
COMPARISON OF ISOFLURANE AND SEVOFLURANE ANESTHESIA FOLLOWING PREMEDICATION WITH BUTORPHANOL IN THE GREEN IGUANA (*Iguana iguana*)

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Abstract

Twenty-three, male captive-bred green iguanas (*Iguana iguana*) weighing 0.51-0.67 kg were anesthetized to determine the effectiveness and the cardiopulmonary effects of butorphanol-sevoflurane and butorphanol-isoflurane. Baseline heart and respiratory rates were recorded on all animals prior to administration of intramuscular butorphanol (2 mg/kg). Thirty minutes after premedication, the heart rate and respiratory rate of each iguana was recorded and 12 animals were induced with isoflurane (5% in 100% oxygen), and 11 were induced with sevoflurane (8% in 100% oxygen), via face mask. Following the loss of the righting reflex, each iguana was intubated and intermittent positive pressure ventilation (IPPV) was provided at 10-12 breaths/min with a small animal ventilator. Mean maintenance isoflurane and sevoflurane concentrations were 2% and 4%, respectively. Heart rate, functional oxygen hemoglobin saturation (SpO₂), and end-tidal CO₂ concentrations were measured every 5 min. The presence or absence of the righting, palpebral, and foot withdrawal reflexes were recorded to judge anesthetic depth. Arterial blood gases (pH, PO₂, PCO₂) were determined at 10 and 30 min following induction. In both groups, anesthesia was maintained for an average of 45 min. Inductions with butorphanol-sevoflurane (6.0 ± 3.0 min; mean ± S.D.) were smoother and significantly shorter than with butorphanol-isoflurane (9.0 ± 4.0 min). During maintenance of anesthesia, cardiopulmonary parameters were well maintained in both groups. In both groups pulse oximeter readings (SpO₂) were < 90% during the first 5 min of anesthesia, indicating potential hypoxemia. For the remainder of the anesthetic period SpO₂ values were > 90%. End-tidal CO₂ concentrations were between 20-40 mm Hg during the first 5 min of anesthesia and decreased to a range between 15-20 mm Hg for the remainder of the anesthetic event. No significant differences in heart rate, oxygen hemoglobin saturation, and end-tidal CO₂ concentrations were noted between the two groups. Mean recovery time was significantly longer (35.0 ± 27.0 min) in the butorphanol-isoflurane group than in the butorphanol-sevoflurane group (7.0 ± 4.0 min). In conclusion, the cardiopulmonary effects of butorphanol-isoflurane and butorphanol-sevoflurane were similar, however quality and speed of induction and recovery was significantly shorter with sevoflurane when compared to isoflurane.

OUTBREAK OF WEST NILE VIRUS IN FARMED ALLIGATORS (*Alligator mississippiensis*) IN FLORIDA

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Abstract

In September and October 2002, an epizootic characterized by neurologic disease occurred at a 9,000-alligator farm in Florida. Approximately 300 alligators (*Alligator mississippiensis*) died during this time. First cases were observed in 1-m long yearlings on 15 October 2002. Three animals were clinically evaluated and exhibited a range of signs that included depression, lethargy and signs of neurologic disease. They were euthanatized and necropsies were performed. Gross lesions consisted of mild coelomic effusions, fibrinonecrotic oral mucosal exudates, and enlarged pale livers with red mottling. The most significant microscopic lesions were a moderate heterophilic to lymphoplasmacytic meningoencephalomyelitis, necrotizing hepatitis and splenitis, myocardial degeneration with necrosis, mild interstitial pneumonia, hepatic lipidosis, adrenalitis, and heterophilic necrotizing stomatitis, and glossitis. Examination of the brain and other tissues was conducted using an immunoperoxidase staining technique and a monoclonal antibody for West Nile virus (WNV) antigen. Positive staining was demonstrated in the brain as well as other tissues. Portions of brain, spinal cord, and other tissues were submitted both to the Florida Department of Health Bureau (FDHB) of Laboratories in Tampa and to the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention (CDC) in Ft. Collins, Colorado, for definitive diagnosis of WNV. Virus was isolated, and polymerase chain reaction (PCR) testing confirmed the presence of WNV. This was the first identification of WNV in a reptile.

ENDOSCOPIC EVALUATION OF THE AMERICAN ALLIGATOR (*Alligator mississippiensis*) RESPIRATORY TRACT

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Abstract

Respiratory diseases are common in captive and wild American alligators (*Alligator mississippiensis*), but are difficult to diagnose ante-mortem. Bronchoscopy permits direct visualization of the respiratory tract and sample collection, but cytologic and microbiologic reference values are lacking for crocodilians. Twelve (three males and nine females) 3-yr-old, 1-m long, 4.5-9.0-kg captive-born alligators were used in this study. Bronchoscopy, using a flexible endoscope (3.3 × 700 mm choledochofiberscope OES, Olympus, Hamburg, Germany), was used to separate the alligators into two groups. The criterion for separating them was the absence or presence of respiratory inflammation, mucous, blood, secretions, parasites and/or masses. Six alligators without any abnormalities were placed in Group A. The other six alligators had respiratory lesions and were placed into Group B. Endoscopy was repeated every 3 mo for 12 mo. A tracheal wash (at the bronchial bifurcation) was collected each time an alligator was evaluated and submitted for *Mycoplasma* spp., aerobic, and fungal cultures. Microscopic slides were prepared for cytologic examination.

Mycoplasma spp. were not isolated from any animal. Low numbers of samples (11 out of 48; 23%) resulted in scant growth of bacterial colonies. Similarly, low numbers of samples (9 out of 48; 19%) resulted in scant growth of fungal agents. These organisms were considered to be nonpathogenic and likely the result of oral contamination. Cytologic evaluation of collected material found most samples (90%) contained only a small amount of mucus, low numbers of ciliated columnar epithelium and keratinized squamous cells. Less than 10% of the samples contained large amounts of mucus, reactive macrophages or heterophils, which would be considered evidence of irritation/inflammation to the airways. No bacteria or parasites were observed in any of the samples.

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HEALTH ASSESSMENT IN THE EASTERN INDIGO SNAKE (*Drymarchon corais couperi*) IN SOUTHEASTERN GEORGIA: PRELIMINARY FINDINGS

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Abstract

The objectives of this on-going study are to develop a database for health assessment and nutritional parameters for free-ranging eastern indigo snakes and evaluate the effects of environmental contaminants on the health and reproductive status of this species.

Health assessment diagnostics performed included a complete blood count, a plasma biochemical profile, plasma bile acid level, and plasma protein electrophoresis, plasma vitamins D, A, and E levels, plasma mineral panel, plasma fatty acid levels, plasma 17 β estradiol and testosterone, heavy metal screen, cholinesterase levels (biomarker for organophosphates) and pesticide screening. A stomach wash, fecal exam, and plasma were samples used for *Cryptosporidia* spp. evaluation. Whole body radiographs were performed on each snake and used to assess reproductive status in females, recently ingested prey items, and bony abnormalities. A complete necropsy was performed on any snake found dead.

Significant findings thus far include a high percentage of snakes with scabby to pustular skin lesions, with a heterophilic inflammation with a mixed bacterial and fungal infection; elevated plasma calcium levels; mild to severe infestations of the RBC with a *Hepatozoon* spp., lower than expected circulating vitamin A concentrations; low to moderate levels of mercury in blood and tissues samples; some snakes were positive for *Cryptosporidium* spp. utilizing the ELISA or IFA testing on various samples; and radiographs and necropsy revealed that prey items are sometimes present in snakes captured from November to mid-December.

HIGH INCIDENCE OF LYMPHOID NEOPLASIA IN A COLONY OF EGYPTIAN SPINY-TAILED LIZARDS (*Uromastix aegyptius*)

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Abstract

Hematopoietic malignancies, particularly lymphoproliferative disorders, are the most common neoplasms in lizards. Although not uncommon, the occurrence of these tumors in reptiles is typically sporadic. This report describes an unusually high incidence of lymphoid neoplasia in Egyptian spiny-tailed lizards (*Uromastix aegyptius*) over an 8-yr period. Between 1993-2001, eight out of 15 (53%) lizards necropsied at the Louisville Zoological Garden were diagnosed with multicentric lymphoma. By light and electron microscopic examination, the neoplasms had plasmacytoid morphology suggesting B-cell origin, although some tumors also had a primitive blast cell component. A concurrent leukemic blood profile was identified in seven out of the eight cases. All were adult animals and no sex predilection was observed. A review of the case study and an environmental survey did not reveal exposure to any known exogenous carcinogens. Some of the lizards were unrelated, suggesting that hereditary factors were unlikely. Although examination via electron microscopy and viral isolation performed on archived tissues and plasma failed to detect viruses, an infectious etiology is suspected. At the time of this writing, immunohistochemical staining is pending to further characterize the neoplastic cell morphology in this case cluster.

