finally, a special thank you goes to our Chilean colleagues: Dr. Braulio Araya, Alejandro Simeone, and Mariano Bernal.

LITERATURE CITED

MEDICAL ASSESSMENT OF RUFFED LEMURS (Varecia variegata) FOR RESTOCKING PROGRAMS IN MADAGASCAR

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Abstract

Conservation activities that involve relocation of animals present unique situations in which disease transmission may occur. As part of a proposed restocking project for black and white ruffed lemurs, a medical evaluation program was designed. Three populations were assessed; the wild population at the release site, the Malagasy captive population, and the North American population. Testing protocols were designed to identify diseases likely to have significant impact - contagious diseases with carrier states, that could become established in wild populations and significantly impact health and population dynamics. Medical evaluation consisted of a complete physical examination, complete blood count, serum biochemical profile, intradermal tuberculin test, serum viral profile (hepatitis A and B, herpes simplex, cytomegalovirus, Ebstein Barr virus, measles, and simian immunodeficiency virus), fecal culture, and fecal parasite examination. No evidence of infectious disease was found in any population; however, the Malagasy captive population had evidence of a possibly compromised nutritional status.

Introduction

The significance of infectious disease to reintroduction projects has become an increasingly important question. As captive propagation and management practices improve and remaining areas of natural habitat are secured, opportunities develop for animals in captivity to be released into native habitat. The movement of animals is accompanied by the inherent risk of exposure to disease in a variety of scenarios. This report describes the rationale and application of a medical evaluation program for a potential restocking program of black and white ruffed lemurs (Varecia variegata variegata) in Madagascar.

The opportunity for restocking of ruffed lemurs occurred due to ongoing projects supported by the Madagascar Fauna Group (MFG). Due in part to support by MFG, a forestry research station near Tamatave, on the east coast of Madagascar, has been converted into a small zoo (Parc Ivoloina). As a result of donations and government confiscations, the number of ruffed lemurs in the collection has grown beyond the carrying capacity. This large population is pushing the limits of both the physical facility and the budget for animal care. At the same time, Reserve Naturelle Integrale No. 1, Betampona, is reasonably accessible and underutilized for research purposes. It is small enough to be well censused and monitored (5500 acres), and has the potential for holding more Varecia than are currently there. The reserve is 30 km from Ivoloina, and is surrounded by degraded forest and agricultural lands. The project proposed by the MFG advisors involves alleviating the overcrowding by releasing selected Varecia from the Ivoloina collection into the Betampona Reserve.

Methods

For the Betampona Restocking Project, three populations were considered. Health assessments were
to be done on each population, using comparisons between the populations to identify significant health issues. The populations were: 1) the potential release population at Ivoloina, 2) the resident population at Betampona, and 3) the North American captive population (provided reference values). To assess the wild population, samples were to be collected at two locations during radiocollaring projects conducted by field researchers: at Betampona, and on the Masoala Peninsula. Although the samples from Masoala were not part of the resident population at the release site (and in fact were a different subspecies, *V. v. rubra*), it was felt that the samples would be useful for comparative purposes.

Diagnostic evaluations were selected based on diseases felt to have potential for significant impact on the restocking project. Exposure to humans played a major part in determination of disease testing. The assumption was made that the captive population had been exposed to many human diseases to which the wild population had not.

Complete health profiles were completed on all lemurs in the study. This consisted of 18 *Varecia variegata variegata* held in captivity at Parc Ivoloina and 4 *Varecia variegata rubra* from the Masoala Peninsula. No animals were captured in the reserve at Betampona. Health profiles were designed to assess the physical health of the animals, as well as to detect exposure to disease.

Each animal was physically or chemically (Telazol) restrained. A complete physical examination was performed. Each captive animal received an intradermal tuberculin test (0.1 ml Cooper’s old tuberculin, upper palpebra). Whole blood was collected for a complete blood cell count and hemoparasite examination, and preserved for genetic evaluation. Blood was collected, separated, and serum saved frozen for serum biochemical profile and viral serology. Fecal samples were collected and preserved in 10% formalin for microscopic examination for endoparasites. Rectal swabs were collected in transport media for enteric pathogen culture. Hair samples and pelage color pattern descriptions were collected for genetic research.

**Results**

*Physical Examinations*

**Ivoloina** - All examined animals were found to be in adequate condition. Body weights and composition seemed appropriate. Hair coats appeared dry and slightly dull. Although some reproduction was occurring, many adults of appropriate reproductive age were not bearing young. No evidence of external parasites, injuries, or other medical problems were detected on physical examination.

**Betampona** - Although no animals were captured, several were visually examined at close distances. All animals observed appeared in good health, and all had dense, lustrous hair coats. All animals were active, and no evidence of compromised health (gait abnormalities, wounds, ocular or nasal discharges, etc.) were detected. Infants were seen on one occasion and heard in a nest on another, indicating that reproduction is occurring.

**Masoala** - All examined animals were found to be in good health. No evidence of compromised health was detected. External parasites, thought to be mites, were present in the ear canals of all four lemurs. Identification of the parasite has not yet been completed. It was felt that these parasites did not present a clinically significant health concern.
**Tuberculin Testing**

All of the captive *Varecia* had negative intradermal tuberculin tests at 72 hr.

**Hematology**

Complete blood cell counts were within normal limits for all animals. With the exception of blood urea nitrogen (BUN), serum chemistry values of captive and wild animals fall within normal ranges as established for the captive North American population. BUN levels in wild (3.8 mg/dL) and Malagasy captive lemurs (7.83 mg/dL) are below the normal range for North American captive ruffed lemurs (mean ± 2SD = 8.3 - 39.9 mg/dL).

No abnormalities were detected for captive animals at Ivoloina that would suggest specific health problem. No hemoparasites were detected in any blood smear.

**Viral Serology**

Serological assays were done for the following viral diseases: hepatitis A (HEP A), hepatitis B (HBsab), herpes simplex 1 (HSV-1), cytomegalovirus (CMV), Epstein Barr virus (EBV), measles, and simian immunodeficiency virus (SIV). These diseases were selected based on several criteria. These diseases are present in the human population of Madagascar. They all have a carrier state, such that they could be maintained latent in a host and shed at a later date. They all have potential to produce significant disease, either by direct mortality or reduced reproduction. The exception is simian immunodeficiency virus. Immunodeficiency viruses are being discovered in a variety of species, including nonhuman primates. Although no immunodeficiency diseases have been documented in lemurs, samples were surveyed for evidence of infection.

None of the 22 Malagasy animals tested positive for any of the viral diseases.

**Fecal Flotations**

Saline flotations of formalin - fixed feces was done for detection of parasite ova. None of the captive lemurs had evidence of internal parasites. These animals are routinely treated with ivermectin (every 4 mo). One of the wild *Varecia* had a single nematode ova detected in the flotation.

**Fecal Cultures**

An enteric pathogen screen was performed on the fecal culture swabs. Each sample was cultured for *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia*. These organisms all cause disease syndromes in humans and a variety of animal species, and have the potential of persisting in a carrier state. All 22 *Varecia* sampled were negative for enteric pathogens.

**Discussion**

Ivoloina - Excess animals present a variety of problems for this collection. Budgetary constraints limit the ability to provide further cage space and may affect provision of a proper diet. Current diets are functional, but may be lacking in vitamins or trace minerals, or lack adequate levels of basic nutrients.
The possibility of a marginal diet is evidenced by the dry, dull hair coats of the animals examined, as well as the decreased reproductive rate. Complete bloodwork does not indicate any specific nutritional deficiencies. No evidence of infectious disease exists in this captive population that would restrict their inclusion in release projects. Although these animals were held as pets by private individuals, no transmissible diseases that occur in the human population in Madagascar were detected in the animals.

Betampona - No specific medical conclusions may be made for the ruffed lemurs in Betampona. In general, the animals appeared in good health.

Masoala - Although the subspecies and location differ from the ruffed lemurs of Betampona, basic health parameters may be used for comparison. Examination of the animals and evaluation of the blood and fecal samples suggest that this group of wild lemurs does not harbor any of the potentially serious infectious diseases surveyed.

The blood urea nitrogen levels of both the Malagasy captive and the wild ruffed lemurs are significantly lower than the (International Species Information System) ISIS values, the wild animals having values roughly half those of the Ivoloina animals. One possible cause of this difference may be diet-related, but this would suggest that the wild animals eat a diet even lower in protein than that fed at Ivoloina. Levels of dietary protein required by lemurs have been discussed, and this result may suggest that dietary levels in captivity are higher than in the wild. The small sample size might also be introducing artifact into the comparison.

Conclusions

No evidence of infectious disease as a limiting factor for release into the wild was identified. Although the captive lemurs live in close contact with humans, there was no evidence of transmission to lemurs of diseases present in the human population (measles, TB, enteric pathogens, hepatitis). However, the animals held at Ivoloina were determined not to be prime candidates for release due to suspected nutritional deficiencies. Subsequently, 4 pairs of these animals were imported into the United States to bolster the US captive population with new founders. These animals quickly improved in condition, and reproduction occurred in the second year.

Healthy, compatible pairs of ruffed lemurs were identified in the captive population to be potential reintroduction candidates. These animals will undergo an extensive medical evaluation and pre-release conditioning, with the goal of releasing them into Betampona in the fall of 1997.

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SOUTHERN AMERICAN PINNIPEDS: IMMobilization, TELemetry, AND HEALTH EVALuations

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Abstract

Over the past 4 yr, immobilizations of South American fur seals (Arctocephalus australis) (FS), South American sea lions (Otaria byronia) (SL) and southern elephant seals (Mirounga leonina) (SES) have been conducted in Peru and Argentina for ecological studies and population health surveys. During the handling procedures, animals were fitted with radio-tracking or behavioral recording devices, individually identified, examined, and blood and fecal samples collected for analysis.

Three hundred and eleven adult FS males, females and their neonates were examined during three pupping seasons from 1992-94 at Punta San Juan, Nazca Department, Peru. Females and pups were manually restrained. Male FS were darted with a CO2 powered pistol using 3 ml plastic darts and 1.5 x 38 mm collared needles (Telinject USA, 9316 Soledad Canyon Rd., Saugus, CA, 91350 USA). Darting was performed after approaching a territorial male and confirming that the animal would not abandon its territory if disturbed. Most males (n = 32) were immobilized using only a premixed combination of equal parts tiletamine hydrochloride and zolazepam (Telazol®, Fort Dodge Laboratories, Fort Dodge, Iowa, 50501 USA) using a mean dosage of 1.43 mg/kg body weight. Four males were immobilized using Telazol® but supplemented with 75 mg or 100 mg i.m. doses (x̄ = 0.81 mg/kg) of ketamine hydrochloride (KET) (Ketaset®, Fort Dodge Laboratories, Fort Dodge, IA) when they were not immobilized enough for safe handling. Additional males (n = 8) were immobilized with a combination of Telazol® (x̄ = 1.15 mg/kg) and KET (x̄ = 0.27 mg/kg) mixed in a single dart. One adult male was immobilized with KET (1 mg/kg) and midazolam (0.1 mg/kg). A benzodiazepine antagonist, flumazenil (Mazicon®, Hoffmann-La Roche, 340 Kingsland St., Nutley, NJ 07110-1199 USA), was administered to most FS (n = 36) which received Telazol®. For 16 FS, flumazenil (0.3-0.5 mg) was given intramuscularly immediately upon induction of anesthesia (x̄ = 10.6 min after darting) to reverse some of the respiratory depressant effects of the zolazepam component of the Telazol®. For 25 FS, flumazenil (3.0-5.0 mg) was given intramuscularly at the end of the handling procedure to antagonize the effects of zolazepam in animals which were still heavily sedated at the end of the handling procedure.

Most of the SL and all of the SES were handled in Chubut and Santa Cruz Provinces of Argentina. For
the SL, 19 adult females were immobilized for radio-tagging. Thirteen were immobilized using Telazol® ($\bar{x} = 2.75$ mg/kg) given intramuscularly with a syringe after restraining the animal in a net. Seven SL were immobilized with isoflurane (Ohmeda PPD, Inc., Liberty Corner, NJ, 07938 USA) after restraining the animal in a net. Atropine hydrochloride (0.02 mg/kg) was given intramuscularly 5 min prior to anesthetic administration for seven of the FS receiving Telazol® and the five receiving isoflurane. Flumazenil was given intramuscularly to females immobilized with Telazol® at the end of procedures using 1 mg of flumazenil for every 20-25 mg of zolazepam administered. Sea lion pups were manually restrained for tagging, examination and sample collection while their dams were immobilized. Elephant seals ($n = 4$) were immobilized with Telazol® (0.6-1.7 mg/kg) given intramuscularly while they were resting or sleeping on the beach.

Immediately upon capture, each animal received a physical examination. A fecal sample was collected manually from the rectum when possible and preserved in 10% formalin. Blood was collected from a tarsal vein in the adult FS and SL or the dorsal vertebral vein/sinus in the neonates and the adult SES and kept on ice until processing.

Two FS were fitted with Standard VHF radio transmitters (Advanced Telemetry Systems, Inc., 470 1st Ave. North, Box 398, Isanti, MN 55040 USA). Sea lions were fitted with transmitters utilizing the ARGOS satellite system and containing instrumentation to record data on diving patterns and behavior (Wildlife Computers, P.O. Box 2211, Woodinville, WA 98072 USA). Elephant seals were fitted with devices that recorded diving behavior but have to be recovered from the animal to download the stored data (Wildlife Computers, P.O. Box 2211, Woodinville, WA 98072 USA). Quick setting epoxy was used to attach all transmitters and recording devices to the pelage of the animal. The devices then fell off at the time of the next molt, or removed at a later date using manual restraint.

Mean time to achieve maximum effect was 10.6 min for Telazol® in the male FS. For the Telazol® and KET combination it was 10.0 min. For Telazol® in the female SL it was 10.7 min and in the SES it was 10.0 min. Telazol® provided predictable and adequate immobilizations in almost all animals for the handling procedures conducted. Respiratory depression associated with benzodiazepams was seen in the FS and SL, but the use of low doses (0.05-1 mg) of flumazenil rapidly improved depth of respiration during the procedures. For complete reversal of benzodiazepams, the recommended dosage...
of flumazenil is 1 mg for every 20-25 mg of benzodiazepam used. The flumazenil appeared to eliminate the “drunken” appearance of animals recovering from anesthesia, and increased alertness and responsiveness within minutes of administration.

Isoflurane resulted in complete immobilization in a mean of 11.7 min. Recovery from isoflurane anesthesia was rapid (3-5 min) and complete.

Blood parasites or ectoparasites were not seen in any of the animals sampled. Ectoparasites or signs of parasitic dermatitis were not observed on any animal. Ascarid ova were found in one of the seven fecal samples from SL. Of the 19 samples from FS, five had strongyle-like ova and two had trematode ova.

Results of FS serum chemistries, enzymes, vitamin, and mineral analyses for the sex and age classes are provided in Table 2. Results for SL are in Table 3. Levels for Al (<1.66 ppm), B (<1.66 ppm), Co (<0.167 ppm), Mn (<0.083 ppm), and Mo (<0.33 ppm) were below accurate detectable limits. All chlorinated pesticide and polychlorinated biphenyl analyses were below detectable limits (0.001 - 0.007 ppm and 0.05 ppm respectively). Serology results are in Table 3.

Histopathology from the yearlings showed lymphoplasmacytic enteritis in nine of the 11 SL with multifocal crypt abscession in seven, and one of the three FS also was found to have lymphoplasmacytic enteritis.

Table 1. Serological tests performed, the number of positive animals, and the number of animals tested in the evaluation of infectious disease agent exposure in free-ranging South American pinnipeds in Argentina and Peru.