SUCCESSFUL RADIATION TREATMENT OF LEUKEMIA IN A SUNGAZER LIZARD (*Cordylus giganteus*)

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Abstract

An adult female sungazer lizard (*Cordylus giganteus*) was presented for routine postshipment quarantine. On physical examination, it appeared in good body condition except for unusually pale mucus membranes and evidence of a previous femoral pore abscess on the left hind leg. A CBC revealed anemia with a hematocrit of 7%, and a WBC of $3.1 \times 10^3/\mu\text{l}$ with a differential of 73% heterophils, 20% lymphocytes, 3% monocytes, and 4% basophils. A few reactive lymphocytes were noted. Strongyle ova were identified on fecal parasitology examination. A deworming treatment was initiated with ivermectin. A subsequent CBC at 3 wk revealed a continued anemia, and large, immature lymphocytes comprising 75% of the leukocytes. This was followed the next week by an increase in the total WBC to $80.1 \times 10^3/\mu\text{l}$, with 87% lymphoblasts. This was considered to be consistent with a diagnosis of acute lymphoblastic leukemia. Treatment with prednisone was initiated at 0.2 mg every 72 hr p.o. scaled from the canine dose of 2 mg/kg every 24 hr. During the following 3 mo of treatment, the WBC remained elevated, ranging between $49\text{--}138 \times 10^3/\mu\text{l}$ with lymphocytes and lymphoblasts comprising over 90% of the total leukocytes. The hematocrit remained between 10-15%. Approximately 6 mo after the initial presentation, the prednisolone treatment was discontinued and a single, low dose, whole body radiation treatment of 1 Gray was administered. One month postradiation, the WBC remained elevated at $34.3 \times 10^3/\mu\text{l}$ with a predominance of lymphoblasts. At 2 mo postradiation, the WBC had decreased to $9.1 \times 10^3/\mu\text{l}$. At 3 mo the WBC was further decreased to $2.9 \times 10^3/\mu\text{l}$ with a normal differential, and the hematocrit was 22%. At 6, 8, and 11 mo posttreatment, the WBC, differential, and hematocrit all remained within normal limits. Approximately 11 mo after radiation treatment, the lizard died of complications arising from reabscessation at the site of the previous femoral pore abscess. The CBC at this time, and subsequent histopathology revealed no evidence of leukemia. There were some areas of squamous metaplasia in the pulmonary parenchyma and the mucosal lining of other organs, as well as some intestinal fibrosis, which were suggestive of postradiation changes in these organs. It is unknown if this animal would have been reproductively viable postradiation, however the treatment maintained her value to the collection as an exhibit animal.

PATHOGENICITY OF THE *Chrysosporium* ANAMORPH OF *Nannizziopsis vriesii* FOR VEILED CHAMELEONS (*Chamaeleo calyptratus*)

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Abstract

During the last decade, a poorly known fungus, the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) has been reported from several cases of cutaneous fungal disease among individual or groups of reptiles, usually with a fatal outcome.^{1,2,4} The CANV is rarely found on the skin of healthy squamate reptiles.³ We conducted a fungal challenge study using an isolate of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) originating from a chameleon to induce infection in veiled chameleons (*Chamaeleo calyptratus*) using noninvasive procedures. Chameleons were exposed to CANV spores in their environment, and were inoculated by direct cutaneous contact on abraded and nonabraded skin. The CANV induced lesions in all experimental groups, suggesting it may act as a primary fungal pathogen in this species of reptile.

This study demonstrates that the CANV may act as a primary pathogen of reptiles by direct cutaneous contact. Animals in which the skin was abraded demonstrated a higher level of infection, suggesting that skin trauma or other changes to the skin may predispose reptiles to infection. Still, the CANV can readily invade the intact skin of veiled chameleons maintained under standard husbandry. The concept of a primary fungal pathogen of squamate reptiles is new and this study firmly documents fungal infection in captive, otherwise healthy reptiles.

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A NOVEL HERPESVIRUS ASSOCIATED WITH HEPATIC NECROSIS IN A SAN ESTEBAN CHUCKWALLA (*Sauromalus varius*)

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Abstract

A San Esteban Chuckwalla (*Sauromalus varius*) died without previous signs of systemic illness. On necropsy, intranuclear inclusion bodies were seen in hepatocytes. The appearance of viral particles on electron microscopy was consistent with a herpesvirus. Degenerate PCR primers targeting a conserved region of herpesvirus DNA-dependent DNA polymerase were used to amplify products from paraffin embedded liver tissue. Nucleotide sequencing of the PCR product showed that the sequence from this lizard was novel. Comparative sequence analysis shows that this virus is a novel member of the subfamily alphaherpesvirinae, and is here termed iguanid herpesvirus 2. Sequence phylogeny has implications for both viral and host evolution.

COELOMIC ENDOSCOPY OF THE GREEN IGUANA (*Iguana iguana*): EVALUATION OF TECHNIQUE AND ORGAN VISUALIZATION

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Abstract

Endoscopic evaluation of the coelom has been recommended as a safe and important diagnostic procedure in reptiles; however, prior to this report no studies had been undertaken to statistically evaluate endoscopic evaluation of organs in any reptilian species. Bilateral coelioscopic technique was performed on 32 juvenile green iguanas (*Iguana iguana*) under general anesthesia using a 2.7-mm telescope. With the anesthetized iguana in right lateral recumbency a 3-mm skin incision was made in the paralumbar region, and the coelomic musculature was bluntly penetrated using closed hemostats. The telescope-sheath unit was introduced into the coelom and coelomic insufflation was achieved using CO₂ at 0.5 L/min to a pressure of 2-4 mm Hg. The pneumocoelom permitted examination of all major structures accessible from the left lateral approach. The levels of difficulty to enter the coelom, insufflate the coelomic cavity, and visualize the viscera were objectively scored by the endoscopists. In addition, the level of difficulty to locate and examine the heart, lung, stomach, small intestine, large intestine, spleen, liver, gall bladder, pancreas, adrenal gland, testis, epididymis, vas deferens, ovary, oviduct, kidney, bladder, and fat body were also objectively scored. Following the procedure, CO₂ was aspirated from the coelom and the skin closed with cyanoacrylic glue. The procedure was repeated for a right lateral approach.

Endoscope entry, insufflation, and general coelomic visualization were simple and straightforward. Results indicated that there was no significant difference between left and right visualization scores for lung, liver, pancreas, small intestine, large intestine, ovary, oviduct, testis, epididymis, vas deferens, bladder, fat body, or kidney. However, a left lateral approach to the heart, stomach, and spleen, and a right lateral approach to the gall bladder and adrenal gland were found to be significantly better ($P < 0.05$). In conclusion, endoscopy is advocated as a safe and effective method for the detailed examination of coelomic viscera in the green iguana.

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IMPLICATIONS OF CARBOHYDRATE FEEDING FOR CAPTIVE HERBIVORE NUTRITION AND WELFARE

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Abstract

Carbohydrates comprise the major portion of most herbivore diets. Research is limited regarding the effect of carbohydrates as provided in commercial feeds, forage, and browse on the nutrition and health of captive herbivores. However, the diversity of carbohydrates, their digestion characteristics, nutrients they supply, impacts of their physical form, and potential impact on animal health strongly recommend that they be evaluated more closely. Feed carbohydrates can be analyzed and partitioned into at least five nutritionally relevant categories:⁷ 1) organic acids from the citric acid cycle and secondary plant compounds may be used by the animal, but offer less energy to rumen microbes than do sugars; 2) sugars (mono- and oligosaccharides) and 3) starch may be digested in the small intestine by some species and offer very fermentable energy sources to rumen microbes; and 4) soluble fiber is comprised of nonstarch polysaccharides (NSP) excluding 5) neutral detergent fiber (NDF). Comprised of pectic substances, gums, and similar NSP, they can be an excellent substrate for rumen microbes, may ferment rapidly, and cannot be digested directly by the animal. Neutral detergent fiber (hemicelluloses + cellulose) serves the dual purpose of supplying fermentable carbohydrate as well as physical form which can enhance rumination, rumen function, and, potentially, rumen outflow. Analysis of a limited number of samples suggests that native browse contains little starch, and relatively greater proportions of sugars and soluble fiber.³ The carbohydrates differ in their yields of microbial protein,⁶ types of fermentation acids (e.g., acetate, propionate, butyrate), and thereby their potential effect on ruminal pH,¹¹ all of which can affect the amount and type of proteinaceous, glucogenic, and lipogenic nutrients available to the animal. The 2001 National Research Council recommendations for dairy cattle¹⁰ recognized that increased consumption of starch could lead to decreased ruminal fiber digestion and possibly to ruminal acidosis, though similar problems are less commonly reported for sugars. Ruminal dysfunction (acidosis and bloat) has been reported in captive browser species⁴ (E. S. Dierenfeld, personal communication).

Physical form of NDF is of particular concern for ruminants. While grazers readily consume and thrive on the long fiber of grass, the folivorous,² frugivorous, and woody plant-eating browsers consume particles of a more polygonal nature. The long particles in grasses enhance the formation of a floating "mat" and stratification in the rumen. Polygonal particles are hypothesized to cause a more amorphous structure of digesta in the rumen.² They have been shown to increase rate of liquid and solid passage from the rumen in cattle.^{1,9} The change in rate of passage as well as

decreased ruminal mat formation may alter digesta mixing and movement in the rumen. This may partly explain reports of decreased papillation of the dorsal surface of the rumen in captive giraffe (*Giraffa camelopardalis*).⁸ Low intake was noted for browsers fed alfalfa or grass hay, but intake was noticeably decreased with the grass hay.⁵ A better understanding must be developed regarding the roles of carbohydrate complement and physical form of the diet if we are to further improve captive herbivore health and nutrition. Combining data from domestic livestock with careful observation of the captive species should offer an excellent start.

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GIRAFFE (*Giraffa camelopardalis*) RESPONSE TO DIETARY FIBER FORM AND CARBOHYDRATE PROFILE: IMPLICATIONS FOR ANIMAL HEALTH

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Abstract

Effects of dietary physical form and carbohydrate profile were evaluated in a modified reversal study using six nonlactating adult female giraffe (*Giraffa camelopardalis reticulata*) in seven 21-day periods. Supplements, equivalent in crude protein, vitamins and minerals, were: an experimental coarse browser supplement (EF); and a mixture of 75% Mazuri Browser Breeder and 25% Omelene 200 (GF; Purina Mills, LCC, St. Louis, Missouri 63166 USA). Individually housed giraffe were fed *ad libitum* alfalfa hay, water, and supplement (EF or GF) each period. Blood collected via jugular venipuncture (day 21) was analyzed for complete blood count and chemistry profile. Observed behavior was recorded every 60 sec (days 13 through 15). Intake of individual feeds was measured days 15 through 21. The statistical model for data analysis included animal, period, and diet. When consuming EF vs. GF, animals had lowered blood glucose (82.3 vs. 99.0 mg/dl, $P = 0.028$) and blood urea nitrogen (BUN, 16.6 vs. 20.6 mg/dl, $P = 0.166$), increased total as-fed intake (1.43 vs. 1.34% body weight/day, $P = 0.128$), and a 2.29 times increase in time spent consuming supplement (277 vs. 121 min/48 hr, $P = 0.064$). Diet-related differences were small but measurable. On EF, glucose approached the range reported for domestic ruminants. Decreased BUN may reflect increased microbial capture and animal use of dietary protein, or decreased degradation of dietary or body protein. Increased eating time may increase salivary rumen buffering. Extended investigations with a larger population are warranted.

ACKNOWLEDGMENTS

We sincerely thank Busch Entertainment Corporation and the Busch Gardens management and staff for their support and assistance, which made this project possible.

COMPARISON OF PARATHYROID HORMONE, CALCIUM, PHOSPHORUS, AND MAGNESIUM IN SERUM AND URINE OF NYALA (*Tragelaphus angasi*) ON CONCENTRATES OR A FORAGE ONLY DIET

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Abstract

The nyala (*Tragelaphus angasi*) herd at Busch Gardens Tampa has historically had one of the highest incidences of Johne's disease (*Mycobacterium avium paratuberculosis*, Mptb) within the collection. A 10-yr epidemiologic review at Busch Gardens Tampa Bay revealed 27/83 (32.5%) Mptb culture positive individuals. At the time of this trial, 20/21 positive animals were also noted to be hypocalcemia. It was decided that the remainder of this herd, nine females, be euthanatized as a control measure. The herd had been maintained on ad libitum alfalfa hay and commercial zoo herbivore pellets (Mazuri® ADF #16 Herbivore, St. Louis, Missouri 63166 USA) fed at the manufactures recommended rate of 10 g/kg body weight. Water was available free choice. The herd was processed through a drop chute for baseline collection of blood and urine. Serum, EDTA, Na citrate, and heparin blood samples were collected from the jugular vein. Complete blood counts (CBC), serum biochemical profiles, ionized calcium (iCa), parathyroid hormone (PTH), and 25-hydroxycholecalciferol (25-OH CC vit D) were performed. The herd was then restricted to ad libitum access to alfalfa hay and browse for 5 wk. At the end of the 5 wk, the herd was again processed through the drop chute over 3 days and the same samples collected. Student T-test was utilized to compare the mean values of serum and urine electrolytes, serum PTH, and serum vitamin D between the two diets.

Results from the first sampling revealed various states of hypocalcemia in all animals, based on both total (range 6.2-8.1 mg/dl, reference range 8.7-11.1 mg/dl, $n = 70$ ISIS) and iCa (range 0.75-0.98 mmol/L). Based on an endocrinologist's evaluation of the iCa and PTH, 8/9 of the animals were thought to have nutritional secondary hyperthyroidism. Results from the terminal collection demonstrated that all animals had some improvement in calcium status. All animals had iCa improvement (0.98-1.17 mmol/L). Changes in mean total calcium and iCa were all higher ($P < 0.05$) between the collections. Serum phosphorus increased ($P < 0.05$) in the group from a mean of 4.2 to 6.2 mg/dl and serum magnesium also increased ($P < 0.05$) from 0.95 to 1.74 mEq/L. Mean parathyroid hormone was less ($P < 0.05$) if the one outlying animal, Number 4, was excluded. Vitamin D (25 OH CC) did not change significantly.

Low serum calcium concentrations have been noted in domestic sheep with Johne's disease. Captive ruminants may be hypocalcemic from various causes; concentrates are known to alter

calcium and phosphorus handling in ruminants and high phosphorus soils may lead to an increase in dietary phosphorus intake, leading to hypocalcemia. We believe that hypocalcemia does not result from Johne's disease and may in fact be a predisposing factor. We also suspect that the hypocalcemia seen in numerous species of zoo browsers/concentrate selectors may be a result of an imbalance of concentrate to forage ratio fed and can potentially be corrected.

HYPOMAGNESEMIA, HYPOCALCEMIA, AND RUMINITIS IN UNGULATES: AN UNDER-RECOGNIZED SYNDROME?

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Abstract

Macro- and microminerals are critical in maintaining normal physiologic processes. Requirements for domestic ungulates have been documented and are available through the National Research Council (NRC).⁴ Normal values have not been established for most wildlife species. Due to specialized feeding habits, collection of baseline data for each species is needed to develop complete diets and detect nutritional diseases.³ Several cases of hypomagnesemia in captive ungulates are described in this report.

Introduction

Formation of a new zoological collection presented a unique opportunity to evaluate animals obtained from many different sources and management conditions. Serum mineral panels were added to the routine bloodwork performed on ungulates to help determine species-specific reference ranges for this collection.

Calcium

The majority of the body's calcium (Ca) is stored in the skeleton. It plays a major role in muscle function, nerve conduction, and enzymatic processes.⁵ In general, the concentrations of Ca in roughages are highest in legumes (alfalfa) and lowest in grains. Absorption of Ca from the diet is affected by multiple factors, including the presence of oxalates, adequate concentrations of Ca and phosphorus (at a 1:1 to 2:1 ratio), and relative balance of parathyroid hormone, calcitonin, and vitamin D. Hypocalcemia may be due to an imbalance in homeostasis, rather than a dietary deficiency.⁵ Acute signs include: stiffness, tetany, weakness, decreased rumen motility, shock and death. Chronic signs of hypocalcemia include reduced feed intake, poor growth, rickets, osteomalacia, lameness, hunched stance, and pathologic fractures.

Phosphorus

Phosphorus (P) is important in skeletal structure and as a source of chemical energy (ATP). Serum concentrations are not as closely regulated as those of Ca and vary with dietary intake and renal

excretion. Concentrations in feedstuff are high in oil seeds, moderate in grains, and lowest in grasses and legumes.⁵ High P diets tend to suppress Ca absorption and predispose to the development of uroliths.