<table>
<thead>
<tr>
<th>Etiologic agent</th>
<th>Fur seals</th>
<th>Sea lions</th>
<th>Elephant seals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># positive/# tested</td>
<td># positive/# tested</td>
<td># positive/# tested</td>
</tr>
<tr>
<td>Brucella abortus mellitensis</td>
<td>2/36</td>
<td>0/27</td>
<td>0/15</td>
</tr>
<tr>
<td>Calycivirus (SMSV)</td>
<td>1/6</td>
<td>0/12</td>
<td>0/2</td>
</tr>
<tr>
<td>Calycivirus (VES)</td>
<td>32/60</td>
<td>1/12</td>
<td>1/2</td>
</tr>
<tr>
<td>Canine herpesvirus</td>
<td></td>
<td>1/9</td>
<td>5/7</td>
</tr>
<tr>
<td>Influenza-A</td>
<td>0/60</td>
<td>0/19</td>
<td>0/7</td>
</tr>
<tr>
<td>Leptospira interrogans</td>
<td>2/60</td>
<td>5/22</td>
<td>4/7</td>
</tr>
<tr>
<td>Morbillivirus (canine)</td>
<td>0/32</td>
<td>1/9</td>
<td>13/17</td>
</tr>
<tr>
<td>Morbillivirus (phocid)</td>
<td>0/60</td>
<td>0/27</td>
<td>12/17</td>
</tr>
<tr>
<td>Morbillivirus (porpoise)</td>
<td></td>
<td></td>
<td>6/15</td>
</tr>
<tr>
<td>Test Name (units)</td>
<td>Males</td>
<td></td>
<td>Females</td>
</tr>
<tr>
<td>------------------</td>
<td>-------</td>
<td>-------</td>
<td>---------</td>
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<tr>
<td>Test Name (units)</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
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<tr>
<td>PCV</td>
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<tr>
<td>Total solids</td>
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<tr>
<td>RBC ($\times 10^6$)</td>
<td>4.1</td>
<td>1.0</td>
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<td>WBC ($\times 10^3$)</td>
<td>18.9</td>
<td>5.6</td>
<td>30</td>
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<tr>
<td>Neutrophils (%)</td>
<td>73.7</td>
<td>6.5</td>
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<tr>
<td>Lymphocytes (%)</td>
<td>22.4</td>
<td>5.7</td>
<td>24</td>
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<tr>
<td>Monocytes (%)</td>
<td>1.9</td>
<td>1.7</td>
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<tr>
<td>Eosinophils (%)</td>
<td>1.9</td>
<td>1.8</td>
<td>24</td>
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<tr>
<td>Basophils (%)</td>
<td>0.2</td>
<td>0.7</td>
<td>24</td>
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<tr>
<td>Glucose (mg/dL)</td>
<td>84.5</td>
<td>35.5</td>
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<td>BUN (mg/dL)</td>
<td>27.1</td>
<td>6.4</td>
<td>33</td>
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<tr>
<td>Creatinine (mg/dL)</td>
<td>2.0</td>
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<td>BUN/creatinine ratio</td>
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<td>33</td>
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<td>Total protein (g/dL)</td>
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<td>33</td>
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<td>Albumin (g/dL)</td>
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<td>0.5</td>
<td>33</td>
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<tr>
<td>Globulin (g/dL)</td>
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<td>0.6</td>
<td>33</td>
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<tr>
<td>Albumin/globulin ratio</td>
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<td>1.2</td>
<td>33</td>
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<tr>
<td>Total bilirubin (mg/dL)</td>
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<td>0.05</td>
<td>33</td>
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<tr>
<td>Alkaline phosphatase (IU/L)</td>
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<td>5.0</td>
<td>33</td>
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<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>18.1</td>
<td>13.0</td>
<td>33</td>
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<tr>
<td>Aspartate aminotransferase (IU/L)</td>
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<td>11.7</td>
<td>33</td>
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<tr>
<td>Lactate dehydrogenase (IU/L)</td>
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<td>156</td>
<td>33</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>77.7</td>
<td>27.7</td>
<td>33</td>
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<tr>
<td>Calcium (mg/dL)</td>
<td>8.9</td>
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<td>33</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>5.5</td>
<td>1.3</td>
<td>33</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>153.4</td>
<td>5.4</td>
<td>33</td>
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<tr>
<td>Potassium (mEq/L)</td>
<td>4.4</td>
<td>0.5</td>
<td>33</td>
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<tr>
<td>Chloride (mEq/L)</td>
<td>114</td>
<td>4.2</td>
<td>33</td>
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<tr>
<td>Copper (µg/ml)</td>
<td>0.9</td>
<td>0.2</td>
<td>27</td>
</tr>
<tr>
<td>Iron (µg/ml)</td>
<td>2.6</td>
<td>3.3</td>
<td>27</td>
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<tr>
<td>Magnesium (µg/ml)</td>
<td>22.7</td>
<td>3.3</td>
<td>27</td>
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<tr>
<td>Zinc (µg/ml)</td>
<td>0.8</td>
<td>0.3</td>
<td>27</td>
</tr>
<tr>
<td>Alpha-tocopherol (µg/ml)</td>
<td>3.5</td>
<td>1.9</td>
<td>17</td>
</tr>
<tr>
<td>Retinol (µg/ml)</td>
<td>0.74</td>
<td>0.31</td>
<td>17</td>
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</table>
Table 3. Hematology, serum chemistry, enzyme, mineral, vitamin and metal values for free-ranging South American sea lion (*Otaria Byronia*) adult females and 1-5-day-old pups.

<table>
<thead>
<tr>
<th>Test Name (units)</th>
<th>Adults</th>
<th>Pups</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>PCV</td>
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<tr>
<td>Total solids</td>
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<tr>
<td>RBC (× 10^6)</td>
<td>4.2</td>
<td>0.4</td>
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<tr>
<td>WBC (× 10^3)</td>
<td>16.1</td>
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<td>Neutrophils (%)</td>
<td>57.8</td>
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<td>Lymphocytes (%)</td>
<td>33.8</td>
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<td>Monocytes (%)</td>
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<td>1.6</td>
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<td>Eosinophils (%)</td>
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<td>Basophils (%)</td>
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<td>Glucose (mg/dL)</td>
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<tr>
<td>Creatinine (mg/dL)</td>
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<td>Albumin (g/dL)</td>
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<td>Globulin (g/dL)</td>
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<td>Albumin/globulin ratio</td>
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<td>Total bilirubin (mg/dL)</td>
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<td>0.08</td>
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<tr>
<td>Alkaline phosphatase (IU/L)</td>
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</tr>
<tr>
<td>Alanine aminotransferase (IU/L)</td>
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<tr>
<td>Aspartate aminotransferase (IU/L)</td>
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<td>Lactate dehydrogenase(IU/L)</td>
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<td>Calcium (mg/dL)</td>
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<td>Sodium (mEq/L)</td>
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<td>Chloride (mEq/L)</td>
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<td>Copper (µg/ml)</td>
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<td>Iron (µg/ml)</td>
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<td>Zinc (µg/ml)</td>
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<td>Alpha-tocopherol (µg/ml)</td>
<td>10.6</td>
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<tr>
<td>Retinol (µg/ml)</td>
<td>0.45</td>
<td>0.05</td>
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ELEPHANTS, BUFFALO, KOB, AND RHINOCEROS: IMMOBILIZATION, TELEMETRY AND HEALTH EVALUATIONS

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Abstract

From 1992 through 1996, African elephants (Loxodonta africana), savanna buffalo (Syncerus caffer), Ugandan kob (Kobus kob), and northern white rhinoceros (Ceratotherium simum cottoni) were immobilized for health evaluations or radio-telemetry studies at Garamba National Park in northeastern Zaire.

Thirty-seven elephants were darted for immobilization using either 50 caliber metal darts and 3.1 or 5.0 × 60 mm (Palmer Chemical & Equipment Co., Inc., Box 867, Douglasville, GA 30133 USA) or 3 ml plastic darts and 2.0 × 60 mm needles (Telinject USA, 9316 Soledad Canyon Rd., Saugus, CA 91350 USA). Four calves, 4-6-yr-old were immobilized using 1 mg of carfentanil hydrochloride (Wildlife Pharmaceuticals, Fort Collins, CO 80524 USA). Adults were immobilized with 3 mg of carfentanil mixed with 1500 IU of hyaluronidase. All animals were reversed with naltrexone hydrochloride (Wildlife Pharmaceuticals, Fort Collins, CO 80524 USA) at a rate of 100 mg for every mg of carfentanil used. Failure of the darts to inject properly occurred in 15 cases. For animals responding completely to the first darting attempt, mean minutes elapsed for initial effect or standing still, recumbency, and recovery following reversal were 5.0 ± 1.6, 10.7 ± 3.9, and 5.9 ± 3.9 respectively.

Sixteen adult buffalo were immobilized using 3 ml Telinject plastic darts with 2.0 × 60 mm needles and 5 mg of carfentanil. Failure of the darts to inject properly occurred in 2 cases. For animals responding completely to the first darting attempt, mean minutes elapsed for initial effect or standing still, recumbency, and recovery following reversal were 2.0 ± 0.6, 4.3 ± 2.4, and 3.9 ± 2.6 respectively.

Eleven adult kob were immobilized 3 ml Telinject plastic darts with 1.5 × 38 mm collared needles and 2 mg of carfentanil. Failure of the darts to inject properly occurred in 5 cases. For animals responding completely to the first darting attempt, mean minutes elapsed for initial effect or standing still, recumbency, and recovery following reversal were 2.7 ± 0.7, 6.5 ± 4.1, and 1.2 ± 0.4 respectively.

Nine rhinoceros were darted with 50 caliber metal darts and 60 mm needles made by the South Africa National Parks Board using a combination of etorphine (2-3.8 mg) and detomidine (9-16 mg). Failure of the darts to inject properly occurred in 3 cases. For animals responding completely to the first darting attempt, mean minutes elapsed for initial effect or standing still, recumbency, and recovery following reversal were 5.2 ± 2.5, 8.0 ± 3.0, and 1.7 ± 0.7 respectively.

Packed cell volumes, total protein, and white blood cell counts were conducted in the field and serum
was frozen in liquid nitrogen. Serum chemistries and enzymes were processed on an automated analyzer. Plasma samples were analyzed for vitamin E (α-tocopherol) and vitamin A (retinol) using high-performance liquid chromatography. Plasma samples were analyzed for aluminum, boron, barium, copper, cobalt, iron, magnesium, manganese, molybdenum, and zinc by inductively coupled argon plasma emission spectroscopy. Plasma was also analyzed for polychlorinated biphenyls and the following chlorinated pesticides: aldrin; alpha - BHC; beta - BHC; O, P' - DDD; P, P' - DDD; P, P' - DDE; O, P' - DDT; P, P' - DDT; Dieldrin; endrin; heptachlor; heptachlor epoxide; and Lindane (Gamma - BHC). Serological tests are listed in Table 1.

Four elephants were fitted with standard VHF radio transmitters (Advanced Telemetry Systems, Inc., 470 1st Ave. North, Box 398, Isanti, MN 55040 USA) mounted on collars made of 75-mm-wide machine belting. One elephant was fitted with a transmitter utilizing the ARGOS satellite system (Telonics, 932 East Impala Dr., Mesa, AZ, 85204 USA). Four rhinoceros were fitted with VHF radio collars (Telonics) and five had VHF radio transmitters (Advanced Telemetry Systems) implanted in their horns. This was accomplished by drilling a 35-mm-diameter hole transversely near the base of the horn and a 5-mm hole from the horn tip down to meet the transverse hole. The transmitter was inserted in the larger hole with the antenna being fed up through the lengthwise hole. Dental acrylic was used to fill and seal the cavities.

No ectoparasites were found on the elephants or kob. No evidence of gastrointestinal parasites was found in fecal samples from the elephants. Samples from kob showed coccidia, strongyles, or trematodes. All buffalo were heavily invested with ticks (Amblyomma cohaerens and Rhipicephalus longus). Fecal samples from buffalo showed coccidia, strongyles, and/or trematodes. Rhinoceros were found to have light infestations of ticks (Amblyomma cohaerens, Dermacentor rhinocerinus, and Rhipicephalus senegalensis), about 20-100 ticks/animal. Fecal samples from the rhinoceros revealed strongyles, strongyloides-like larvae, coccidia, and in one individual sample, ascarid ova.

Hematologic, biochemical, vitamin and mineral tests results for buffalo and kob, and for elephant and rhinoceros in Tables 2 and 3 respectively. Levels for Al (<1.66 ppm), B (<1.66 ppm), Co (<0.167 ppm), Mn (<0.083 ppm), and Mo (<0.33 ppm) were below accurate detectable limits. All chlorinated pesticide and polychlorinated biphenyl analyses were below detectable limits (0.001-0.007 ppm and 0.05 ppm respectively). Infectious disease serology results are provided in Table 1.
Table 1. Serological tests performed, the number of positive animals, and the number of animals tested in the evaluation of infectious disease agent exposure in free-ranging buffalo, kob, elephants, and white rhinoceros in Zaire.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Buffalo</th>
<th>Kob</th>
<th>Elephant</th>
<th>Rhinoceros</th>
</tr>
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<tbody>
<tr>
<td>African horse sickness</td>
<td>15/16</td>
<td>0/8</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Anaplasmosis</td>
<td>0/16</td>
<td>0/8</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Bluetongue</td>
<td>4/7</td>
<td>9/16</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Bovine viral diarrhea</td>
<td>7/16</td>
<td>0/8</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Brucellosis</td>
<td>4/16</td>
<td>2/8</td>
<td>0/5</td>
<td>4/16</td>
</tr>
<tr>
<td>Epizootic hemorrhagic disease virus</td>
<td>10/10</td>
<td>8/8</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Equine adenovirus</td>
<td></td>
<td></td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Equine herpes virus (-1,-2,-3)</td>
<td></td>
<td></td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Equine influenza</td>
<td></td>
<td></td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Equine rhinovirus (-1,-2)</td>
<td></td>
<td></td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Foot-and-mouth disease</td>
<td>14/16</td>
<td>1/8</td>
<td>0/16</td>
<td>0/5</td>
</tr>
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<td>Infectious bovine rhinotracheitis</td>
<td>16/16</td>
<td>8/8</td>
<td>0/16</td>
<td>0/5</td>
</tr>
<tr>
<td>Johne’s disease</td>
<td>0/7</td>
<td>0/16</td>
<td>0/5</td>
<td></td>
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<tr>
<td>Leptospira interrogans</td>
<td>7/16</td>
<td>3/8</td>
<td>16/16</td>
<td>5/5</td>
</tr>
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<td>16/16</td>
<td>2/8</td>
<td>0/16</td>
<td>0/5</td>
</tr>
<tr>
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<td>0/16</td>
<td>0/8</td>
<td>0/16</td>
<td>0/5</td>
</tr>
<tr>
<td>Rinderpest</td>
<td>0/16</td>
<td>0/8</td>
<td>0/16</td>
<td>0/5</td>
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Table 2. Hematology, serum chemistry, enzyme, mineral, vitamin and metal values for free-ranging buffalo and kob in Zaire.

<table>
<thead>
<tr>
<th>Test Name (units)</th>
<th>Buffalo</th>
<th></th>
<th></th>
<th>Kob</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
</tr>
<tr>
<td>PCV</td>
<td>36.9</td>
<td>7.25</td>
<td>16</td>
<td>49.1</td>
<td>4.2</td>
<td>9</td>
</tr>
<tr>
<td>Total solids</td>
<td>9.3</td>
<td>1.0</td>
<td>16</td>
<td>8.3</td>
<td>0.7</td>
<td>8</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>99.0</td>
<td>38.4</td>
<td>16</td>
<td>112</td>
<td>48.8</td>
<td>8</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>12.8</td>
<td>3.8</td>
<td>16</td>
<td>23.5</td>
<td>2.8</td>
<td>8</td>
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<tr>
<td>Albumin (g/dL)</td>
<td>2.3</td>
<td>0.5</td>
<td>16</td>
<td>3.9</td>
<td>0.2</td>
<td>8</td>
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<tr>
<td>Globulin (g/dL)</td>
<td>5.8</td>
<td>1.0</td>
<td>16</td>
<td>3.1</td>
<td>0.5</td>
<td>8</td>
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<tr>
<td>Albumin/globulin ratio</td>
<td>0.4</td>
<td>0.1</td>
<td>16</td>
<td>1.3</td>
<td>0.3</td>
<td>0.3</td>
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<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.1</td>
<td>0.0</td>
<td>16</td>
<td>0.4</td>
<td>0.2</td>
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<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>78.3</td>
<td>56.5</td>
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<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>22.0</td>
<td>5.0</td>
<td>16</td>
<td>36.2</td>
<td>12.1</td>
<td>8</td>
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<tr>
<td>Aspartate aminotransferase (IU/L)</td>
<td>139</td>
<td>37.7</td>
<td>16</td>
<td>99.7</td>
<td>11.7</td>
<td>8</td>
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<td>Lactate dehydrogenase (IU/L)</td>
<td>464</td>
<td>65.7</td>
<td>16</td>
<td>685</td>
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<td>Cholesterol (mg/dL)</td>
<td>53.6</td>
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<td>16</td>
<td>37.6</td>
<td>8.3</td>
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<td>Calcium (mg/dL)</td>
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<td>0.8</td>
<td>16</td>
<td>8.6</td>
<td>0.4</td>
<td>8</td>
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<td>Phosphorus (mg/dL)</td>
<td>5.3</td>
<td>1.2</td>
<td>16</td>
<td>6.2</td>
<td>1.0</td>
<td>8</td>
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<td>Sodium (mEq/L)</td>
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<td>3.4</td>
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<td>144</td>
<td>4.4</td>
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<td>Potassium (mEq/L)</td>
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<td>16</td>
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<td>Chloride (mEq/L)</td>
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<td>16</td>
<td>104</td>
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<td>Copper (µg/ml)</td>
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<td>16</td>
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<td>16</td>
<td>2.2</td>
<td>0.7</td>
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<tr>
<td>Magnesium (µg/ml)</td>
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<td>2.8</td>
<td>16</td>
<td>35.5</td>
<td>6.1</td>
<td>8</td>
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<td>Zinc (µg/ml)</td>
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<td>0.2</td>
<td>16</td>
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<td>0.2</td>
<td>8</td>
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<tr>
<td>Alpha-tocopherol (µg/ml)</td>
<td>1.86</td>
<td>0.47</td>
<td>15</td>
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<td>0.4</td>
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<tr>
<td>Retinol (µg/ml)</td>
<td>0.25</td>
<td>0.08</td>
<td>15</td>
<td>0.70</td>
<td>0.11</td>
<td>8</td>
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</table>
Table 3. Hematology, serum chemistry, enzyme, mineral, vitamin and metal values for free-ranging elephants, and white rhinoceros in Zaire.