Magnesium

Seventy percent of the total body magnesium (Mg) is found in bone. It also plays a role in muscle contraction, energy metabolism, and Ca metabolism.⁵ Serum concentrations are not under close hormonal control, but vary with dietary intake and renal excretion. The availability is lower in forages than grains. Multiple factors affect absorption including serum Ca and rumen pH. Serum Mg concentrations depend on constant dietary absorption. Magnesium is primarily absorbed in the rumen, and efficiency of absorption tends to decrease with age. Clinical signs of hypomagnesemia are seen when concentrations drop below 18 ppm in cattle.¹ There may be a considerable lag between development of hypomagnesemia and appearance of clinical signs. Signs of tetany often coincide with development of acute hypocalcemia with preexisting hypomagnesemia.¹ Acute signs are observed with a rapid decline in serum Mg, such as when a ruminant is put on lush pasture ("grass staggers"). Chronic hypomagnesemia usually develops over months in response to a marginal diet.⁵ Clinical signs include: poor growth rate or body condition, nervous signs (twitching ears, kicking at abdomen, hyperexcitability, chomping jaws, frothy salivation), tetany, and seizures. Signs can be precipitated by stress, and may progress to death. Although the whole herd may be involved, usually only a few animals show clinical signs during any given period.

Role of Feed Management

Rumen acidosis can be a cause of mineral imbalances. Improper feed management can lead to subacute and chronic rumen acidosis. Decreased rumen pH is associated with intake of fermentable carbohydrates, decreased effective fiber intake, stress, and overall decreased feed intake.² Signs associated with rumen acidosis are: laminitis, poor body condition, variable feed intake, abnormal rumen motility, and changes in fecal consistency. Factors that may prevent chronic rumen acidosis include: avoiding limited access time to feed concentrates or competition for feeders; providing adequate roughage (stemmy hay); addition of buffers and fiber to the diet; providing a balanced diet; and minimizing stress.

Methods

Serum samples for mineral analyses were collected over 4 yr during routine exams (animals were either in restraint device or immobilized) and submitted to Michigan State Animal Health Diagnostic Laboratory (Lansing, Michigan 48909 USA). Most ungulates were fed a commercial pelleted diet (Mazuri ADF 16 or 25, St. Louis, Missouri 63166 USA), small amounts of produce, alfalfa and/or Bermuda grass hay, and fresh cut browse (average percentage DM: 1% pellets; 1.5-2% hay; 0.3% browse). Nutrient and mineral concentrations of feed are analyzed as part of the quality control program. Animals were fed in barns or holding areas. In addition, animals had access during the day to a habitat containing multiple species of grasses, shrubs, and trees. Although data regarding

consumption of diet fed is available, amount of habitat vegetation consumed is unknown.

Results and Discussion

Initially, results were compiled to determine this collection's reference ranges for each species. The first indication of possible clinical signs associated with hypomagnesemia occurred in an adult female kudu (*Tragelaphus strepsiceros*) in January 1999. Initially this animal presented for weight loss, rough hair coat, and retained placenta following birth of a weak calf. She later had an acute onset of muscle fasciculations, hunched posture with stiff gait, and lethargy. Values at that time showed hypocalcemia and hypomagnesemia (Ca = 4.8 mg/dl, P = 8.2 mg/dl, Mg = 12 ppm).

A second female kudu showed tetanic muscle contractions during the first trimester of pregnancy. This animal had serum Ca = 6.0 mg/dl, P = 10.0 mg/dl, and Mg = 8.06 ppm. A review of records showed that 6 mo previously this animal had a dystocia and bloodwork at that time revealed serum Ca = 7.1 mg/dl, P = 8.7 mg/dl, and Mg = 9.34 ppm.

Three adult male kudu presented with bilaterally symmetric facial/cervical alopecia and loss of body condition. Initial diagnostics showed no significant abnormalities except an inverse Ca:P ratio and hypomagnesemia. One male kudu began exhibiting a hunched posture with shifting and stretching of its hindlimbs when standing. Follow-up examination showed a progression in loss of condition, evidence of laminitis, hypocalcemia (Ca = 6.7 mg/dl), hyperphosphatemia (P = 14.6 mg/dl), and hypomagnesemia (Mg = 10.3 ppm).

A second male kudu was immobilized for examination. Although the anesthetic procedure was uneventful, approximately 3.5 hr after reversal, this kudu was observed to suddenly stiffen and fall into lateral recumbency with muscle fasciculations. The animal's condition deteriorated rapidly despite supportive care, and it died within 20 min of initial signs. Bloodwork revealed severe hypocalcemia (Ca = 4.5 mg/dl), hyperphosphatemia (P = 13.5 mg/dl), and hypomagnesemia (Mg = 7.75 ppm). Necropsy findings included evidence of chronic/active rumenitis and laminitis.

The third, less severely affected, male kudu was immobilized to further investigate the syndrome. Laboratory results were similar to the other cases (Ca = 6.8 mg/dl, P = 12.0 mg/dl, Mg = 9 ppm). Due to evidence of a herd problem, diet changes were implemented (addition of chelated minerals (4-Plex, Zinpro, Eden Prairie, MN), buffers (sodium bicarbonate, magnesium oxide), and increased effective fiber (white oak, soy hulls, citrus pulp). Six months after the diet change, this kudu was immobilized for a follow-up exam. General body condition and hoof and hair growth had improved. However, despite an uneventful anesthetic procedure, the animal developed stiff gait, hunched posture, and muscle fasciculations the following day. Results from the initial immobilization showed hypocalcemia (Ca = 6.4 mg/dl), hyperphosphatemia (P = 13.6 mg/dl), and hypomagnesemia (Mg = 7 ppm). This animal was treated with 23% calcium gluconate (250 ml s.c., 125 ml i.v.; Phoenix Scientific Inc., St. Joseph, MO 64506), 50% magnesium sulfate (2 ml i.v., 35 ml s.c.; American Regent Laboratories Inc., Shirley, NY 11967), and i.v. fluids (25 L LRS, 375 ml 5% dextrose in LRS). The animal's condition continued to deteriorate and it was euthanatized.

Necropsy findings confirmed chronic rumenitis.

Although the kudu herd appeared to be most severely affected, other species also were at risk based on review of medical records. Several eland (*Taurotragus oryx*) had developed evidence of laminitis and one showed facial tremors and stiffness postimmobilization. This individual had serum Ca = 8.9 mg/dl, P = 9.2 mg/dl, and Mg = 13 ppm. Other affected eland typically had magnesium values in the range of 12-16 ppm, often associated with inverse Ca:P ratios. Nyala (*Tragelaphus angasii*) also appeared likely to develop clinical problems associated with mineral imbalances. An adult male nyala developed facial and flank alopecia. Serum minerals were Mg = 12.1 ppm, Ca = 6.9 mg/dl, and P = 8.9 mg/dl. An additional immobilization occurred 6 mo later. The procedure was uneventful, however the animal developed signs of shifting leg lameness and hypermetria the afternoon of the immobilization. These signs rapidly progressed to recumbency, seizures, and death. Lab results (Ca = 7.1 mg/dl, P = 9.8 mg/dl, Mg = 8 ppm) and clinical signs were consistent with hypomagnesemia. Chronic rumenitis was found at necropsy.

A compilation of mineral results for various ungulates was made and species grouped as grazers, browsers, or intermediate feeders. In most of the grazers, ranges and mean values for serum Mg fell in expected ranges (Table 1). Occasional low values were seen in individual gemsbok (*Oryx gazella gazella*) and scimitar-horned oryx (*Oryx dammah*). Among the browsers, kudu and nyala herd mean Mg values were lower than the other species (Table 2). Bongo (*Tragelaphus eurycerus*) may also be a potential concern with slightly lower mean Mg values. Eland appear to be at risk among the intermediate feeders (Table 3). The eland herd's mean value was also lower than other species.

It is speculated that feeding concentrates may play a role in the development of rumen acidosis/chronic rumenitis and subsequently affect Ca:P balance and Mg absorption. Addition of buffers and chelated minerals may ameliorate some of these changes, however, the primary underlying etiology is still incompletely understood. Preliminary data suggest that Ca:P balance and Mg concentrations may be improved if treated early.

Mineral imbalance may result from inappropriate diets, concentrate feeding resulting in alterations in rumen pH, and concurrent disease. Measurement of trace minerals in exotic ungulates may be an underutilized diagnostic tool in assessing herd health. Clinical signs of alopecia, loss of body condition, lameness, muscle fasciculations, and seizures have been observed with hypocalcemia concurrent with hypomagnesemia in kudu, nyala, and eland. Evaluation of diet and feed management, along with serum mineral concentrations, should be performed to allow veterinarians and nutritionists the ability to recognize, treat, and prevent mineral imbalances leading to clinical signs.