<table>
<thead>
<tr>
<th>Test Name (units)</th>
<th>Elephant</th>
<th>Rhinoceros</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>PCV</td>
<td>42.8</td>
<td>2.5</td>
</tr>
<tr>
<td>Total solids</td>
<td>8.9</td>
<td>0.6</td>
</tr>
<tr>
<td>WBC ($\times 10^3$)</td>
<td>12.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Neutrophils ($\times 10^3$)</td>
<td>5.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Lymphocytes ($\times 10^3$)</td>
<td>4.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Monocytes ($\times 10^3$)</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Eosinophils ($\times 10^3$)</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Basophils ($\times 10^3$)</td>
<td>5.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>70.1</td>
<td>18.1</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>9.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>5.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Albumin/globulin ratio</td>
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<td>0.1</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.17</td>
<td>0.1</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>150.6</td>
<td>85.4</td>
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<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>10.2</td>
<td>6.1</td>
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<tr>
<td>Aspartate aminotransferase (IU/L)</td>
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<td>18.6</td>
</tr>
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<td>Lactate dehydrogenase (IU/L)</td>
<td>621</td>
<td>393</td>
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<td>Cholesterol (mg/dL)</td>
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<td>Calcium (mg/dL)</td>
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<td>0.55</td>
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<tr>
<td>Phosphorus (mg/dL)</td>
<td>5.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
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<tr>
<td>Potassium (mEq/L)</td>
<td>4.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>86.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Copper (µg/ml)</td>
<td>0.76</td>
<td>0.22</td>
</tr>
<tr>
<td>Iron (µg/ml)</td>
<td>1.3</td>
<td>0.13</td>
</tr>
<tr>
<td>Magnesium (µg/ml)</td>
<td>29.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Zinc (µg/ml)</td>
<td>1.9</td>
<td>0.3</td>
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<td>Retinol (µg/ml)</td>
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TRANSLOCATION OF WILD ORANGUTANS (*Pongo pygmaeus pygmaeus*) IN SABAH, MALAYSIA

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Abstract

Habitat destruction and forest fragmentation due to agricultural expansion are leaving orangutans (*Pongo pygmaeus pygmaeus*) scattered in populations of questionable viability. The Sabah Wildlife Department is capturing these stranded wild animals and translocating them to protected habitat. The goals of this project are numerous; to provide these stranded animals with a chance of survival, to continually improve capture techniques, to establish a permanent identification protocol for translocated animals, and to evaluate the health status of these wild orangutans via physical examination and blood sample collection. Telazol® (tiletamine HCl and zolazepam HCl) at mean dosages of 2.92 mg/kg or a 5:1 Ketamil:Xylazil combination (ketamine HCl and xylazine HCl) at mean dosages of 8.24 mg/kg of ketamine are used as immobilization drugs. In general, the Telazol® protocol offers several advantages including shorter induction times and a smaller dart volume.

Captured animals are identified with a tattoo on the inner thigh, a Trovan transponder chip, a freeze-brand and photographs. Blood samples are collected at the time of capture to establish values for serum chemistries, vitamins, minerals, metals selected toxicological agents, and infectious diseases. When possible, white blood cell counts, PCV and total serum proteins are evaluated in the field. Physical examinations are performed, physiological data is collected, and fur is collected for genetic analysis. Unfortunately, freeze-branding in orangutans under field conditions did not give the desired effect and has therefore been temporarily discontinued. Evaluation of collected samples is currently in progress.
TRANSLOCATION OF WILD ASIAN ELEPHANTS (Elephas maximus) IN SABAH, MALAYSIA

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Abstract

The East Malaysian State of Sabah is believed to be home to about 1000 wild Asian elephants (Elephas maximus). Some forest habitat has been lost through agricultural development. In some cases, elephants are stranded in small pockets of forest which are unable to sustain them. The Wildlife Department of Sabah has adopted a policy of capturing and translocating these animals to wildlife forest reserves. The capture of these wild animals is made possible using chemicals such as Immobilon® (etorphine HCl and acepromazine maleate) and Xylazil-100® (xylazine HCl). The reversal agents are Revivon (Diprenorphine) and Reverzine (Yohimbine) respectively. A recent capture and translocation exercise carried out involving eight wild elephants employed xylazine hydrochloride. The dose of xylazine used was calculated based on the diameter of the front footprint which provides information on body dimensions when actual weights are not available. Xylazine doses used ranged from 100-550 mg with a mean of 0.209 mg/kg body weight. Sedation was observed within 26 min after the darting. The animals were then shackled and tethered. The time for the capture operations ranged from 27-150 min, with a mean of 72 min. Xylazine is used again during the loading of the animals onto the lorries. It is an effective sedative for wild elephants which can be adjusted or reversed. The choice and used of this drug depends entirely on the ability to track the animal after darting and the ability to maneuver the captive elephants into suitable locations for tethering prior to loading. Heavy machinery is required to load the animals, unlike most other wild Asian elephant translocations were trained elephants are used to facilitate loading.
A COMPARATIVE ANALYSIS OF FECAL CORTISOL CONCENTRATIONS BETWEEN FOUR POPULATIONS OF WOOLLY MONKEYS (Lagothrix lagotricha) LIVING UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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Abstract

Over 16,000 woolly monkeys, Lagothrix lagotricha, were imported into the United States beginning in the 1960’s. They have an extremely high mortality rate in captivity and it is estimated that there are less than 25 remaining in zoos and in private ownership in the United States today. Studies have indicated that woolly monkeys in captivity have a very high incidence of hypertension and related disorders.1,2,3

It is unknown whether woolly monkeys develop hypertension under natural conditions or if it is in some way related to captivity. One possible explanation for the high incidence of hypertension is that this species may be in some way hypersensitive to physiological and/or psychological stressors affiliated with a captive environment resulting in chronic stress response activity and an associated hypertensive state. If abnormally high stress associated with captivity is a primary factor in the development of hypertension in this species, then measurable indices of stress response activity would be expected to be significantly higher in captive populations, especially in traditional zoological settings, than they would in wild populations.

The primary purpose of this pilot study is to investigate whether differences exist in fecal cortisol levels from woolly monkeys living under different environmental conditions and to evaluate fecal cortisol as a potential hormonal indicator of chronic stress response activity in this species. Fecal cortisol analysis is a very new area of research, especially in nonhuman primates. In the future, fecal cortisol analysis may prove to be a safe and noninvasive method for measuring cortisol levels and assessing stress in animals.

Mean fecal cortisol concentrations were determined from four populations of woolly monkeys living under different conditions to discern if significant differences exist between them. The four populations consist of one from a zoo setting (the Louisville Zoo), one from a less conventional captive environment (the Monkey Sanctuary), a population of orphans who have daily human contact but live freely at the Caparu Biological Station in the Colombian Amazon Rainforest and a wild population living in the vicinity of the field station. The Caparu Biological Station is located deep in the Amazon Rainforest far away from any human populations so it is expected that these monkeys will not have any additional stress brought about by proximity to human populations such as hunting or habitat loss, which is the case elsewhere.

Fecal cortisol concentrations were determined using a cortisol enzyme immunoassay (EIA) at the Assay Services Laboratory of the Wisconsin Regional Primate Research Center. Prior to assaying the samples, two woolly monkey samples were separated through high performance liquid chromatography (HPLC):
a thoroughly mixed pool of wild woolly monkey feces (wfp) and a sample from a captive male 3-yr-old. UV detection indicated that both cortisol and cortisone were identified in the feces of the woolly monkey. HPLC elutes were fractionated into 0.025 or 1 ml aliquots. The aliquots were then assayed for cortisol by enzyme immunoassay (EIA) using the technique described by Ziegler et al. with minor modifications. Results indicated that both cortisol and cortisone were present in the feces of the woolly monkey and that the cortisol EIA antibody cross reacted with both steroids (100% with cortisol and 60% with cortisone) giving a complete profile of cortisol metabolites. Assayed fractions indicated that over 80% of cortisol activity occurred at the retention times of cortisol and cortisone.

The enzyme immunoassay was used to determine cortisol concentrations in fecal samples collected from individuals from all four populations. Fecal samples were either collected in ethanol and later frozen or first frozen and later placed in ethanol prior to processing. The alcohol was evaporated off and samples were lyophilized to complete dryness. Prior to assaying, a portion of each sample was weighed, extracted with water/ethanol and put through solvolysis to remove all conjugates following previously reported methods.

Mean cortisol levels were calculated for individual animals for which there were multiple samples. Mean population cortisol levels were then calculated. Statistical analysis using the Kruskal-Wallis test showed significant differences in mean population cortisol levels between all population comparisons except between the wild and orphan populations ($P < 0.01$). The cortisol mean from the Louisville Zoo was higher than the mean from the Monkey Sanctuary and both captive population cortisol means were significantly higher than wild and orphan means.

Further research will continue to investigate the possibility that woolly monkeys living in traditional captive settings are subjected to greater stress than woolly monkeys living in more stimulating captive habitats or in natural environments to which they are evolutionarily adapted and whether fecal cortisol measurements can accurately be used for assessing stress response activity in this species. This research project has many potential implications in terms of ecosystem health, animal welfare and captive breeding programs. If zoological parks or other institutions decide that woolly monkeys should be kept in an enriched or otherwise improved captive environment, then fecal cortisol measurement may prove useful in the future as a simple and noninvasive method for continually assessing the risk of hypertensive disease. This method would allow for the early identification of inadequate captive habitat and living conditions so that efforts could be made to improve them prior to the onset of disease.

This study also has the potential for identifying fecal cortisol in the future as an easily measured, early, sensitive indicator of environmental changes in habitats in which woolly monkeys occur. Future study in this area will involve field investigations in which woolly monkey fecal cortisol concentrations are compared at different levels of environmental perturbation, ranging from areas with little or no human population pressures to areas where human population pressures are great. The purpose of this will be to investigate the suitability of the fecal cortisol parameter as an indicator of habitat health in neotropical rainforests.

ACKNOWLEDGMENTS

The authors wish to thank Drs. Tom and Sara Defler of the Caparu Biological Station; Dr. Roy Burns, Silvia Logsdon and the Louisville Zoo; Dr. Jordi Casamitjana and the Monkey Sanctuary; and Dr. Robert Yaffee of New York University for all of their essential contributions to this project. We also wish to acknowledge the helpful input of Dr. Brent White, Daniel Wittwer, Nicholas Wolfinger and Frank LoPresti. We are grateful to the Geraldine R. Dodge Foundation and the National...
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LITERATURE CITED


HEAD STARTING BLANDING'S TURTLES (*Emydoidea blandingii*): A STRATEGY FOR ENHANCEMENT OF A FRAGMENTED POPULATION

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Abstract

Blanding’s turtles (*Emydoidea blandingii*), historically a common species in New York State, are now threatened. Population numbers of adults (16+ yr) are known to exist in two distinctly separate geographic locations in the state, and total approximately 600 individuals. In 1993 a plan was developed by the New York State Department of Environmental Conservation, Nature Conservancy, and Cornell University to further assess both of the populations and to use Head Starting as a strategy to enhance the population located in the southeastern part of the state. This strategy was selected because predation was known to be an important factor limiting immigration of hatchlings into the population. To date, two groups of turtles have been raised in captivity and released back into their natal nesting areas.

In the late summer and early fall of 1994 and 1995 turtles were brought into captivity 24-72 hr after hatching. They were housed in small groups of one to five individuals, and were fed two different commercially available aquatic turtle diets on a daily basis in 1994 and a single diet in 1995. Turtles were housed initially in plastic feeding troughs in 4-6 in of water and later in 30-gal aquaria which were both maintained at 78°-82°F. Submerged filters were utilized to maintain water quality. Floating plastic plants provided hiding places for the turtles. Rocks were stacked in the tanks so as to allow for turtles to leave the water as desired, and incandescent lamps were used to stimulate natural basking behavior and were fixed at a 12/12 hr light/dark cycle. Full spectrum lighting was not used, and did not appear to be required for proper growth. Common problems associated with rapid growth in turtles, such as shell and long bone malformations were not observed.

Because of the high predation rate of free-ranging hatchling turtles, a target release size was predetermined based on previous field observations. At the same time, an effort was made to minimize the time the turtles were to be retained in captivity. Nine to 10 mo was required for turtles to reach the appropriate size. Preparation for release of the turtles included live prey trials to assure prey recognition, screening the turtles for selected infectious agents, gradually decreasing water temperatures, and conditioning the turtles to seasonal photoperiods.

For the first group of turtles, mass, carapace length, and width measurements were recorded regularly on all individuals. In addition, mid-line plastron length and height measurements were made starting shortly after the project began. Upon entering captivity in the fall of 1994, the average measurements of hatchling turtles in the first group were: mass: 9 g (range 8-10 g); carapace length: 36.8 mm (range 35.1-37.8 mm); width: 33.1 mm (range 32.0-34.4 mm). At release in the summer of 1995, average turtle measurements for this group were as follows: mass: 158 g (range 129-185 g); carapace length: 100.1 mm (range 90.1-106.3 mm); mid-line plastron length: 98.4 mm (range 91.5-103.9 mm); height 39.8 mm (range 36.0-43.0 mm); width: 76.2 mm (range 71.9-79.2 mm).
The following year, the mid-line plastron length of all turtles was measured regularly in addition to the other measurements listed above. Upon entering captivity in the fall of 1995, the average hatchling measurements for the second group of turtles were: mass: 9 g (range 8-11 g); maximum carapace length: 39.0 mm (range 34.8-41.8 mm); mid-line carapace length: 34.6 mm (range 31.3-37.7 mm); height: 16.0 mm (range 14.6-18.2 mm); width: 34.4 mm (range 32.5-36.0 mm). One month prior to release in the summer of 1996, average turtle measurements for this group were as follows: mass: 109 g (range 97-131 g); carapace length: 87.2 mm (range 82.5-94.7 mm); mid-line carapace length: 85.5 mm (range 80.0-92.9 mm); mid-line plastron length: 81.4 mm (range 76.7-89.2 mm); height: 35.1 mm (range 33.0-37.0 mm); width: 68.8 mm (range 66.2-72.1 mm). The slightly lower growth rate of this group is attributed to a more restricted caloric intake and food type, which was imposed because of concerns that turtles from the first group may have been fed excessive quantities of food at certain times during the 10-mo period in captivity.

Prior to this project, it was unclear how rapidly the turtles might grow in a 10-12-mo period. In this study, the size attained in 10 mo is comparable to that of 5-6-yr-old free-ranging Blanding’s turtles. Presently, it can be recommended that the morphometrics recorded in the 1995-1996 season be used as target parameters for future groups of Blanding’s turtles being raised within a similar time frame. Based on limited retrapping studies, survival rate appears to be high at 1 and 2 yr post-release.
TEAM APPROACHES TO THE CONSERVATION OF ENDANGERED SPECIES: THE SIBERIAN TIGER AS A PARADIGM

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Abstract

The role of science in biodiversity and endangered species conservation is dynamic; the role of veterinarians in this scheme - although essential - is only part of performing a successful effort. Only through integrated, comprehensive approaches will conservation projects be truly successful, both in the short term and long term. We present evidence from the first ever U.S./Russian field study of the ecology of the endangered Siberian tiger, and efforts to secure a long-term framework for its conservation.

Siberian tigers face two main threats to their continued existence: poaching for the Asian medicine market, and logging or habitat alteration. Our project, begun in 1992, proceeded in two phases. The primary focus of phase one was to obtain ecological and biological information from the wild population. To date, 14 tigers have been radio-collared, and tracked through both ground and aerial telemetry. Biological data was collected, including blood and tissue samples for genetic assessment, and over 500 locations have been recorded from these study animals. The emerging picture of land use patterns, habitat requirements, land tenure structures, and individual and population health assessments is invaluable. Preliminary data indicate primary prey species for tigers to be Manchurian elk, and Russian wild boar. Our preliminary data also indicate male home ranges are much larger than for females, between 400-500 and 200-300 km² respectively. Female study animals appear to reproduce every other year, after 4-yr-old, averaging two offspring/litter.

Field work is ongoing, but once this picture was sufficiently complete, phase two, conservation activities for the Siberian tiger, was initiated. There are two main areas of focus: development of a zoning plan to provide a framework for a tiger corridor throughout the tiger range, and environmental education to increase awareness of the threats that tigers face. Project efforts are making a difference for the future of Siberian tigers. Poaching, unchecked at the beginning of the project, with an estimated 50 animals killed each winter has decreased to less than 20 animals/yr. Anti-poaching teams, a recent addition to the forests, are an effective deterrent, and only two study tigers have been lost to poachers. The Siberian tiger population is stable, estimated at 330-380 adult individuals.

Poaching activities can create difficult situations, where linkages with zoological parks can be helpful. In 1992, one study tigress was poached, leaving four nursing orphans. Two cubs died, but the surviving two were sent to zoos in Omaha and Indianapolis, and added to the breeding pool of captive Siberian tigers.

The integrated role of zoo biology, veterinary science, wildlife field biology, conservation biology, genetics, and environmental education - along with successful cultural integration and long term-commitment - form the essentials for successful conservation.
VETERINARY INVOLVEMENT IN BLACK-FOOTED FERRET RECOVERY

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Abstract

Veterinary care and intervention have played and continue to play a prominent role in the recovery and reintroduction of the black-footed ferret. From the last 18 ferrets (7 founder animals) salvaged from plague and distemper outbreaks in Wyoming, we now have over 500 captive individuals. Approximately 240 of these are breeding adults. The United States Fish and Wildlife Service oversees the re-introduction of ferrets and coordinates with the six AZA breeding facilities to allocate appropriate animals for release. Over 500 individuals have been re-introduced to parts of their former range. With the current average success rate of approximately 30%, there are at least 50 free-ranging ferrets in Montana, South Dakota, Arizona and Wyoming. The primary threat to initial survival is predation from coyotes and raptors. All released ferrets have passive transponders and most are fitted with radio-collars. Post-release monitoring includes radio telemetry and spotlighting from all-terrain vehicles. Once a ferret is located, the passive transponders can be read remotely from burrows to identify individuals. Juvenile kits of the year are trapped, serologically evaluated for exposure to plague and distemper and implanted with a transponder.

Veterinarians such as Tom Thorne, Beth Williams and JoGayle Howard have played integral roles in the success of the black-footed ferret program. Veterinary involvement is still just as critical today. In addition to loss of its habitat and primary prey base from prairie dog eradication, disease continues to have a significant effect on the ferret’s endangered status. Although there have been no documented deaths in released ferrets attributable to disease, silvatic plague and canine distemper are two of the most serious diseases that the ferrets will likely face upon reintroduction. Preliminary studies are pending with regard to ways to prevent plague in reintroduced black-footed ferrets and their offspring. Possibilities include the use of an insect growth regulator, pyriproxifen for use in prairie dogs and/or developing an oral plague vaccine for use in ferrets.

Canine distemper has long been known to be a fatal disease of black-footed ferrets. Work is currently underway by Dr. Beth Williams, Dr. Dick Montali, Dr. Werner Heuschle and others to re-develop a killed virus or a recombinant canine distemper vaccine for use with all black-footed ferrets. At present, Dr. Max Appel has come out of retirement to once again provide his killed virus vaccine until a safe and effective replacement can be manufactured and distributed in the United States.

Dr. JoGayle Howard continues to use her successful artificial insemination technique on black-footed ferrets to help maximize their existing genetic diversity by circumventing behavioral problems with genetically optimal pairings and by inseminating females that come into estrus after the male has gone out of breeding condition. She is also creating a genome resource bank for the black-footed ferret.

As more ferrets are released and reproduce in the wild, there is an increased need to capture, anesthetize, transponder, collar and serologically evaluate the free-ranging animals. New techniques are developing for practical and safe methods of field anesthesia. Dr. Terry Kreeger is investigating the use of ketamine/medetomidine and atipamezole. The Montana and South Dakota release sites also have been
using portable isoflurane anesthetic machines to achieve the brief immobilization needed for processing free-ranging black-footed ferrets.
CIRCULATING FIBRINOGEN AND HAPTOGLOBIN VALUES IN FARmed WAPITI (Cervus elaphus)

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Abstract

The early identification and staging of inflammatory disease in animals has been enhanced by knowledge of acute phase protein responses in various species. Fibrinogen and haptoglobin have been identified as useful indicators of inflammation in domestic ruminants and cervids. Major acute phase proteins (APP) should either have low concentrations or be nonexistent in serum or plasma of healthy animals. This study was conducted to ascertain normal values of fibrinogen and haptoglobin in wapiti. Serum and plasma samples were taken from 41 wapiti ranging from 8 mo to several years of age.

Introduction

Acute phase proteins are a group of primarily glycoproteins that are produced by the liver in response to inflammation. They have been further defined as plasma proteins that increase in concentration by 25% or more in the first 7 days following tissue damage. Their production is directly stimulated by mediators produced by leucocytes and macrophages during episodes of infection or inflammation. These mediators include interferon, and cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor. Major APPs of various animal species quickly reach high circulating values after inflammatory disease commences. Their degree of response is directly related to the amount of tissue damage. Likewise, they decline rapidly when the disease process diminishes.

Species differences are evident with regard to the magnitude of production of each APP. An example of this is the prevalence of haptoglobin in bovine and equine inflammatory diseases. It is an excellent indicator of pulmonary inflammation in domestic cattle and appears to be the major APP in all ruminants. However, it is a poor indicator of respiratory disease in horses. In contrast, fibrinogen is recognized as the most universal APP in animal species. It has been used in combination with haptoglobin to improve the detection of tuberculosis in deer. This emphasizes the point that APP combinations are superior to single proteins in identifying inflammation and its various stages. Acute phase proteins used in combination may indicate the magnitude of tissue damage, the length of time during which inflammation continues, and the response to treatment.

Methods

The wapiti sampled in this project were deemed physically healthy by visual examination and by limited physical examination while in a drop-floor restraint chute. Blood was collected from a jugular vein of each animal during physical restraint and placed in CaEDTA and clot tubes. Fibrinogen is a component of blood coagulation and is removed from plasma during the clotting process. For this reason,
fibrinogen samples were collected in CaEDTA tubes.

Plasma fibrinogen was evaluated using the heat precipitation method. In this method, whole blood is centrifuged in paired capillary tubes. One tube is used for plasma protein quantification with a refractometer. The other capillary tube is immersed in a water bath at 56 °C. This process causes coagulation of fibrinogen. Fibrinogen is the only plasma protein that coagulates at this relatively low temperature. This tube is centrifuged once again, separating the coagulated fibrinogen from the remaining plasma protein. The plasma protein is again determined with a refractometer and this value is subtracted from the first plasma protein value. The result of this subtraction is the fibrinogen value.10

Haptoglobin was determined by using an indirect assay, rather than using an immunological method.2,7,9,11 This avoided the necessity of having to use a species-specific antibody to haptoglobin.4,5 The assay was run on an Abbott Series II automated biochemical analyzer at 37 °C, using primary and secondary wavelengths of 404 and 380 nm. This procedure makes use of the ability of haptoglobin-bound cyanmethemoglobin to resist the effects of incubation in an acid environment. For this reason, it is important to use unhemolyzed serum samples. However, hemolyzed samples containing up to 200 mg/dL of hemoglobin can be successfully analyzed.

Results

Forty-one samples were evaluated for haptoglobin levels. The mean value was 7.7 mg/dL with a range of 31 mg/dL. Only 37% (3/8) of the adult wapiti had circulating levels of haptoglobin. Most of the wapiti 1-yr-old and under had haptoglobin present in their serum (31/33; 94%). The mean haptoglobin for adults was 1.25 mg/dL as compared to 9.18 mg/dL in animals 1-yr-old and younger. Twenty animals less than 1-yr-old had a mean haptoglobin value of 11.7 mg/dL.

Forty-one samples were evaluated for fibrinogen levels. The mean value for all animals was 165 mg/dL with a range of 400 mg/dL. Only two wapiti in this study, both yearling animals, had no plasma fibrinogen levels (2/40; 5%). Animals 1-yr-old and under averaged 169 mg/dL while adults averaged 150 mg/dL. It is noteworthy that 19 wapiti under the age of 1-yr had a mean fibrinogen value of 221 mg/dL.

Discussion

It was evident from this study that both haptoglobin and fibrinogen are present in wapiti and are found in low blood concentrations in healthy animals. This research also revealed some significant age-related information. Normal haptoglobin levels were higher in juvenile wapiti (11.7 mg/dL) than adults (1.25 mg/dL). Similarly, juvenile wapiti had higher resting levels of fibrinogen (221 mg/dL) than adult animals (150 mg/dL).

Haptoglobin and fibrinogen may individually be useful in identifying inflammatory processes in wapiti. It is also likely that tandem use of these acute phase proteins may assist the staging of inflammatory processes in this species as has been seen in domestic animals. These proteins should be further evaluated in wapiti by using cytokines to induce inflammatory events and then measuring the blood concentration and duration of response.

LITERATURE CITED
USE OF DRENCHRITE® TO DETECT ANTHELMINTIC RESISTANCE IN CAPTIVE WILD RUMINANTS

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Abstract

Farming of wild ruminants in the United States, and particularly in Texas, has been on the increase over the past several years. Little is known of anthelmintic resistance in wild ruminants, with the majority of research being conducted with sheep and goats. The availability of an assay to detect resistant larvae to specific anthelmintics has been limited. DrenchRite®, a larval development assay, tests for resistance against benzimidazoles, levamisole, benzimidazole/levamisole combination, and avermectins/milbemycins, has been produced by CSIRO (Australian Commonwealth Scientific and Industrial Research Organization, McMaster Laboratory) through Horizon Technology in New South Wales, Australia. Using DrenchRite®, anthelmintic resistance has been diagnosed for two herds of wild ruminants, one of elk (Cervus elaphus) and one of Armenian red sheep (Ovis orientalis).

Introduction

In both Texas and Australia, anthelmintic resistance is a major problem, with Haemonchus sp. being the main contributor to this problem in Texas.2,8,10 Having an anthelmintic resistance screen which evaluates different anthelmintics in one test would be beneficial not only to the veterinarian but to the producer who will have the results in about 1 wk. For positive results, the appropriate measures can be taken to curb resistant worms in the population by decreasing their numbers while improving the health of the herd.

Methods

The protocol that was followed was adapted from the DrenchRite® User Manual.1 Separate fecal samples were taken from the two populations, one rectally (sheep) and the other collected from the ground (elk). A trichostrongyle eggs/g of feces (epg) count was taken for both populations. Since the epg count was above 100, a sufficient amount of eggs were present for evaluation. A fecal slurry was prepared and rinsed through a 210 µm sieve. The filtrate was then washed free-flow through additional sieves of 70 and 37 µm. The eggs were recovered on the 37 µm sieve. A sugar gradient was prepared to remove any extra debris and recover the eggs. After centrifugation the eggs were pipetted from the gradient and recovered onto a 37 µm sieve. A sugar gradient was prepared to remove any extra debris and recover the eggs. After centrifugation the eggs were pipetted from the gradient and recovered onto a 37 µm sieve and rinsed with water to remove any sugar solution. The eggs were recovered, counted and divided according to the manufacturer’s instructions1 so that each of the 96 wells in the assay plate would contain 40 to 70 eggs. Nystatin (Fungizone) was added to combat any fungus that might have disrupted the parasite’s development.

The DrenchRite® plate contains 12 columns with 96 wells that are color-coded: a clear lane for the control wells, 4 green lanes for susceptible, 3 yellow lanes for weak to intermediate resistance, and 4
red lanes for highly resistant larvae. The 8 rows of drug-containing wells have increasing concentrations from each anthelmintic class. After dispensing the eggs, the plates were incubated for a period of 6.5 days at a range of 25 to 26 °C. During this incubation time, a growth medium was added in order to provide nutrients to the developing larvae. Also the plates were checked periodically for loss of hydration within the wells. Larvae that had hatched and developed to the third stage were identified to genus and counted.9,11

Results

No prior anthelmintic use was documented with this particular herd of elk. The predominant genera present from the elk were *Trichostrongylus* and *Ostertagia*. For *Ostertagia*, there was a 99% efficacy found with the benzimidazoles (Table 1). With levamisole, 4.17% of the population was seen as highly resistant, and 95.83% being moderately or not resistant. A 68% efficacy was found for the moderate/no resistance population while a 32% was found for the highly resistant population. An overall population efficacy was found to be 65.16%. The predominant genera found for this data was *Trichostrongylus*. For the benzimidazole/levamisole combination, *Ostertagia* was present along with *Trichostrongylus* in the control wells, but when the critical well (LD<sub>50</sub>) was reached, only one or the other species was identified. *Ostertagia* was the only genera seen with the efficacy being estimated at 100%. The genera of *Trichostrongylus* was eliminated before reaching the critical well (LD<sub>50</sub>) so an assumption of 100% efficacy was estimated for this genera with the benzimidazole/levamisole combination (Table 1).

*Trichostrongylus* was the only genera that displayed a suspected resistance to the avermectins/milbemycins with *Ostertagia* having an assumed susceptibility. It should be noted that in the DrenchRite® manual, it states that resistance to the avermectin/milbemycins is rare in the field by comparison with the first broad spectrum anthelmintics (e.g., thiabendazole, levamisole) introduced. DrenchRite® can be used to detect the presence of resistance but not as yet, to quantify efficacy of the avermectins/milbemycins.1

Anthelmintic treatment history in the Armenian red sheep was known for the last 6 yr. This group of red sheep consisted of eight females and two males that originated from west Texas. The females had been given pyrantel tartrate in the feed and also ivermectin. This regimen was alternated 4 times/yr. The males had also been given pyrantel tartrate in their feed but the last time ivermectin was given was in July 1994.

The predominant genus found from these red sheep was *Haemonchus*. Forty-six percent efficacy was seen with the benzimidazoles (Table 2). With levamisole, 14.6% of the population was considered highly resistant, while 85.4% were seen as moderately or not resistant. A 90% efficacy was estimated for the moderate/no resistance population while a 10% efficacy was found for the highly resistant population. An overall population efficacy was estimated at 76.9%. A 98% efficacy was seen with the benzimidazole/levamisole combination. For the avermectins/milbemycins, no resistance was suspected (Table 2).

Discussion

DrenchRite® has been tested mainly with sheep and goats. This research shows that it has application in wild sheep and cervid species. It can likely be applied to all exotic ruminant species because this test is independent of the host species and many exotic hoofstock are medicated with
anthelmintics (e.g., zoos, game parks, deer farms, etc.).

Note: This is in partial fulfillment of a master’s thesis.

ACKNOWLEDGMENTS

Acknowledgments and thanks to the following individuals for their help and assistance with this research: Mr. Jeff Craven, Gabriella Foxworth, DVM, Donald Davis, PhD, and Mr. Rob McCook.