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Table 1. Selected mineral values in grazer species at Disney's Animal Kingdom.

Grazer species	Ca (ppm)	P (ppm)	Mg (ppm)		SD	n
			range	mean		
Goat ^a (<i>Capra hircus</i>)	90-116	37-97	21-29			
Cattle ^a (<i>Bos taurus</i>)	85-110	45-80	20-35			
Ankole (<i>Bos taurus</i>)	91-105	76-109	18-24	20.6	1.9	8
Banteng (<i>Bos javanicus</i>)	87-119	65-114	17-27	21.7	2.7	10
Gemsbok (<i>Oryx gazella gazella</i>)	71-119	43-124	13-26	19.2	2.9	19
Scimitar horned oryx (<i>Oryx dammah</i>)	69-113	59-124	12-26	20.6	3.5	29
Sable antelope (<i>Hippotragus niger</i>)	73-113	40-104	15-25	21.3	2.4	24
Wildebeest (<i>Connochaetes taurinus</i>)	91-109	41-124	18-25	19.9	2.4	11

^aLaboratory reference ranges.

Table 2. Selected mineral values in browser species at Disney's Animal Kingdom.

Browser species	Ca (ppm)	P (ppm)	Mg (ppm)		SD	n
			range	mean		
Elk ^a (<i>Cervus elaphus</i>)	68-108	50-93	17-28			
Blesbok (<i>Damaliscus pygargus phillipsi</i>)	82-111	74-121	17-26	22.6	2.7	14
Bongo(<i>Tragelaphus eurycerus</i>)	75-90	45-106	15-23	18.6	2.5	10
Axis deer (<i>Axis axis</i>)	82-96	30-146	15-22	19.5	2.7	7
Elds deer (<i>Cervus eldii</i>)	85-119	54-112	17-25	22.9	2.4	10
Gerenuk (<i>Litocranius walleri</i>)	87-124	23-155	17-33	23.8	5.1	17
Giraffe (<i>Giraffa camelopardalis</i>)	78-97	54-119	20-25	22.9	2.1	4
Kudu (<i>Tragelaphus strepsiceros</i>)	46-109	58-172	8-25	15	5	26
Nyala (<i>Tragelaphus angasii</i>)	70-103	46-164	9-27	15.1	5.4	15

^aLaboratory reference ranges.**Table 3.** Selected mineral values in intermediate feeder species at Disney's Animal Kingdom.

Intermediate feeder species	Ca (ppm)	P (ppm)	Mg (ppm)		SD	n
			range	mean		
Blackbuck (<i>Antilope cervicapra</i>)	81-107	49-200	19-42	28.8	5.4	37
Mhorr gazelle (<i>Gazella dama mhorr</i>)	76-106	59-123	16-30	21	3.9	12
Waterbuck (<i>Korbus ellipsiprymnus</i>)	100-123	56-80	14-24	19.3	4	5
Eland (<i>Taurotragus oryx</i>)	90-109	38-111	14-21	17	1.9	13
Thomson gazelle (<i>Gazella thomsonii</i>)	84-117	44-169	19-35	26.8	3.7	20
Impala (<i>Aepyceros melampus</i>)	81-106	45-118	13-26	19.6	3.6	22

SUSPECTED VITAMIN D₃ TOXICITY IN A GROUP OF BLACK RHINOCEROS (*Diceros bicornis*)

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Abstract

Three black rhinoceros (two males, one female, *Diceros bicornis*) in a group of 10 black rhinoceros, (two male and two female long-term wild-caught adults and six male captive-born juveniles) began to show hypercalcemia and partial anorexia over a 5-mo time frame. These signs were more pronounced in the adult rhinoceros, serum sampling in the juveniles were inconsistent due to behavioural conditioning problems. During this period the three black rhinoceros died. Clinical signs were similar in all three: weight loss (15-20%), weakness, stiffness in hind legs, hypercalcemia (13-18 mg/dl, normal 12.4 mg/dl), and normophosphotemia. All three rhinoceros that died had elevated BUN and creatinine (near death, within 24 hr). These three rhinoceros were unable to stand with progressive weakness starting in the hind quarters and moving forward. Necropsy on the two adult rhinoceros revealed a varying degree of mineralization of the major vessels, heart valves, heart muscle, stomach, lungs, and kidneys consistent with vitamin D₃ toxicity.² The youngest rhinoceros (3-yr-old) did not have gross mineralization of soft tissue and had no other abnormalities. Shortly after the first death, vitamin D₃ concentrations were determined on tissue, feed (two pelleted diets), and serum samples. In the original pelleted diet A, vitamin D₃ was formulated at 250 IU/kg of feed, however follow-up routine testing for vitamin D₃ is not standard. A total of four pelleted lots were tested, two during the crisis and two after the crisis. Rhinoceros are fed approximately 8-9 kg of pelleted diet A, two flakes of alfalfa hay, four flakes of timothy hay, two apples, two carrots, 6.8 kg of browse and 30 ml of vitamin E oil daily in two feedings. Most food was eaten with the exception of 20-30% of timothy hay. Pelleted diet B was originally fed and was used as a comparison. Other portions of the diet were not tested as samples of the original hay and browse were not available.

Concentrations of vitamin D₃ in pelleted diet A were 9500 IU/kg, and pelleted diet B 3282 IU/kg feed (as-fed basis). Toxic concentrations in horses are above 2200 IU/kg of feed and recommended feeding concentrations are 500 IU/kg of feed.³ Serum samples from the first two rhinoceros that died were submitted and 25-hydroxy vitamin D₃ concentrations were mildly elevated, 138 nmol/L and 203 nmol/L, when compared to concentrations of 25-hydroxy vitamin D₃ in wild black rhinoceros at 139 nmol/L.¹ Tissue concentrations of 25-hydroxy vitamin D₃ of the liver of the first rhinoceros to die were elevated at 515 ng/g (normal in sheep 3.4-10 ng/g)² and the second and third rhinoceros to die had normal liver concentrations of 16 and 14 nmol/L, respectively. Additional serum and tissue samples from a female black rhinoceros that died 1 yr previously were submitted for analysis. This female rhinoceros also had soft tissue mineralization of the major vessels, stomach and lungs. Additionally the female was normocalcemic, had an elevated serum 25-hydroxy vitamin D₃ of 544 nmol/L, and normal liver concentration of 25-hydroxy vitamin D₃ of 35 nmol/L. Normal

concentrations of 25-hydroxy vitamin D₃ in black rhinoceros tissue have not been established, however this concentration appears normal when compared to concentrations in domestic animals.² Additional samples of serum 25-hydroxy vitamin D₃ in other black rhinoceros and white rhinoceros (*Ceratotherium simum*) were elevated concurrently at the same time that the feed concentrations of 25-hydroxy vitamin D₃ were elevated. Once the same pelleted diet was obtained from a different feed mill, vitamin D₃ concentrations have decreased and clinical signs are now abating.

It is unclear if the vitamin D₃ was toxic enough to cause death, however clinical signs of vitamin D₃ toxicity and elevated serum calcium concentration and soft-tissue mineralization were present. Weight loss may have been exacerbated by two factors: removing the pelleted feed from the diet for a period of 1 mo, and unusually cold weather (many nights below freezing). It is recommended that both feed and serum concentrations of 25-hydroxy vitamin D₃ are tested on a routine basis in addition to serum parathyroid hormone (PTH), calcium and phosphorus (Table 1).

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Table 1. Information for feed and serum testing.

Conversion Factor

From ng/ml to nmol/L: $\text{ng/ml} \times 2.496 = \text{nmol/L}$

Tissue and Serum Samples

Animal Health Diagnostic Laboratory

629 West Fee Hall B

Michigan State University

East Lansing, MI

48824-1315

(517)355-0281 (tissue samples)

(517)353-0621 (serum samples)

Feed Samples

Woodson-Tenent Laboratories, Inc.