LITERATURE CITED

**Table 1.** Efficacy seen with larvae from elk.

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<th>Species of larvae</th>
<th>Drugs</th>
<th>Benzimidazoles</th>
<th>Levamisole</th>
<th>BZ/LEV</th>
<th>AVM/MIL</th>
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<td>Haemonchus</td>
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<td>NP</td>
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<tr>
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<td>100%</td>
<td>S</td>
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<tr>
<td>Trichostrongylus</td>
<td>100%</td>
<td>65.16%</td>
<td>100%</td>
<td>SR</td>
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</tr>
</tbody>
</table>

SR: suspected resistance; S: susceptible

**Table 2.** Efficacy seen with larvae from red sheep.

<table>
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<th>Species of larvae</th>
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<td>Trichostrongylus</td>
<td>NP</td>
<td>NP</td>
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NSR: no suspected resistance; NP: not present
AN OUTBREAK OF CRYPTOSPORIDIOSIS IN A COLONY OF CAPTIVE SNAKES: EPIDEMIOLOGIC CONSIDERATIONS

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Abstract

Cryptosporidiosis is an illness of which very little is known in Mexican wildlife; no reports of this illness in reptiles have been found in our country. This paper describes the first outbreak of cryptosporidiosis in the collection of reptiles in the Guadalajara Zoo. In November of 1996 a Pituophis deppei jani was diagnosed with the illness by analyzing gastric washings. Oocysts of Cryptosporidium spp. were observed using the modified acid fast stain technique. From that case in November through February 1997, nine deaths caused by this protozoan have occurred. Also, five asymptomatic carriers have been located, two of which have died from other causes although the presence of this protozoan was detected.

Introduction

Cryptosporidiosis is an illness caused by a small protozoan that belongs to the genus Apicomplexa. The cryptosporidium has been found in birds, mammals, reptiles, amphibians and fish. In reptiles, Cryptosporidium serpentis is a relatively recently identified parasite that causes considerable mortality,1-5,7,8 and Cryptosporidium infection may last years in a latent or subclinical state. Infection may also cause chronic debilitation of the reptile or shedding of the organism in response to stress or immune suppression.3 Cryptosporidium has sometimes been associated with animals that have recently arrived at an institution. This protozoan has also been detected in wild reptiles but in a much lower percentage than captive animals.1 In the clinical illness, the parasite infects the stomach causing mucosal cell inflammation, edema and hypertrophy, as well as hemorrhage and focal necrosis. The typical clinical signs are chronic regurgitation, weight loss and emaciation, and secondary bacterial infections.1,2,4,5 Diagnosis is made by endoscopy, ultrasonography, or radiography, and it can be confirmed by gastric biopsy or cytology of gastric washings, regurgitated prey, cloacal/colon washings and feces. Cytology specimens are stained with modified acid alcohol resistant stain and scanned for oocysts.1,2,4 Recent studies have shown that the use of monoclonal antibody tests for Giardia and human Cryptosporidium have a cross reaction with C. serpentis and may be useful in detecting infection in reptiles.

False negative results with cytology or other ante-mortem tests are not uncommon. Necropsy and histopathological findings can confirm the ante-mortem diagnosis.

There is no known effective treatment. The disease is considered to have zoonotic potential even though no confirmed zoonoses have been seen.1,2 This is a report of an outbreak of cryptosporidiosis and its epidemiologic implications in the reptile collection at the Guadalajara Zoo. The zoo has maintained a collection of 350-500 specimens of reptiles since its opening in 1988. To our knowledge, this is the first report of cryptosporidiosis in a Mexican zoo.
Case Report

Case 1

In November of 1996 a 2-yr-old snake of the species *P. deppei jani*, born in this institution, was presented to the zoo clinic. History included regurgitation of 6 wk duration, weight loss and emaciation. The physical examination revealed dehydration and mid-body swelling. Two weeks previously, copro-parasitoscopic studies by flotation were negative. A gastric wash was performed and material stained by modified kinyoun technique. An abundance of acid alcohol resistant oval forms were seen. A tentative diagnosis of cryptosporidiosis was made. The serpent had been housed individually inside a room with 35 other reptiles. The snake was euthanatized 2 days after presentation. The necropsy showed poor nutritional condition and mild gastric mucosa erythema. Histopathologic findings included inflammation of the gastric mucosal cells with hemorrhage and slight necrosis.

Case 2

One day following the euthanasia of the snake in Case 1, a *Boa constrictor constrictor* died. The clinical history included intermittent periods of anorexia and regurgitation for more than a year. The snake had been donated to the zoo from an unknown source. Routine fecal screening for parasites had been previously negative. It had been kept in the same area as the snake from Case 1.

Necropsy findings revealed petechial hemorrhages and necrosis of the stomach. Cytology of the stomach contents confirmed the presence of *Cryptosporidium* using the same stain technique as in Case 1. Histopathology showed gastric submucosa edema, mild gastric epithelial degeneration, cellular infiltration of heterophils and eosinophils, atrophy and loss of granular cells, necrosis of the apical surface of the enterocytes and many spherical to ovoid organisms adherent to microvillar borders of surface, pit and glandular epithelium suggestive of *Cryptosporidium*. Other findings were renal amyloidosis with hydropic and fatty degeneration of the tubular epithelium and severe hepatic lipidosis.

Case 3

In December of 1996 a specimen of *Crotalus atrox* presented with weight loss and regurgitation of 1 mo duration. Gastric washing found the presence of *Cryptosporidium*. The animal died 2 wk later. The necropsy and histopathological findings were similar to those in Case 2. This snake had been donated to the zoo in 1989. In June of 1995 it had presented with an illness characterized by regurgitation and dysecdysis. Treatment was initiated with Metronidazole (Flagyl, Rhone-Poulenc Rorer, 03100 Mexico D.F.) p.o., Kaobiotic (The Upjohn Company, 04879 Mexico D.F.) p.o., Baytril (Bayer of Mexico, 11520 Mexico D.F.) i.m. and fluid therapy. Furthermore, this snake was isolated in the same room in which the snakes in Cases 1 and 2 were later isolated. The snake made a clinical recovery by April 1996. In November 1996 regurgitation recurred and the snake was transferred again to the same isolation room.

During the following 2 mo nine animals died with the presence of *Cryptosporidium*: *B. constrictor constrictor* (*n* = 1), *Pituophis deppei jani* (*n* = 1), *Trimeresurus gramineus* (*n* = 1), *Sistrurus ravus ravus* (*n* = 1), *C. atrox* (*n* = 1), *Crotalus molosus nigrensis* (*n* = 1), *Crotalus aquillus* (*n* = 1), and *Masticophis flagellus* (*n* = 1). Two other animals that died of other causes were found to be...
infected with *Cryptosporidium*. Three asymptomatic carriers have been found in a group of five rattlesnakes. The protozoan has now been found in all areas of the serpentarium.

**Discussion**

This is the first finding of *Cryptosporidium* in the Guadalajara Zoo. There are no reports of necropsy filed prior to 1994. Previously no tests for *Cryptosporidium* were done in quarantine since there had been no reports of disease in Mexico.

The two factors that are necessary for the onset of cryptosporidiosis are the reproduction of the organism and a state of stress or immune suppression in the host. The snake in Case 3 likely reproduced and disseminated the protozoan during the illness it suffered in 1995. The source of the organism in this outbreak is unknown but it is probable that *Cryptosporidium* is widely distributed in Mexico. Most of the cases of *Cryptosporidium* at our zoo have occurred in the two places occupied by the snake of Case 3.

Besides the fact that they are in captivity which in itself offers some stress, there are other factors in our serpentarium that affect the immune status of the animals.

The temperature and humidity in the shelters of some of these snakes is inadequate. Some are not provided with ultraviolet light of sun baths. However, the factor that we consider the most important in predilection for stress and infection is obesity. The snakes in our collection tend to be overfed with varying degrees of obesity. The presence of fatty livers on necropsies has been a frequent finding on necropsies since 1994 (in some occasions the only finding). A few months before Case 1, the bioterium, (the area of the zoo that provides rodents to the serpentarium) began substituting commercial dog food with a high fat content for the rodents instead of the previous rodent food. This added to the overfeeding of the reptiles, may have caused some hepatic lipidosis in the snakes. Vitamin E deficiency may have played a role as well as the unsaturated fats of the rodents competed for this essential antioxidant. These factors may have decreased the general state of health of the reptiles, diminished their immune status making them susceptible to illness including cryptosporidiosis.

Preventative measures have been initiated to inhibit the spread of parasites. Quality, quantity and frequency of food items has been altered. Vitamin E supplements are also being used. Hygiene procedures are being used in the serpentarium to prevent dissemination of *Cryptosporidium*. Quarantine procedures have been reinforced and testing for *Cryptosporidium* during quarantine initiated. Carriers or sick animals are isolated and evaluated so that in the case of a severe illness they may be euthanatized. The control of temperature and humidity has been improved. Testing of animals from other areas of the country will aid us in understanding the state of *Cryptosporidium* in Mexico. To this date no treatment has been initiated for sick species. Special care is taken against the risk of zoonosis for the workers as well as disinfection of utensils and work areas.

**ACKNOWLEDGMENTS**

We thank Dr. Francisco Rodriguez Herrejon, Director of the Guadalajara Zoo; Dr. Pablo Varela, Head, Veterinarian Services of the Guadalajara Zoo; the staff of Area Tecnica and Herpetario of the Guadalajara Zoo; Dr. Oscar H. Sanchez Molgado, Pathologist, for his assistance; and especially Mrs. Barbara Keenan for the translation of the manuscript.
LITERATURE CITED


DERMATOPHILOSIS IN A FREE-RANGING ROE DEER (Capreolus capreolus) IN SWITZERLAND: A CASE REPORT

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Abstract

Dermatophilus is a skin disease caused by the bacterial parasite Dermatophilus congoensis. It affects a wide range of animal species including man.1,2,9 This zoonotic disease is world wide in distribution but more prevalent in the humid tropical and subtropical regions.

Dermatophilosis has been described in many wild ungulate species including antelope, eland, gazelle, kudu, buffalo, giraffe,1 white-tailed deer3,7 and kafue lechwe.9 In chamois, the disease sporadically occurs in the European Alps; it was diagnosed for the first time in 1967.5

A 2-mo-old male free-ranging roe deer was found dead in the vicinity of Berne, Switzerland. The skin showed severe lesions and because of suspicion of mange, the animal was sent for necropsy to the Institute of Animal Pathology, University of Berne.

The lesions were characterized by thickening of the skin and coalescing crusts which could be easily detached, leaving a moist hairloss patch on the skin covered with a yellowish sticky exudate. Almost all parts of the body were affected. In addition, the animal was infested with a high number of ticks (Ixodes ricinus).

The direct examination with KOH revealed neither mites nor mycotic organisms. The bacterial culture showed an infection with Staphylococcus sp. Histologically, there was diffuse acanthosis with hydropic degeneration in the upper spinous layer and marked palisading ortho- and parakeratotic hyperkeratosis alternating with inflammatory exudate. Characteristic large ramified filaments with compartmented structures resembling “rolls of coins,” positive with Grocott stain, were found in the crusts as well as in the follicles and identified as D. congoensis. Aggregates of coccoid bacteria were also present.

A diagnosis of dermatophilosis was made, based on the characteristic morphology of the etiologic agent revealed by histological examination. The infection with Staphylococcus sp. was considered to be secondary. According to Morrow & Compton4 and Walker & Lloyd,8 the tick infestation was a predisposing factor in the pathogenesis of the D. congoensis dermatitis. The exceptional wet weather in the summer of 1996 may have favored the infection as well.

To our knowledge, this is the first reported case of dermatophilosis in roe deer.

LITERATURE CITED

SURGICAL CORRECTION OF PALATINE BONE LUXATION IN A BLUE AND GOLD MACAW: ANATOMIC AND SURGICAL TECHNIQUE DESCRIPTION

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Abstract

Palatine bone luxation was diagnosed in a blue and gold macaw (Ara ararauna) based on history and physical examination findings. The bird presented with a history of recent head trauma and subsequent inability to prehend food. Physical examination findings were normal except for a hyperextended maxillary beak. Simple reduction was unsuccessful.

Detailed analysis of radiographs, anatomic descriptions and cadaver dissections revealed that the hyperextended maxillary beak was due to the dorsal luxation of the palatine bones. Surgical reduction of this luxation is achieved by introducing an intramedullary pin transversely across the infraorbital sinus dorsal to the palatine bones and hyperextending the maxillary beak while concurrently reducing the luxated palatine bones ventrally to their anatomic position. The luxation is stabilized by passing absorbable suture around the suborbital arch and jugal bones bilaterally. Reduction and stabilization was successful, but unfortunately the macaw described in this report died of anesthetic complications after surgery.

Palatine bone luxation causing hyperextension of the maxillary beak is a rare condition previously reported in only one other blue and gold macaw. It can present a challenging problem since it is difficult to radiographically conceptualize the luxation and there are few sources that describe the macaw skull or the treatment for palatine luxation in detail. The objective of this paper is to provide a detailed anatomic description of the mechanism of palatine bone luxation and surgical technique for reduction.

Clinical Report

A 2-yr-old blue and gold macaw of unknown sex presented to Sonora Veterinary Surgery and Oncology in Scottsdale, Arizona with a history of head trauma 2 days previous to presentation and inability to prehend food since the traumatic incident. On physical examination the bird weighed 900 g and individual system examinations were normal except for the musculoskeletal system. The maxillary beak was locked in a hyperextended position preventing occlusion with the lower beak. There were no other outward signs of trauma. Simple manipulation did not reduce the maxillary beak.

Radiographs taken with the bird anesthetized revealed a hyperextended maxilla but the exact cause of the hyperextension was unclear. Attempts to reduce the beak were again unsuccessful. The bird was recovered from anesthesia and surgery was postponed to permit further review of the anatomic features and mechanism of palatine bone luxation. The owners were instructed to continue handfeeding soft foods until surgery was scheduled.
Anatomy

Even the most sophisticated avian anatomy texts are lacking detailed descriptions of the macaw skull. Among birds, there is great variation in skull and jaw structure. Macaws exhibit a type of upper jaw movement called prokinesis in which there is a flexible junction between the maxilla and the braincase. This junction, termed the craniofacial hinge, is an actual synovial articulation. There are several bones in the upper jaw and palate that are responsible for upward movement of the maxillary beak. The palatine bone articulates rostrally with the premaxilla and caudally with the pterygoid bone which forms a hinge joint with the quadrate bone. The jugal arch also articulates with the quadrate bone and rostrally with the upper maxilla. Thus, the quadrate bone articulates with the lower jaw, the braincase, the jugal arch and the pterygoid bone. It is the rotation of the quadrate bones rostrally which causes a gliding motion of the pterygoid, the palatine and the jugal bones allowing the maxilla to swing upward. The upper beak then extends at the craniofacial hinge to give macaws their versatile beaks.3,6,7

In the event of severe maxillary hyperextension due to trauma, the palatine bones luxate dorsally and become hooked on the interorbital septum which is located on the ventral aspect of the braincase. In psittacines, this septum is longer, wider and thinner than the septum in domestic fowl. As the maxilla is hyperextended, the palatines slide rostrally. If a simultaneous traumatic force is applied dorsally, the palatines become trapped dorsally on the rostral edge of the interorbital septum. Once this occurs, the palatines are unable to slide caudally and the maxilla remains in the hyperextended position.

Surgical Technique

The bird is placed for surgery in dorsal recumbency, the feathers are plucked bilaterally cranial and ventral to the orbit and the skin overlying the infraorbital sinus and jugal bone is aseptically prepared. Using radiographs to estimate the location of the infraorbital sinus, a stab incision approximately 2 mm ventral and 3 cm rostral to the medial canthus of the eye is made with a #15 surgical blade. A 3/32-diameter or smaller intramedullary pin is introduced through the right infraorbital sinus and exited through the opposite side using a hand chuck. The maxilla is hyperextended and simultaneous ventral pressure is exerted on the intramedullary pin displacing the palatine bones ventrally. This allows the maxilla and palatine bones to slide caudally to their anatomic positions beneath the interorbital bone. The intramedullary pin is then removed. To stabilize reduction a lateral approach is made to the jugal bone at the level of the ventral bony orbit and a 3-0 polydioxanone suture is passed around the jugal bone anchoring it to the suborbital arch. The incisions are closed with 3-0 polyglactin.4 Postoperative instructions include feeding soft foods and placing the bird in an enclosure that would discourage it from using its beak to climb for approximately 2 to 3 wk.

The bird in this case was treated as described above and the luxation was readily reduced and stabilized. Unfortunately, during anesthesia recovery the bird underwent cardiopulmonary arrest and despite resuscitation attempts, it died approximately 15 min after the surgical procedure concluded. Gross necropsy confirmed the initial diagnosis and ruled out that the intramedullary pin placement was the cause of death.

Conclusion
Palatine bone luxations are rare and maybe a species-specific problem which occurs only in blue and gold macaws. In the first case reported the palatine luxation was caused by the bird attempting to bite a large wooden dowel. In this case, the owner accidentally threw the bird down after it attempted to bite him. This suggests that a dorsal traumatic force simultaneous with maxillary hyperextension is necessary in order for the luxation to occur. Closed reduction is not successful and prehension of food is not possible with the degree of maxillary hyperextension created by the luxated palatine bones. Surgical reduction and stabilization is indicated. Once familiar with the anatomy and pathophysiology of palatine bone luxations, the surgical procedure for reduction is simple and fast. The prognosis for normal function is seemingly excellent.

ACKNOWLEDGMENTS

We would like to thank Greg J. Harrison, DVM for sharing his personal experience with a similar case. We would also like to thank Drs. Kathy Orr and Dan Burke for contributing psittacine skulls.

LITERATURE CITED

4. Personal communication. Greg Harrison, DVM.
DERMATOPHYTOSIS IN RED PANDAS (*Ailurus fulgens fulgens*): A REVIEW OF 14 CASES

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Abstract

Medical records of red pandas (*Ailurus fulgens fulgens*) housed at the Knoxville Zoo between 1980 and 1996 were reviewed. Cases were included in this report if clinical signs were suggestive of dermatophytosis and if the diagnosis was confirmed by cytology, culture, or histopathology. Parameters analyzed included signalment, clinical signs, etiologic agents, presence of secondary infection, treatment regimens and duration of disease. The median age of affected animals on initial presentation was 8.5 wk (range = 3 wk to 11 mo). All but one were < 4 mo-old. No gender predilection was noted.

Clinical signs included crusting (*n* = 8), purulent exudate (*n* = 6), alopecia (*n* = 3), thickening of affected skin (*n* = 3), ulceration (*n* = 3), and necrosis (*n* = 3). Seven animals had mild lesions with signs restricted to crusting and/or alopecia and 6 animals had more severe infections, with ulceration, skin necrosis, and purulent exudate indicating presence of secondary bacterial invaders. Lesions occurred on the extremities (*n* = 7), tail (*n* = 6), muzzle (*n* = 4), and ear (*n* = 1), with 6 animals having signs in two or more locations.

Diagnosis of dermatophytosis was confirmed by culture (*n* = 9), cytology (*n* = 1), histopathology (*n* = 2), or culture followed by histopathology (*n* = 2). *Microsporum gypseum* was the only organism cultured from any fungal culture (*n* = 11).

Of six animals with mild disease (signs restricted to alopecia, crusting, and/or mild inflammation), two received topical therapy alone, one received topical therapy and systemic antibiotics, and three received topical therapy and systemic antifungal therapies. All six animals had full resolution of clinical signs.

Of the six animals with severe lesions, five had tail involvement. Partial tail amputation was required as part of the treatment regimen for two animals, and two others had ulcerated tail lesions which left circumferential scarring following resolution of infection. Three of these animals received topical antibiotic/antifungal therapy and systemic antibiotic therapy. The other three animals received combinations of topical antifungal agents with concurrent systemic antibiotic and/or antifungal therapy.

Although clinical presentation and treatment regimen varied widely among these cases, the most severe lesions appeared on the tail and required partial amputation and/or systemic antifungal therapy.
IN-ZOO VETERINARY COMMUNICATIONS: WHAT WORKS FOR US!

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Abstract

Poor communication remains a key stumbling block to efficiency and progress in any zoological facility. For a number of years we have used several forms of communication that have improved the quality of care and efficiency of service for the veterinary care of the animals housed at the Kansas City Zoological Gardens.

Communication, by definition, is the exchange of thoughts, messages, etc. In addition to the standard International Species Information System (ISIS) and MedArks systems, we have developed several novel approaches to inform staff of veterinary aspects regarding their animals, and develop quality working relationships which have improved all aspects of management and care for the animals. These approaches consist of a select number of standardized forms, and videotaped presentations to staff.

Written Communications

Pre-Procedure Forms

This standardized form (orange) is filled out by the veterinary staff and is forwarded to the curator or technician 1 wk prior to any immobilization, vaccination, or detailed veterinary work. This form informs the staff on what day and time the procedure will take place, how many hours to withhold food and water, what specific procedure will be performed (surgery, EKG, etc.), how many staff are needed, and any medications or dietary changes that are needed prior to the procedure. Each item that is necessary is simply checked. This saves time for the veterinary staff in filling out the forms.

Post-Procedure Forms

This standardized form (blue) is also filled in by the veterinary staff, and is left with the responsible keeper or technician after the procedure. It details, in check-off form, what the person can expect from the animal. For example, if the procedure performed was a fracture repair, the form could be checked “lethargic for 24-48 hr,” “withhold food for 4 hr,” etc. Any diet changes are indicated on the form, as well as any medications dispensed. What to look for at the incision site, or what the bandage should look like is also on the one-page form. In addition, a statement on when the animal can return to the exhibit (immediately, 2 hr, 4 hr, 5-7 days, etc.) or if it must be rechecked by the veterinarian is also included. At the bottom of each of these forms is a space for additional comments, since there are obviously many scenarios that cannot be addressed with just one form.

Post-Quarantine Forms

This green form details all aspects of what tests were performed during quarantine (and the outcome...
of those tests, in a check-off format), quarantine length, identifying features, attitude and behavior while in quarantine, “fecal day,” treatments performed during quarantine, and the diet the animal was on while it was in quarantine.

**Neonatal Checklist**

This white form is a simple checklist that is copied to curators regarding the status of the newborn. Physical exam findings, antibiotics and wormers, vitamins, and any other injections that were given, animal heart rate, animal weight, etc. A space is included for any additional comments.

**Animal Immobilization Worksheet**

This pink form, for in-hospital use, allows the veterinarian to properly inform the veterinary technician, student, or intern of what drug will be used, its concentration and amounts to be used, the reversal drug to use, the mg and amounts of reversal, and how it is to be administered. In addition, the predetermined amounts of emergency drugs to have on hand are also included in this one-page sheet.

**Animal Anesthetic “To Do” List**

This white form allows the veterinarian to simply check what procedures are to be accomplished while the animal is anesthetized. Heart rate, weight, rectal swabs, ear swabs, what type of antibiotic, amount of blood and what is to be done with it, etc. This allows the veterinarian, technicians, and students to be on the same level, and accomplish all goals in the shortest amount of time.

**AHC Calendar**

This in-house wall board denotes days when major procedures are to occur, what animals need to be examined, as well as what animals are coming in or going out. This allows the veterinary and technician staff adequate time to prepare for the procedure, order products necessary (vaccines, etc.) and schedule their time appropriately.

**AHC Quarantine Board**

This in-house wall board schedules the animals coming in for each quarantine room, and how long they will be there. If the quarantine space is filled, animal supervisors and curators are informed, and timetables for quarantine are discussed.

**Medical Treatment Board**

This in-house wall board displays specific treatment protocols for individual animals either in the hospital or in the zoo. This information helps ensure proper administration of medications by the veterinarians, technicians or students.

**Oral Communication**

**Video programs**
Every veterinary procedure performed during the week is videotaped. The video is presented to the management staff, and then the keeper staff on a weekly basis to allow them to witness exactly what is happening with the animals from a veterinary standpoint. From this, decisions can be made regarding long term care, exhibitry, possible zoonoses, etc. Video presentations are kept to less than 10 min for each session, and a brief question and answer period is allowed regarding animal concerns.

*Curators Meetings*

An essential, down to earth, meeting among all curators takes place weekly. During these meetings, major concerns and events occurring in the zoo are discussed.

*Staff Meetings*

This weekly meeting involves all management staff, including public relations, concessions, security, education, veterinary and animal supervisors. Zoo-wide concerns, events, and updates are discussed. It is at this meeting that videotape presentations are made regarding veterinary procedures and concerns.

*Animal Management Meetings*

This weekly meeting discusses specific animal concerns and procedures. Shipments in or out, short and long term exhibit goals, and animal health issues or procedures are just a few of the topics discussed. Veterinarians, animal supervisors, and animal curators are involved in this 1-hr meeting.

*Summary*

The development and use of these approaches to communication is what currently works for us. It is not a panacea, and minor communication problems still persist, but overall, veterinary aspects of communication work well, providing another avenue to ensure quality care and efficiency in this zoological setting.
HEMATOLOGY, SERUM CHEMISTRY, AND SELECTED NUTRITIONAL VALUES OF THE WILD KOMODO DRAGON (Varanus komodoensis)

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Abstract

The Komodo dragon (Varanus komodoensis) is the world’s largest lizard and occupies the smallest range of any large carnivore in the world. Currently this varanid is listed in Appendix I (species threatened with extinction) of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). In various books and media presentations the bite of this species is supposed to transmit deadly bacteria with death resulting from subsequent sepsis. As part of a comprehensive plan to evaluate this possibility further, normal physiologic values were collected on 11 animals during November 1996. Samples were assayed for vitamin A, vitamin D, vitamin E, trace mineral levels, and routine serum chemistries were performed. Complete hemograms were also performed on each animal. Data collected can be used for better captive management and to help determine potential problems in the wild population. Based on limited sample size and concurrent field biology studies, no significant toxic, infectious, or nutritional problems have been identified in Komodo dragons on the island of Komodo. These data will be used as part of a larger study including virology, parasitology, and microbiology to explore the immune system of this species. Further field studies are planned to expand sample size and for comparison with normal values of captive specimens.

ACKNOWLEDGMENTS

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THE NOVEL USE OF HINGED BRACES AS EXTERNAL SUPPORT DEVICES FOR SOFT TISSUE JOINT INJURIES IN LONG-LEGGED BIRDS

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Abstract

Management of soft tissue joint injuries in long-legged birds is often frustrating and unrewarding due, in part, to the lack of appropriate external stabilization devices that allow for the continued use of both legs during the healing process. Birds that are immobilized for a period of time often become fractious, intolerant of the restraint, and generally depressed. Skeletal muscle atrophy complicates the medical management of these birds, often resulting in an unsuccessful recovery, unless a rigorous physical therapy program is instituted. In an attempt to ameliorate these complications, the authors have adapted hinged brace assemblies as novel external support devices for soft tissue injuries to tarsometatarsal (hock) joints in several species of long-legged birds.

A 2-yr-old, endangered Mississippi sandhill crane (Grus canadensis pulla) presented with an acute grade 3 lameness of the right leg with swelling of the medial hock. Radiographs demonstrated soft tissue swelling without evidence of fractures or luxations. There was moderate mediolateral laxity of the joint on palpation suggesting a medial collateral ligament rupture, however. The joint was initially stabilized with a support bandage of cast padding and elastic wrap (Vet Wrap®) to allow the bird restricted function of its leg. Mild hock swelling, medial joint laxity, and a grade 2 lameness persisted after 2 wk. Sequential radiographs demonstrated no significant change in the initial findings.

The crane was anesthetized with isoflurane and a Phoenix® adjustable hinged elbow brace (Smith & Nephew) was fitted as an external support of the right hock. The brace was constructed of a lightweight aluminum alloy with a ball-bearing hinge to allow friction-free movement within a normal range of motion (ROM). Adjustable static bracing was possible if metal stops were inserted in the hinge. This restricted movement feature was not necessary in this case. Microfoam® tape was applied as padding to the leg proximal and distal to the affected joint to protect the skin surface from friction rubbing. The brace was properly aligned along the medial and lateral aspects of the hock so that the center of the hinge matched the point of flexion and extension of the joint. Stainless steel surgical wire was looped through each end of the brace armature and subsequently interwoven between the layers of adhesive tape that secured the brace to the Microfoam® tape on the leg. Upon recovery from anesthesia, the crane began walking immediately without appreciable lameness. There was normal ROM with flexion and extension of the hock during walking, sitting, and rising. The crane tolerated the presence of the support brace for 4 wk. Once the brace was removed, a soft bandage was placed on the right hock to provide minimal support for one additional week. At that time, the crane had regained normal ambulatory function and it was returned to its field pen.
This type of brace apparatus has been used successfully for similar injuries in several species of cranes, flamingos, and storks. A substitute brace of similar design, but of lesser stability, was constructed from the hinged ribs of an umbrella frame. Padding the arms of the umbrella ribs in an adhesive tape wrap, and securing the apparatus medially and/or laterally to the leg, significantly increased the stability of the joint. This device was used successfully on an adult green magpie (*Sissa chinensis*) which sustained a ruptured medial collateral ligament with complete joint instability. The brace was well tolerated for 4 wk with the subsequent achievement of good joint stability and ROM after removal.

The novel adaptation of hinged devices for use as external support braces for soft tissue injuries of joints in birds has been remarkably successful. A dynamic hinged brace designed for human elbow injuries was fitted to the leg of a Mississippi sandhill crane. It permitted normal ambulation with full ROM of the affected hock, and afforded mediolateral stability to the joint until the soft tissue injury resolved. A similar hinged brace constructed from umbrella ribs provided external support to the luxated hock of a green magpie with resultant return of joint function. These birds were completely tolerant of the hinged brace assemblies without interruption of their normal behavioral and motoring activities.

**LITERATURE CITED**

SUCCESSFUL ARTIFICIAL INSEMINATION AFTER EXOGENOUS GONADOTROPIN TREATMENT IN THE OCELOT (Leopardus pardalis) AND TIGRINA (Leopardus tigrina)

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Abstract

The ocelot (Leopardus pardalis), margay (Leopardus wiedii) and tigrina (Leopardus tigrina), are three endangered South American small cat species whose long term survival depends upon both in situ and ex situ conservation actions. Application of assisted reproductive technology, such as artificial insemination (AI) with fresh or frozen-thawed sperm, has considerable potential for improving captive propagation and management of endangered felids with low natural breeding success. Exchange of genetic material between captive and wild populations also could be achieved by these means. To date, offspring have been produced by laparoscopic AI in two small-sized nondomestic cat species, the leopard cat and the ocelot. 2,3 In this study we assessed the feasibility of exogenous gonadotropin treatment and laparoscopic AI for inducing ovulation and producing offspring in wild-caught female margays, tigrinas and ocelots maintained at two institutions in southern Brazil. Females were treated with a sequential combination of equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG); hCG was administered 80 hr after eCG. Ocelots (n = 4) were injected (i.m.) with 400 IU eCG/200 IU hCG whereas tigrinas (n = 2) and a margay (n = 1) received two treatments each of 100 IU eCG/75 IU hCG (September 1995) and 200 IU eCG/100 IU hCG (April 1996). Ovaries of each animal were evaluated laparoscopically 39-44 hr after hCG to determine number of unovulated follicles (≥ 2 mm) and corpora lutea. 4 Blood samples were collected by jugular venipuncture and recovered sera were assessed for estradiol and progesterone concentrations using double-antibody 125I-estradiol and solid-phase 125I-progesterone radioimmunoassay kits (Diagnostic Products Corp., Los Angeles, CA). Females with recent ovulation sites (n = 9) were inseminated in utero using fresh semen obtained by electroejaculation. 1 Inseminated volume was 180-200μl (3.7 - 50.4 × 10⁶ motile spermatozoa) in ocelots and 70-180 μl (2.7 - 35.7 × 10⁶ motile spermatozoa) in margays and tigrinas. Number of ovarian structures, serum hormone levels and results of AI are presented for each female in Table 1. One female ocelot conceived and gave birth to six healthy kittens after a gestation period of 78 days. One tigrina also conceived and gave birth to three kittens after a 76 days gestation. These preliminary results suggest that tigrinas and margays, as previously reported for ocelots, 2 are relatively insensitive to exogenous gonadotropins, requiring higher dosages (on a per body weight basis) than other cat species to induce follicular growth and ovulation. Nonetheless, two ovulating females became pregnant and carried offspring to term, representing the first successful artificial inseminations of nondomestic felids in Latin America and the first tigrina kittens produced by AI. In conclusion, although additional studies are needed to improve the efficiency of ovarian stimulation and increase conception rates, this AI technique has proven feasible to produce viable kittens in these small, endangered spotted cats. These findings suggest that laparoscopic AI may be useful as an adjunct to
natural breeding for conservation of small Latin American felids.

ACKNOWLEDGMENTS

The authors thank the Curitiba Zoo and Itaipu Binacional for providing animals and assistance with sampling. This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), the New Opportunities in Animal Health Sciences (NOAHS) Center, Friends of National Zoo (FONZ), British Airways and The Philip Reed Foundation.