PO Box 1292

3507 Delaware Ave

Des Moines, IA

50305

(515)265-1461

IMPROVEMENT OF MALE FERTILITY BY LONG-TERM BETA-CAROTENE SUPPLEMENTATION IN WHITE RHINOCEROSSES (*Ceratotherium simum*)

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Abstract

Substantial knowledge on the reproductive physiology of the female white rhinoceros (*Ceratotherium simum*) has been gathered over the past years. However, little emphasis has been put on the evaluation of male fertility as a possible contributing factor to the demographic crisis of the captive population considering that 81% of male captive white rhinoceros have not sired offspring. Objective of this study was the reproductive assessment of male white rhinoceros to determine their semen characteristics. Ultrasound and electroejaculation were conducted in 20 anesthetized white rhinoceros, which had not sired offspring. Electroejaculation represented a semen collection method, which allowed a repeatable semen assessment applicable to the different management systems and training standards. Semen quality was evaluated on the basis of one to four semen samples collected during this 4-yr study. Fifty percent of the males examined showed suboptimal and inconsistent semen characteristics. Social stress and vitamin A insufficiency were discussed as potential causes for the reduced semen quality. Beta-carotene supplementation used in the stallion and boar has shown to improve semen characteristics. In a preliminary study, one single-housed individual was supplemented with 1,200 mg/day beta-carotene over period of 14 mo. Beta-carotene improved sperm motility from presupplementary 50-80% after supplementation. From the large proportion of intermittent and infertile males assessed in this study, increased efforts should be made to improve breeding fitness of nonrepresented male white rhinoceros. Dietary beta-carotene supplementation may represent an easy-to-apply measure to improve spermatogenesis and fertility in the white rhinoceros.

CONSERVATION MEDICINE: THE SCIENCE AND PRACTICE OF ECOLOGICAL HEALTH

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Abstract

In recent years, the term conservation medicine has been used in several contexts within different scientific communities, national/international organizations and research groups. Last year the book *Conservation Medicine: Ecological Health in Practice* was published in an attempt to define a new discipline that links human and animal health with ecosystem health and global environmental change.¹ This novel approach in the protection of biologic diversity challenged scientists and practitioners in the health, natural and social sciences to think about new, collaborative ways to address ecological health concerns in this deteriorating world. The objectives of this presentation are to concisely overview and update the concepts related to conservation medicine outlined in that book.

Conservation medicine is a transdisciplinary scientific field devoted to understanding the interactions among: (1) human-induced and natural changes in climate and habitat structure; (2) emergence of pathogens, parasites, and pollutants; (3) biodiversity and health within animal communities; and (4) health of humans. Conservation medicine has both scientific and applied elements, and many endeavors in this field combine aspects of both. Simply stated, conservation medicine is the science and practice of ecological health and is especially relevant in today's human-modified landscapes, where habitat destruction and degradation and episodes of emerging human and wildlife diseases are increasing. In this context, ecological health is at the nexus of the fields of human health, animal health and ecosystem health. Conservation medicine embraces participation by scientists and practitioners in the fields of ecology; organismal, cellular, molecular, and conservation biology; toxicology; epidemiology; veterinary and human medicine; and public health. In addition, perspectives from the social and political sciences are fundamental in understanding the underlying causes of human-induced changes in climate, habitat, and the use of terrestrial and marine ecosystems. Although conservation medicine is a scientific discipline, it may provide the basis for political positions on the conservation and management of species and ecosystems. The hope is that once armed with the appropriate knowledge, public policymakers and scientists will proactively devise and implement epidemiologic strategies to better ensure ecological health.

Conceptually, conservation medicine is at the nexus of the fields of human health, animal health and ecosystem health. And yet, it is more than interdisciplinary because it represents an integration of knowledge between disciplines. The term transdisciplinary is being advanced to describe this intellectual trend.² Conservation medicine requires professionals from diverse disciplines to work

together in addressing the complex aspects of the intersection of health and the environment. In essence, conservation medicine is practiced through collaborations beyond the individual, the institutional, and the interdisciplinary levels.

In comparison to human and veterinary medicine, conservation medicine addresses the examination of ecological health concerns beyond the species-specific approach. Clearly, in some respects, human health holds a greater priority than the health of other species. To date, human medicine has been limited in looking at the health connections between species and the environment (i.e., ecosystem health). Human health intervention has been focused on the downstream effects of environmental impacts (e.g., health consequences of landscape changes or pollution emissions) rather than encompassing a preventive approach in looking at upstream events (e.g., prevention of massive ecological degradation or pollution prevention).^{1,3}

There are no simple solutions to address global environmental problems. A multi-pronged strategy is required. By bringing disciplines together, conservation medicine can contribute to solving environmental problems by improving problem definition requiring new tools for assessing and monitoring ecological health concerns. Perhaps, incorporating aspects of environmental indicator studies with specific biomedical diagnostic tools can develop integrated ecological health assessment. Tools include the development of noninvasive physiologic and behavioral monitoring techniques; the adaptation of modern molecular biologic and biomedical techniques; the design of population level disease monitoring strategies; the creation of ecosystem-based health and sentinel species surveillance approaches; and the adaptation of health monitoring systems for appropriate developing country situations. Beyond monitoring and assessment is action. Improving medical and ecological education and the need for public policy development in promoting ecological health are fundamental goals of conservation medicine.¹

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WEAPONS OF MARSH DESTRUCTION: VETERINARY INVOLVEMENT IN EVALUATING THE IMPACT OF NUTRIA (*Myocastor coypus*) ON THE UNIQUE FLOTANT MARSH ECOSYSTEM IN SOUTHERN LOUISIANA

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Abstract

The nutria (*Myocastor coypus*) is a large aquatic rodent endemic to the Patagonian subregion of South America. It has been widely introduced around the world for the fur industry.² In most places, this invasive species has been very successful, even to the extent that population control measures have been instituted.^{1,6,8}

The nutria has a voracious appetite, eating 25% of its body weight per day.⁵ additionally, it is a destructive forager often uprooting plants and consumes preferred parts while the rest of the vegetative biomass is discarded.³ this kills the plants, resulting in habitat loss.⁹

In coastal Louisiana, this rodent is considered a nuisance in part due to its detrimental ecological impact on the unique floatant marsh habitat found in southern Louisiana. This type of freshwater tidal floating marsh is only found in delta systems of the Mississippi and Danube rivers.¹¹ the overall ecological impact of nutria on coastal wetlands is so severe that the state of Louisiana initiated a bounty on nutria in the fall of 2002 (D. Bounds, personal communication, 2003).

No study to date has ever investigated the habitat selection of nutria on the Louisiana floatant marsh. This investigative study explores how this rodent utilizes and impacts its nonnative plant community over the course of 1 yr at the Barataria unit of Jean Lafitte National Historical Park and Preserve (Jean Lafitte Park), Jefferson Parish, Louisiana USA.

Radiotelemetry is invaluable in this study because it allows the tracking of individual animals not only during the winter, but also during the summer when the dense vegetation does not normally allow for direct observation. This will facilitate the evaluation of the nutria's seasonal impact.

Implantable transmitters were chosen primarily due to the high morbidity and mortality associated with using radio tracking collars on nutria.⁴ Abdominal radio transmitters have been used successfully for tracking nutria in Maryland (D. Bounds, personal communication, 2003) and other aquatic mammals.^{7,10}

As part of a pilot study, 13 nutria were implanted with transmitters. Animals were captured in Salvadore Wildlife Management Area about 6-7 aquatic miles from Jean Lafitte Park due to the ease of capture at this locale. These nutria were immobilized once with tiletamine-zolazepam (telazol®, Ft. Dodge Animal Health, Ft. Dodge, IA 50501 USA, i.m., 100 mg/ml, 10.0 mg/kg, range of 7.8 - 11.7 mg/kg) by hand-injection with the aid of a 3-ft animal control pole (Tomahawk®, Ben Meadows Company, PO Box 5277, Janesville, WI 53547-5277 USA). Surgeries were performed in the wet lab of the education building. Radiotransmitters were placed into the abdomen of nutria using a standard ventral midline approach. Mean duration for the surgical implantation was 38 min, and no surgery exceeded 60 min. No mortalities were associated with the surgical procedure. The animals were left in pens to recover overnight and were subsequently released into the marsh of Jean Lafitte Park.

None of the pilot animals suffered weight loss or observable behavioral changes due to the presence of the implants when the transmitters were retrieved up to 3 mo later. Preliminary results show that female nutria can conceive a pregnancy and sustain a pregnancy to full term if implanted during mid-gestation.

Although in its nascent stage, it is hoped this study will elucidate the feeding habits and preferences of nutria in the flotant marsh in southern Louisiana. This in turn will help direct more focused control efforts of the nutria population over all seasons. Preliminary results demonstrate that tiletamine-zolazepam anesthesia is an excellent method for the handling and surgical implantation of radiotransmitters into the abdomen of nutria. It also shows that this method of implantation has low morbidity and mortality and has a minimal effect on nutria behavior.