LITERATURE CITED

Table 1. Ovarian responses, serum hormone concentrations and AI results in female ocelots (Lpa), tigrinas (Lti) and margays (Lwi) treated with exogenous gonadotropins and laparoscopically inseminated in utero.

<table>
<thead>
<tr>
<th>Female (kg)</th>
<th>Gonadotropin dosage</th>
<th>Ovarian response</th>
<th>Serum hormones</th>
<th>Inseminated sperm</th>
<th>Pregnancy (yes/no)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lpa54 (17.0)</td>
<td>400 IU eCG/200 IU hCG</td>
<td>Number of follicles (diameter-mm)</td>
<td>Number of corpora lutea (diameter-mm)</td>
<td>Estradiol (pg/ml)</td>
<td>Progesterone (ng/ml)</td>
</tr>
<tr>
<td>Lpa56 (10.0)</td>
<td>400 IU eCG/200 IU hCG</td>
<td>5 (7.2 ± 1.3)</td>
<td>4 (4.9 ± 0.4)</td>
<td>307.4</td>
<td>5.1</td>
</tr>
<tr>
<td>Lpa57 (14.5)</td>
<td>400 IU eCG/200 IU hCG</td>
<td>6 (4.7 ± 0.8)</td>
<td>2 (3.0 ± 1.0)</td>
<td>115.9</td>
<td>4.0</td>
</tr>
<tr>
<td>Lpa58 (9.5)</td>
<td>400 IU eCG/200 IU hCG</td>
<td>2 (2.0 ± 0.0)</td>
<td>12 (3.7 ± 0.2)</td>
<td>844.5</td>
<td>2.5</td>
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<td>Lti21 (2.5)</td>
<td>100 IU eCG/75 IU hCG</td>
<td>2 (1.5 ± 0.5)</td>
<td>1 (4.0)</td>
<td>121.0</td>
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</tr>
<tr>
<td>Lti21 (2.4)</td>
<td>200 IU eCG/150 IU hCG</td>
<td>0</td>
<td>13 (2.1 ± 0.1)</td>
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<td>3.0</td>
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<td>Lti22 (2.2)</td>
<td>100 IU eCG/75 IU hCG</td>
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<td>60.1</td>
<td>3.7</td>
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<td>Lti22 (2.3)</td>
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<td>5 (3.6 ± 0.7)</td>
<td>110.5</td>
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<td>Lwi31 (2.8)</td>
<td>100 IU eCG/75 IU hCG</td>
<td>4 (4.9 ± 1.4)</td>
<td>4 (8.0 ± 0.0)</td>
<td>90.3</td>
<td>2.9</td>
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<tr>
<td>Lwi31 (3.0)</td>
<td>200 IU eCG/150 IU hCG</td>
<td>7 (5.0 ± 0.0)</td>
<td>3 (4.0 ± 0.0)</td>
<td>159.7</td>
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<table>
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<tr>
<th>Estradiol (pg/ml)</th>
<th>Progesterone (ng/ml)</th>
<th>Inseminated sperm</th>
<th>Pregnancy (yes/no)</th>
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<tr>
<td>5.1</td>
<td>3.7</td>
<td>NOT inseminated</td>
<td>NO</td>
</tr>
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<td>4.0</td>
<td>40.2</td>
<td>NOT</td>
<td>NO</td>
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<tr>
<td>2.6</td>
<td>6.1</td>
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</tr>
</tbody>
</table>

a Body weight
b mean ± SEM
c number of motile sperm × 10⁶
HERPESVIRUS INFECTION IN FREE-LIVING BLACK-TUFTED-EAR MARMOSET (Callithrix penicillata, E. GEOFFROYI 1812) AT THE SERRA DA TIRIRICA STATE PARK, NITERÓI, RIO DE JANEIRO, BRAZIL

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Abstract

Some herpesviruses can cause disease in nonhuman primates, especially the Herpes simplex, whose only natural host is the human. Herpes simplex can cause lethal disease in marmosets. Symptoms typical of a herpesvirus infection are vesicular and necrotic plaques, erosion and ulceration of oral mucosa and the mucocutaneous border. These alterations are associated with ceratitis and conjunctivitis. Additionally, some animals develop meningitis and meningoencephalitis. There have been descriptions in the literature of marmosets with Herpes simplex or putative cases only from captivity. These animals died within 2-5 days after the appearance of the ulcerations described above.

Methods

In 1995, a very sick C. penicillata from Serra da Tiririca State Park was brought to the Polyclinic of the Veterinary School of UFF. According to the case history, many more sick animals could be found at the same site. A herpesvirus infection was diagnosed through clinical findings, necropsy, histopathological investigations and electron microscopy.

Results

During clinical examination and necropsy, small blisters and ulcers of the tongue, soft palate and infra-ocular area were found. The retropharyngeal lymph nodes showed hyperplasia. In the gastrointestinal tract, parasitosis could be diagnosed. The lung showed multiple tumors. In the histological examination, we found shallow ulcers with inflammatory reaction and intranuclear inclusion bodies, typical of herpes of the tongue, soft palate and skin. We also observed follicular hyperplasia in spleen as well as retropharyngeal lymph node and necrosis of the liver. The lungs showed emphysema, alveolar edema, acute alveolar hemorrhagia, parasites and tumor masses. Under electron microscopy, the virus could be seen in skin and mucosa. It was located intranuclearly as well as intracytoplasmatically and showed characteristics of herpesvirus, suggesting Herpes simplex.

Discussion

As mentioned above Callithrix spp. are susceptible to Herpes simplex. There is, however, no evidence for such an infection in wild marmosets, specifically C. penicillata. The most probable means of transmission of herpesvirus is direct contact with the saliva of the infected subjects. Nevertheless, such a virus can also be transmitted by, for example, domestic utensils a short time...
The marmosets in the park have substantial contact with the man who feeds them. A transmission of *Herpes simplex* is therefore likely to have occurred through this vector.

**ACKNOWLEDGMENTS**

Many thanks to Instituto Estadual de Florestas (IEF) and Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) for their cooperation.

**LITERATURE CITED**


IMMUNE COMPLEX GLOMERULOPATHY IN MAMMOSETS AT THE GERMAN PRIMATE CENTER

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Abstract

Marmosets (Platyrrhini: C. Callitrichidae) are small New World monkeys well suited for laboratory research because of their close relationship to man and their low maintenance costs. Marmosets have a low immunological competence compared to other primate species which may be due to defective B-cell activity.8,9 When kept in captivity, marmosets are very susceptible to several disorders such as wasting marmoset syndrome (WMS),4,13 jejunitis and colitis,5 colon carcinoma,1 and nephritis,3,15 characterized as IgM-nephropathy.2 Furthermore a high incidence of subclinical chronic urinary tract disease in a colony of marmosets at the German Primate Center (GPC) was reported.7

For further information on chronic glomerulopathy in marmosets triple diagnostic (light microscopy, immunofluorescence microscopy and electron microscopy) was done and similarities to IgA-nephropathy found. The degree of glomerular IgA-deposition was correlated to hematuria and proteinuria.

Methods

The kidneys of the 127 marmosets and tamarins from the GPC were investigated for histologic lesions, IgA- and IgM-deposits. All animals were submitted by other departments for routine necropsy after natural death or following sacrifice. The control group consisted of 13 Old World monkeys (5 Macaca mulatta, 5 Papio hamadryas, 1 Macaca fascicularis, 1 Papio sphinx, 1 Presbytis entellus).

The light microscopical and immunopathological techniques applied to the renal tissues have been previously in principle described.2,3 Because there are no antisera to marmoset immunoglobulins available, fluorescin-labeled polyclonal antisera to human IgA and IgM (Dako, Hamburg) were used. These antibodies were checked for cross-reactivity with agarose diffusion techniques.11 According to the results of the cross-reactivity tests, working dilutions of 1:20 (in phosphate buffered saline) for the antiserum to human IgA and 1:50 for the antiserum to human IgM were used. The histological lesions and glomerular deposits were graded from “negative” to “weakly positive” and “moderate to heavy positive.”

For electron microscopy, 1 mm³ fragments of kidney tissue were immediately placed in 2,5% glutaraldehyde, post-fixed in phosphate buffered 1% osmium tetroxide, dehydrated in graded alcohols and propylene oxide and Epon embedded. Sections were cut on an ultramicrotome (Leica, Bensheim) and stained with uranyl acetate and lead citrate. For electron microscopy a transmission electron microscope EM 10 (Zeiss, Oberkochen) was used.

Hematuria and proteinuria were determined by puncturing the separated bladder during necropsy, and the bladder urine was checked for blood and protein with Test-sticks (Boehringer, Mannheim).
Statistical evaluation: Statistical significant differences between the different monkey groups (p< 0.5) were evaluated by one-way-analysis of variances to compare more than 2 groups followed by Dunn’s test for pairwise multiple comparison procedures.

Results

Histology
Typical signs of nephropathy in marmosets were: increased mesangium (cells and matrix), adhesions between Bowman’s capsule and glomerular tufts, glomerulosclerosis, segmental sclerosis and interstitial fibrosis often accompanied by tubulointerstitial nephritis. According to these criteria 36 of 127 animals showed distinctive signs of mesangioproliferative glomerulonephropathy (mesGN).

Electron microscopy
In kidneys with minor glomerular IgA-deposits frequently thickened peripheral capillary basement membranes and scattered electron dense deposits “humps” subepithelial in the peripheral capillary walls were found. In kidneys with heavy glomerular IgA-deposits, large electron dense deposits in the mesangial and paramesangial areas were present.

Immunopathology
Compared to previous descriptions2,3 more frequent and more prominent IgA deposits were found in the mesangial areas. Twenty-nine of the 127 marmosets showed moderate to heavy granular IgA-deposits in the glomerulum, mostly in the mesangium and rarely along the glomerular tufts. Moderate to heavy IgM-deposits were found in the glomeruli of 56 of the 127 marmosets of the same group in mesangial pattern.

In contrast in 13 Old World monkeys, no animal showed signs of chronic nephropathy or moderate to strong IgA-deposits. In 4 of 13 of this group moderate IgM-deposits were found, but in minor amounts and along the capillary loops and not in the glomerular mesangium.

Hematuria and Proteinuria
Thirty-one marmosets of the German Primate Center, previously checked for glomerular IgA-deposits were tested for hematuria and proteinuria, and the results compared with the graduation of nephropathy. The marmosets were graded into three groups: those without glomerular IgA-deposits (group I, n = 7), those with slight (group II, n = 15) and those with strong glomerular IgA-deposits (group III, n = 9).

Significant differences in proteinuria were found between the three groups by one-way- analysis of variances and between group I and group II by pairwise multiple comparison. Also significant differences in hematuria were found between the three groups by one-way-analysis of variances and between group I and group II and between group I and group III.

Discussion

Using triple-diagnostic procedures${}^{6}$ (histology, immunopathology, electron microscopy) in the present study, the immune-mediated nephropathy in marmosets was found to be Immune-complex-glomerulopathy (IC-GP) showing striking similarities to IgA-nephropathy (Berger’s Disease) in humans. The pathology of disease consists of mesangial proliferation, segmental sclerosis,
interstitial fibrosis and tubulointerstitial nephritis and electron-dense deposits and is very similar to human IgA-nephropathy. Because of the weak cross-reactivity of the antibody to human IgA with marmosets IgA, for immunofluorescence technique a 1:20 dilution of antibody instead of 1:50 in previous studies was used and thereby IgA was found more frequently and more prominent in marmoset glomeruli than previously described.\textsuperscript{2,3} Considering the weak cross-reactivity of the antibody with marmoset IgA even a higher incidence and more prominent IgA-deposits in marmoset glomeruli might be expected using an antibody to marmoset IgA. IgM was a frequent finding in the glomeruli of marmosets with IC-GP, but occurently it was also found in minor amounts and different pattern in the kidneys of monkeys from other primate species not suffering from the disease.

The marmosets of the German Primate Center (GPC) showed a spontaneous onset of disease and clinical features typical for Berger’s Disease like hematuria and proteinuria. There was a good correlation between hematuria and proteinuria and the glomerular IgA-deposition in marmosets suffering from IC-GP. The IC-GP in marmosets might be a good and suitable animal model for Berger’s Disease. Chronic tubulointerstitial nephritis and glomerulopathy in marmosets were reported several times.\textsuperscript{2,3,14,15} Given the diagnostic obstacles, requiring special histological staining methods (Movat, PAS-McManus, immunofluorescence techniques) and therefore a higher incidence of IC-GP in marmoset colonies might be expected.

The diet in captivity as well as intestinal lesions, infections and their immunologic and metabolic situation were considered as a possible reason for enteritis and nephropathy in marmosets.\textsuperscript{2} Also a certain role of gliadin from gluten in etiology and pathogenesis of IC-GP as well as in WMS in marmosets was assumed.\textsuperscript{12} Their low immunological competence might be important for the pathogenesis of immune complex glomerulopathy in marmosets as is assumed the disease in humans.\textsuperscript{10}

There are further studies necessary about the occurrence and genetics of IC-GP in marmoset colonies. Having a suitable animal model now, there might be better chances to get informations about the still enigmatic etiology and pathogenesis of Berger’s Disease in humans.

**LITERATURE CITED**

INFRARED-THERMOGRAPHY IN ZOO ANIMALS: PRELIMINARY EXPERIENCES WITH ITS USE IN MAMMALIAN PREGNANCY DIAGNOSIS AND AVIAN AND REPTILIAN EGG CONTROL

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Abstract

A preliminary report of the use of infrared-thermography in zoo and wild animals was given by Eulenberger at the thirty-sixth International Symposium on the Diseases of Zoo and Wild Animals. In this report, new areas of application of infrared light, measurement of normal body surface temperature pictures and alterations through inflammations, are presented.

Thermography offers the possibility to investigate an animal from a distance of one to a maximum of 20 m without having to sedate or anesthetize the animal. This technique measures the heat-radiation (between 3 and 5 μm wavelength) a body reflects on a surface into the environment.

Methods

The infrared-camera THERMOVISION 550 from AGEMA is cooled electrically to -80°C. The lens has a silicon surface, a semiconductor, which converts infrared radiation into photo-optical signals in an optimum way. This signal is then converted to digital black and white pictures, which then can be converted into color. At a wavelength of 10⁻⁴ to 10⁻⁶ μm and a frequency of 10¹⁴ Hz infrared-cameras today are able to differentiate surface-temperatures of 0.1 °C. High resolution monitors and computer-graphic-hardware produce an accordingly precise presentation of the pictures. Measurements are only executed in the long wave band, since these rays are not damped so quickly in their intensity as short waved ones, so that measured values can be achieved over long distances. Long wave infrared radiation (λ = 3 -5 μm) is reflected by a coefficient of emission εs of nearly 1 from hairless skin, which is comparable to a black body. Due to the more or less thick hair of animal bodies thermography has its limits. In eggs these problems do not exist.

The best results can be achieved if the investigated structure is placed within a region of 2 cm under the skin-surface, which applies, for example, to skin-tumors and abscesses. But also greater structures situated deeper inside a body can be investigated with infrared, as long as they transmit heat to the surface, which then is measured as heat-area or -spots. This feature is used here to diagnose pregnancies in mammals or investigate avian or reptilian eggs for growth or fertilization.

In thermoregulatory processes, small inflammations or heat-producing tumors, more or less heat is transmitted to the body surface, so that in a thermogram a temperature difference can be measured. In the thermogram, yellow and red colors show warmer areas, green and blue cooler areas. In black and white graphic representations, the warmer areas are light, the cooler dark. In human medicine infrared thermography is mainly used in neurology, orthopedic diagnostics,
rheumatology, oncology (especially skin- and mammatumor-diagnostic), as well as in pain research.¹

**Results**

In the search of new noninvasive technologies in veterinary medicine, at the Zoological Garden of Berlin and Leipzig, Germany, we started a new research project with the goal of developing an easy-to-apply method for pregnancy diagnosis in zoo animals. Our preliminary results with using this new method revealed, that animals with no or short hair are well-suited for this technique. A uterus filled with a fetus and its fluids will be in contact with the body surface layers. The fetus produces heat from its metabolism that the mother has to cope with and try to eliminate. The shortest way of doing this is the direct way through the muscle and skin layers to the outside. In animals where there is no hair (e.g., rhinoceros) or short hair (e.g., giraffes) on the outside to prevent thermoregulation, the uterine heat can escape directly to the surface. In animals, with a lot of hair on the surface (e.g., camels) the method is, as of now, not applicable, because these animals thermoregulate entirely via the inside of their legs and the ventral body surface.

Another handicap with this technique is the lack of knowledge concerning the anatomic base of thermoregulation in exotic animals. For example, in babirussas, there seem to be thermoregulatory heat windows on the body surface, specific to each individual.

This technique was used on several zoo animals and the results are described below. Individual diagrams are provided during presentation of these results.

**Pregnancy diagnosis in black rhinoceros (*Diceros bicornis*)**. Diagram 1 illustrates the approximate location of the uterus with an 11-mo-old rhinoceros fetus in situ. Diagram 2 shows a non-pregnant black rhinoceros. Diagrams 3-5 show a pregnant black rhinoceros. In diagram 3 the female is approximately 6 mo pregnant, in diagram 4 approximately 9 mo, and in diagram 5 approximately 11 mo. The diagrams show the volume increase of the uterus as the fetus grows over time. The light colored area indicates the heat transmission area of the uterus to the body surface. Over time, the heat transmission area grows larger, because greater parts of the uterus come in contact with the body surface. Towards the end of pregnancy, the heat area covers the entire main body surface.

**Pregnancy diagnosis in elephants (*Elephas maximus*)**. Diagram 6 illustrates the approximate location of a 20-mo-old fetus in situ. Diagram 7 shows a non-pregnant elephant cow, diagram 8 an approximately 15-mo, and diagram 8 an approximately 20-mo pregnant cow. At mid-pregnancy, the animal shows a higher body temperature and also a local heat area on one or both sides of the body. In late pregnancy, the local heat area becomes less obvious as the uterus spreads over a large body surface area and hence the cow must put more general effort into thermoregulation and is not able any more to give up heat just locally.

**Egg control in reptiles and birds**. As an attempt to observe the continuous growth of reptiles and birds inside their eggs, infrared thermography also showed its usefulness. In birds, the infrared heat measurement gives a quick overview over great amounts of eggs from either the incubator or natural sitting birds, when they rise from their nest. In reptiles it is especially important to be able to look at the thin-shelled eggs without having to touch them. In diagram 9, eggs are shown from a shelf of an incubator 3 min after removal from the incubator. Intact eggs are able to keep their temperature more constant to that of the incubator than are unfertilized or dead eggs. In reptiles, the area not
covered by the hatching-material is available for temperature measurement with the infrared camera. Here the same principles apply as in bird eggs: eggs with live embryos show higher temperatures than those with dead embryos or unfertilized eggs. Diagram 10 shows the eggs of *Heloderma* spp. in an incubator box partially covered by hatching material. The dark area inside the eggshell indicates that it is not a live egg.

**Discussion**

As the results presented above indicate, infrared-thermography is a useful technique for zoo and wild animal medicine. It could be used for population management in rhinoceros and elephants by helping the wildlife biologist or veterinarian to diagnose pregnancies at a distance and hence give a better overview of happenings in populations. To date, the method is still in the process of being standardized, but once this is concluded, it is obviously a very useful tool in zoo and wildlife medicine. No narcosis or sedation is necessary to do this test and hence it is well suited for flighty animals as well. The only disadvantage is the limits to species with short or no hair. Also the person using this method has to learn to differentiate between surface structures, such as slight scratches, intense sunshine, or wet areas etc., and true heat areas from uterus thermoregulation. Once the method is established, the distance to the object can be increased and tele-lenses used. The method for investigating bird eggs, especially thick- or dark-shelled ones, as well as reptilian eggs seems to be another useful application of this technique. It allows for observation of many eggs at once as well as allowing one to look at eggs from naturally sitting birds when they rise from the nest. This gives new possibilities for population management and gaining of easy information concerning rates of pregnancies in mammals, and fertilization in birds and reptiles. Finally, this would be a method to acquire more basic data concerning the reproductive biology and performance of wild animals.

**LITERATURE CITED**

USE OF CT IMAGING AND THREE-DIMENSIONAL COLOR RECONSTRUCTION FOR COMPARISON OF CRANIAL ANATOMY AND DENSITY IN CAPTIVE AND WILD CHEETAHS (Acinonyx jubatus)

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Abstract

Cheetahs are declining in the wild and do not prosper in captivity.2,3 They have notoriously low fertility and high infant mortality in captivity.2,4 Though it is tempting to attribute their problems to low genetic diversity, disorders caused by husbandry must not be ignored, as these are more likely to be prevented. In 1982 Fitch and Fagan described a condition in captive cheetahs called Focal Palatine Erosion (FPE).1 The etiology of the disorder remains unknown, though it has been suggested that it is a disease of captivity and thus environmental, not genetic, causes.1,5 The hypothesis put forth by Fitch and Fagan explained the condition as a defect in occlusion of the molar, caused by lack of the “hassle factor” during development. The “hassle factor” is absent in captive raised animals because they are fed soft commercial diets, and thus are not exercising their masticatory apparatus by manipulating tendons, ligaments, and bone. Affected animals have clinical signs of dysphagia and osteomyelitis, and on physical examination they have a defect in the hard palate caused by traumatic insult by the lower molar. To further investigate this disorder, we scanned seven skulls using computed tomography technology and three-dimensional color reconstruction. We looked at two skulls from wild cheetahs killed in Kenya in 1910, two skulls from captive cheetahs at the San Diego Wild Animal Park who died in the 1980’s, and three skulls from wild cheetahs killed in Namibia in the 1990’s. Preliminary results showed that all the wild cheetahs examined have greater bone density through their skulls when compared to the captive animals. If this is the case, it suggests that FPE is not an isolated problem of occlusion, but a generalized disorder of the skeletal components of the head. We are hypothesizing that lack of the “hassle factor” causes a failure of adequate development of the bony elements of the jaws. Because the size of the teeth is determined prenatally and not influenced by diet, the teeth are too large for the underdeveloped jaw and malocclusion results. In addition, the bones are not stressed sufficiently, leading to less mineral deposition and thus lower density. The malocclusion is the immediate cause of the trauma to the hard palate, but the lesions are exacerbated by the poor bone density. If this hypothesis is correct, it is likely that altering the diet of young cheetahs in captivity would prevent FPE and its associated morbidity and mortality.

LITERATURE CITED

CLINICAL SIGNIFICANCE OF THE MOLAR DENTITION OF THE WARTHOG

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Abstract

The warthog is a species within the Suidae family indigenous to the northern and southern African savanna plains. Warthogs differ from many of the Suidae in their dental formula. The formula of most Suidae is I:3/3, C:1/1, PM:4/4, M:3/3, while the warthog is described as I:0-1/3, C:1/1, PM:3/2, M:3/3.\textsuperscript{3} However, examination of an adult warthog’s dentition usually reveals a reduced number of teeth which will surprise the first time observer. In addition, the structure of the molars, and in particular the third molar, is unique and has clinical implications for dental procedures.

There is a recently clarified species division in warthogs.\textsuperscript{7} If incisors are present in the maxilla, the species is considered to be the common warthog (\textit{Phacochoerus africanus}). Individuals that do not possess upper incisors, or have very small, or non erupted incisors are considered to be the desert warthog (\textit{Phacochoerus aethiopicus}) and is distributed in parts of Ethiopia, Somalia and Kenya. The desert warthog is also considered to be smaller in body size. Within the 2 species there are 6 subspecies divisions, however these show no apparent trends in dentition.

In addition to the species differences, the present study and others have documented a considerable individual variation in teeth numbers. In a study which examined over 1200 warthog skulls in Zimbabwe, the majority contained 2 premolars, some had 3 premolars and 1 single skull showed a first deciduous premolar. Thus, the dental formula of \textit{P. africanus} is I:1-2/2-3, C:1/1, PM:2-4/1-2, M3/3 1 and that of \textit{P. aethiopicus} is I:0/0-2, C:1/1, PM:2-3/1-2, M:3/3 (J.P. d’Huart, personal communication). Lastly a process of normal permanent teeth loss is apparent where by premolars and anterior molars are progressively lost as the third molar grows.\textsuperscript{1} Examination of the oral cavity will reveal a spectrum of normal dental formulas and differences must not be viewed with alarm. In many very old animals the only teeth remaining were the canines and third molars giving a geriatric dental formal of I:0/0, C: 1/1, PM: 0/0, M:1/1.

At Ol Jogi Ltd, Laikipia District, Kenya, 18 warthog maxillas and 9 mandibles were opportunistically collected and examined for their dental formula. All skulls showed distinct upper incisors identifying these warthogs in Laikipia as belonging to the \textit{P. africanus} species. All skulls had complete permanent dentition indicating they were over 24-mo-old.\textsuperscript{1}

Anatomy of Premolars and Anterior Molars

The premolars and molars are generally squarish teeth that are composed of irregular shaped tubules of dentine bonded together by cement. The number of tubules that form each tooth varies between individuals. Tubules may be bifurcated and originate from common roots or individual tubules may have single roots. As teeth wear down the coalescing of tubules becomes noticeable. The number and structure of roots also varies between individuals and the process of dental change. As the third molar applies pressure to the anterior teeth roots become twisted, curved caudally, constricted and
then disappear. Some teeth have virtually no roots at all and pulled away from the skull with a minimum of force.

**Anatomy of the Third Molar**

The third molar is composed of a large number of closely set cylinders of dentine embedded in cement of different sizes. The M3 of the upper jaw has an average length of 44 mm, a width of 12 mm and a depth of 60 mm \((n = 12)\). Third molars of up to 70 mm in length have been recorded.\(^1\) The tubules from the third molar curve slightly caudally. It has been considered the M3 is growing through most of the animals' life. The first portion of the tooth to erupt forms the anterior edge and additional tubules continue to erupt at the posterior aspect of the tooth until well into adulthood.\(^1\) Cutting the molar vertically and horizontally shows that the greater majority of dentine cylinders do not branch or fuse together into common roots. Each tubule has its own root. However, in the posterior aspects as many as 3-4 tubules may originate from common roots. Several molars were noted to have individually rooted tubules buried within a group of tubules with a common root.

**Changing With Age**

Growth over time of the third molar has been described.\(^4\) Continued eruption of tubules from the posterior aspect of the tooth causes forward movement of M3. As the premolars do not move, pressure is put against M1 and M2. This changes the teeth’s shape and their root’s direction eventually constricting the roots causing loss of contact with the socket and thus, loss of the tooth. Following, M3 applies pressure to the premolars which are in turn lost by a similar process.

Wearing of any tooth does not always follow from front to back. On 5 skulls, the first or second molar of the maxilla were completely worn down to a smooth surface, while their anterior neighbor, the 3rd PM or 1st molar, had not been worn down to such extent. The opposing tooth of the mandible was also worn down to the same extent so that when looking at the lateral surface of the skull a window like gap of 5-8 mm is seen. In addition, another abnormal wear pattern was noted on two skulls. In both of these a large hook extending 1 cm above the level of the molar ridge on one side was present. In one case the tooth was M3 on the mandible, the only remaining tooth and in the other the second molar of the maxilla which was still within the premolar/molar row.

Lastly incisors are also lost over time.\(^1\) The process is not clear but appears due to wear over time. However, the loss of incisors does not seem to affect the animals' ability to feed.

**Possible Dental Procedures?**

It will be important to determine the degree of bifurcation and the root pattern in a diseased molar. Use of endodontal probing and dental radiography, possibly with contrast fluids being injected into the root canal\(^2,6\) will be of vital importance. If the radiographs show the root canal to be separated from the surrounding cylinders it might be possible to drill the single cylinder loose or to remove fractured remnants of cylinders. However, the length of the roots and their slight curve will make this procedure difficult and risky as the root or drill might fracture in the depths while working on it.

If more cylinders are involved in the disease process or a fracture is present, a split in the multi rooted molar for partial removal of the diseased area could be considered.\(^6\) Drill assistance will be needed. Cleaving of the tooth with a chisel will not be possible. While working on postmortem
specimens it was noted that the cylinders do not break along the boundary lines but make jagged 
cuts through adjacent dentine and root cavities.

Total extraction of a molar may not be feasible in aged animals as the only remaining grinding teeth 
could be the third molars.\(^1\) There should be great concern regarding the affect on the opposing tooth 
and the ability of the animal to masticate.

Any oral approach will be difficult due to limited access for instruments. A total extraction of the 
third molar will require a surgical approach through the buccal wall on the lateral surface, keeping in 
mind that the large masseter muscle will make access difficult. Osteotomy procedures will follow to 
gain access to the roots of the third molar.

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ENHANCED REPRODUCTIVE EFFICIENCY AND PREGNANCIES AFTER ARTIFICIAL INSEMINATION IN BLACK-FOOTED FERRETS

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Abstract

The black-footed ferret (Mustela nigripes) is an endangered species, once considered extinct until a remnant population was discovered in Wyoming in 1981. Between 1985 and 1987, the last remaining 18 black-footed ferrets were captured to begin a multi-institutional propagation program. Since 1987, ~2,200 ferrets have been produced by natural breeding, and ~300 animals currently reside in 7 breeding facilities (National Black-Footed Ferret Conservation Center, Laramie, WY; National Zoological Park’s Conservation & Research Center, Front Royal, VA; Omaha’s Henry Doorly Zoo, Omaha, NE; Cheyenne Mountain Zoological Park, Colorado Springs, CO; Louisville Zoological Garden, Louisville, KY; The Phoenix Zoo, Phoenix, AZ; Metropolitan Toronto Zoo, Ontario, Canada). The ability to produce ferrets in captivity has allowed for the reintroduction of black-footed ferrets in four states (Wyoming, Montana, South Dakota, Arizona). To date, ~80 black-footed ferrets survive in the wild and young have been produced from reintroduced animals. Although captive breeding is successful, the goal of the recovery program (1,500 breeding ferrets in >10 free-ranging locations by year 2010) will not be achieved at the current rate of propagation. Recent assessment of breeding records revealed that a remarkably high proportion of males (~50%) fail to reproduce in captive breeding situations due to behavioral incompatibility. Additionally, certain original ferret “founders” are poorly represented in the current population, and some of their descendants have never sired young due to aggression towards females. These issues prompted the use of assisted reproduction to improve black-footed ferret propagation to meet reintroduction demands and maintain maximum genetic diversity. A Black-footed Ferret Genome Resource Bank (repository of cryopreserved sperm) was established to preserve valuable germ plasm. The objectives of the program are to use an intrauterine artificial insemination (AI) technique with fresh or cryopreserved semen to: 1) breed behaviorally incompatible animals; 2) produce offspring from genetically-valuable ‘non-proven’ males that have failed to reproduce; and 3) enhance founder representation in under-represented lineages. As a research tool, AI also is being applied to Siberian polecat (Mustela eversmanni) females using black-footed ferret semen to produce hybrids for testing the efficacy of a new “killed” canine distemper vaccine. For these procedures, electroejaculates are diluted in an egg-yolk cryodiluent and used either immediately for AI or pellet frozen on dry ice. Females in natural estrus with maximum vulvar swelling and >90% cornified vaginal epithelial cells are given 90 IU human chorionic gonadotropin to induce ovulation. For AI, females are laparoscopically inseminated in utero with fresh or frozen-thawed semen. In 1996 and 1997, a total of 20 females (17 black-footed ferrets and 3 Siberian polecats) maintained at the Conservation & Research Center (14 black-footed ferret females) or National Black-footed Ferret Conservation Center (3 black-footed ferret and 3 Siberian polecat females) were inseminated with fresh (n = 16) or frozen-thawed (n = 4) semen from black-footed ferret males meeting at least one criterion listed in the objectives above. In spring of 1996, 5 of 6 (83.3%) black-footed ferrets inseminated with
fresh semen became pregnant and produced 16 kits (mean litter size, 3.2). In spring of 1997, 6 of 8 (75.0%) black-footed ferrets inseminated with fresh semen and 2 of 3 (66.7%) inseminated with frozen-thawed semen became pregnant, resulting in a total of 19 kits (mean litter size, 2.4). Pregnancies also were achieved in 2 of 3 (66.7%) Siberian polecats inseminated with fresh or frozen-thawed black-footed ferret semen; a total of 10 hybrid kits (5 kits/litter) were produced for the vaccine research. These results demonstrate that: 1) laparoscopic intrauterine AI using fresh or cryopreserved black-footed ferret semen is successful (overall pregnancy rate, 75%) for enhancing reproductive efficiency; and 2) the integration of assisted reproduction and a Genome Resource Bank can play a beneficial role in the management and recovery of the endangered black-footed ferret.