ACKNOWLEDGMENTS

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ENDANGERED SPECIES RECOVERY PLANS: CONSIDERING PARASITE CONSERVATION

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Abstract

The extinction of host-specific parasites occurs with the extinction of their hosts—two species of host-specific lice were lost with extinction of the passenger pigeon (*Ectopistes migratorius*).³ When a host is either rare or removed from its ecosystem, parasite transmission may diminish to a level that results in the extinction of host-specific parasites. Two previously recorded species of parasites (*Neotrichodectes* mite and eimerian protozoa), possibly host-specific, are no longer observed in the endangered black-footed ferret (*Mustela nigripes*) population.² The ethical and moral arguments used to justify the conservation of individual host species also apply to their co-evolved parasites.^{2,6} Utilitarian justifications for parasite conservation include: 1) the loss of one parasite can alter the competitive interactions of remaining parasite species, 2) parasitism in captivity may maintain the integrity of host immunity to pathogens still present in wild populations, and 3) the potential medicinal and biomedical research value of parasites.^{1,2,6}

The United States Fish and Wildlife Service's consideration of parasites was evaluated by examination of the Threatened and Endangered Species System's Recovery Plans. All Recovery Plans for mammals available online (www.fws.gov) in March 2003 were reviewed for their mention of parasites. Seventy-six mammals were listed as either threatened or endangered. Twenty-eight Recovery Plans representing 37 endangered and four threatened mammals were reviewed. For the purpose of this analysis, parasites were defined as the resident fauna of a host animal without implying that pathologic changes result from the association. Only metazoan and protozoan parasites were included.

None of the 28 Recovery Plans specified actions to conserve parasites. Unique or species-specific parasites were discussed in two of the 28 (7.1%) Recovery Plans—the Point Arena mountain beaver (*Aplodontia rufa nigra*)⁵ and the Mexican long-nosed bat (*Leptonycteris nivalis*).⁴ Although the Recovery Plan for the Point Arena mountain beaver indicates awareness that mountain beavers are host to several host-specific mites and the largest known species of flea (*Hystricopsylla schefferi*), the parasite community of the Point Arena subspecies has not been investigated.⁵ The Mexican long-nosed bat (*Leptonycteris nivalis*) hosts a macronyssid mite that resides in the tissue surrounding the molar roots—the characteristic damage to the bone caused by the mites has been used to distinguish this bat species from others in the genus.⁴ Two of 28 (7.1%) plans listed, by either species or family name, three or more parasites found in the host. Thirteen of 28 (46.4%) plans

either mentioned one or two specific parasitic diseases, or made a nonspecific reference to parasitism. Eleven of 28 (39.3%) plans made no reference to parasites as they affect either individuals or populations.

It is unlikely that government programs will be initiated to conserve parasites because of economic obstacles and/or negative public perceptions.^{2,6} Therefore, veterinarians and other ecological health professionals must be cognizant of the issue, as they are among those individuals most likely to determine the fate of parasites in a host. Suggested means of parasite conservation include a survey for host-specific parasites prior to implementing single-species conservation programs, use of alternative hosts to maintain the parasite population, establish programs that conserve the host-parasite interaction, and emphasize community rather than species-level conservation programs.² Resources permitting, all host-specific biologic entities, including bacteria and viruses, should be given similar considerations.

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MAPPING AND MODELING WILDLIFE AND HUMAN DISEASE OVER AN ECOLOGICAL LANDSCAPE

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Abstract

The Yellowstone to Yukon Conservation Medicine Program is a conservation-based research initiative designed to investigate issues related to wildlife disease throughout a region spanning from the greater Yellowstone Ecosystem to the MacKenzie Mountains of the Yukon Territories. The Yellowstone to Yukon (Y2Y) Wildlife Disease Mapping project is an innovative initiative to bring wildlife and ecosystem health into the conservation arena by documenting the distribution of important diseases, training future veterinary and human health professions in the concepts of conservation-based medicine, and using project information in the formulation of management decisions. Our program is dedicated to exploring methods of conserving health in wildlife populations and the habitats they reside in through synthesis of existing disease information via agency, university and international collaborations. We are using this information to model disease transmission via habitat corridors as they are planned and designed for the Yellowstone to Yukon Conservation Initiative (Fig. 1). Habitat design scenarios providing the least negative effect to current land use activities can be planned using present and past disease information and related and disparate variables. Using geographic information system mapping technology, we have mapped important wildlife diseases including brucellosis (Fig. 2), bovine tuberculosis, chronic wasting disease, sylvatic plague, Hantavirus infection, anthrax, tularemia, and rabies. Corresponding human infection is also mapped. The information is presented to the public and agency users via an electronic information system called the National Biological Information Infrastructure Wildlife Disease Node (<http://wildlivedisease.nbii.gov/>). Predictive analyses in this study are used as tools in habitat corridor design. Within our program we are also placing veterinary, medical, and conservation biology students in exciting new experiential learning opportunities involving globally important emerging diseases including using wildlife as sentinels for emerging disease and projects involving anthrax, plague among others.

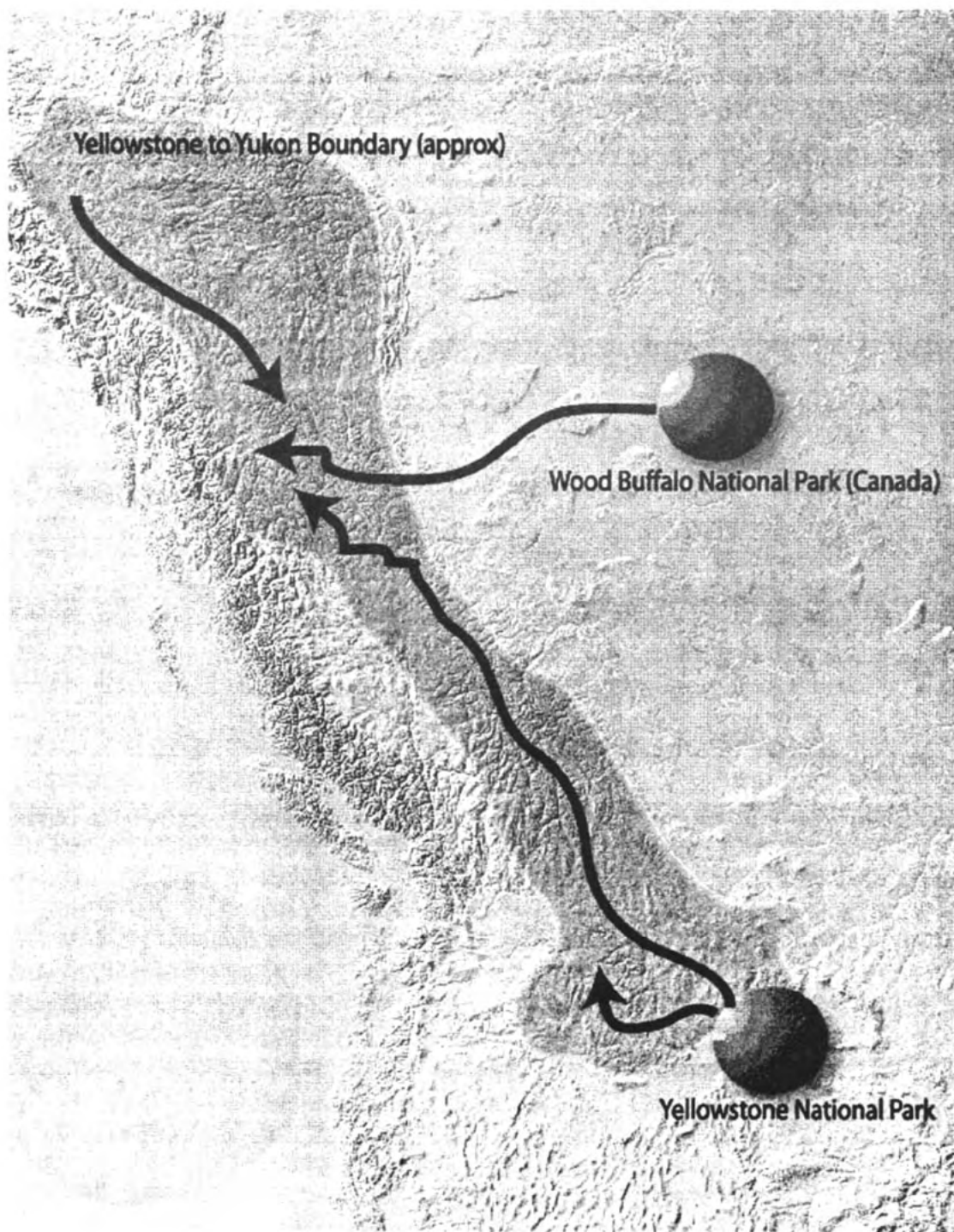


Figure 1. Hypothetical wildlife disease transmission pathways within and outside the Yellowstone to Yukon bioregion.

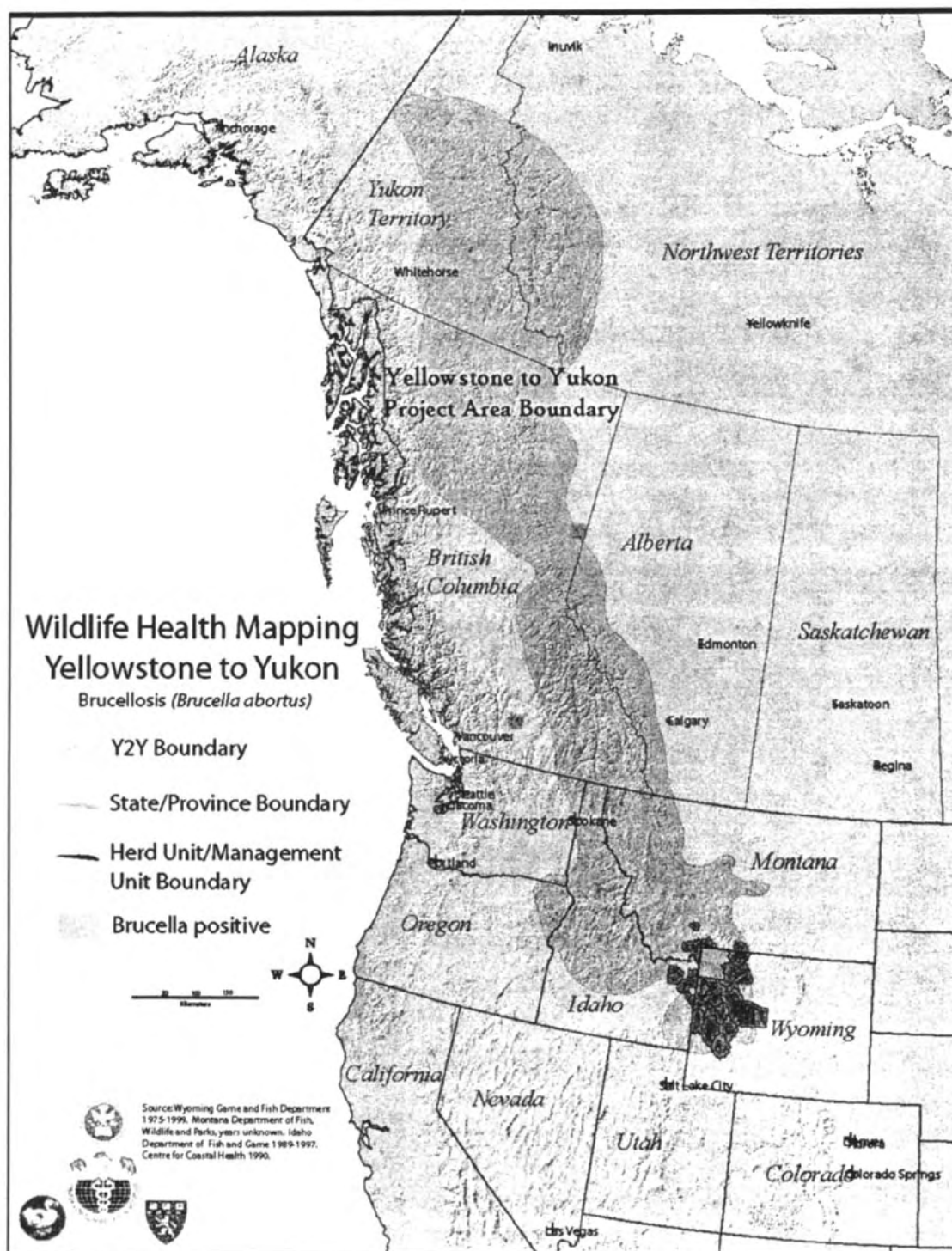


Figure 2. Areas testing positive for brucellosis by elk and bison within the Yellowstone to Yukon bioregion 1970-2002.

THE ZOO VETERINARIAN'S ROLE IN DEVELOPING CONSERVATION MEDICINE PARTNERSHIPS

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Abstract

The new discipline of conservation medicine involves the study of the relationship among animal health, human health and the environment. Emerging infectious diseases, ecosystem health and the role of disease in wildlife population biology are all important aspects of this field of study. The study and application of conservation medicine requires the work of veterinarians, physicians and biologists in a variety of disciplines. Historically there has not been a strong relationship among experts in these different areas. Zoo veterinarians are an exception to this rule. In order to meet the challenge associated with the wide variety of species in our care, zoo veterinarians must apply knowledge from veterinary science, human medicine and wildlife biology. The increasing emphasis on immersion exhibits in park-like settings exposes zoo animals to free-ranging wildlife. The resident wildlife, as well as the millions of human visitors and staff provide many opportunities for the movement of emerging diseases to the animal collection. Studies of the biology of animals in nature must be incorporated into captive husbandry practices in order to maintain animal health.

In-situ conservation efforts by many zoos are supported or lead by veterinary staff. Zoo veterinarians must form partnerships with a range of experts in order to practice effectively. Multidisciplinary teams must be formed to address complex medical issues. Zoo veterinarians are skilled in gathering information from disparate sources as well as team building and problem solving. These are the requisite skills for the field of conservation medicine.

The Conservation Medicine Center of Chicago (CMCC) represents a focusing of existing partnerships to meet the challenges of this new discipline. These collaborations evolved into an education and research center. Capitalizing on strong links that already existed among the Brookfield Zoo, the University of Illinois College of Veterinary Medicine and the Loyola University Stritch School of Medicine, the CMCC was formed in 1999. The CMCC has endeavored to foster an exchange of expertise and enhanced collaboration to address conservation medicine issues. The CMCC consists of research and education committees under the direction of a steering committee. The research committee funded new studies as well as additional components of ongoing studies. New research included investigations of the molecular genetic ecology of a Midwest focus of lyme disease, immunology of *Callimico goeldii*, anthropogenic factors affecting the emergence of largemouth bass virus and the molecular characterization of canine distemper virus isolates from free-ranging raccoons. Additions to ongoing research efforts included multidisciplinary health assessments of felids in Namibia and effects of organochlorine contaminants on bottlenose dolphin

health and reproduction. The CMCC has also provided direction and research project approval for a unique program of wildlife disease research funded locally by the Cook County Department of Animal and Rabies Control. These projects have included the development of a model for the spread of raccoon rabies, zoonotic diseases and their relationship to local deer ecology and study of urban coyote biology. The CMCC also helped, through a federally funded grant to develop a molecular diagnostic laboratory. The education committee has developed a course in conservation medicine that is offered on three different university campuses through the use of teleconferencing facilities. The primary challenge of the CMCC has been to develop programs that do not compete with efforts of the partner institutions that may also further the goals of conservation medicine. The intent is to serve as a catalyst to research and education programs that would not have received support in any other way.

There is a clear and growing recognition that science needs to address issues of health from a broader perspective. The role of wildlife and the environment in human public health are being discussed in the scientific and popular press. This attention can serve to benefit, or hinder wildlife and ecosystem conservation. In addition to veterinary staff, zoos must possess expertise in education, development and government affairs. This expertise is vital to the formation of programs addressing conservation medicine. Zoos can formalize partnerships to expand the field of conservation medicine. The CMCC serves as an example of how this can be accomplished without creating a completely independent organization.

THE ROLE OF ZOOS IN ENDOCRINE DISRUPTION STUDIES: A CASE MODEL

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Abstract

A well managed zoo represents a closed community with controlled animal movements. The variety of Taxa gathered in one place provide a unique opportunity to study the effects of environmental agents on a great number of species. Environmental agents with adverse effects on humans also have similar adverse effects in a variety of other species. Endocrine disrupting chemicals are environmental agents that work by mimicking or blocking the hormone signals within the endocrine system. Serum samples obtained from the Audubon Zoo's reptiles, birds, and mammals have been assayed utilizing a strain of *Sacharomyces cerevisiae* yeast that, in turn, has been transformed to express wild type human estrogen receptor (hER) and a reporter plasmid with two estrogen response elements (ERE) linked to a lacZ gene. Incubation of the transformed yeast with a positive control, 17 β -Estradiol and appropriate β -galactosidase substrate (ONPG) has resulted in a chromogenic change that is quantified via spectrophotometry at A420. This system has been utilized to detect changes in lacZ reporter activity by incubating the transformed yeast with 17 β -Estradiol in the presence of the zoo serum. Further studies are required to more clearly elucidate the degree and pattern of these changes.

Introduction

Modern open plan zoos with naturalistic environments, water systems, grasslands, and abundant flora provide extremely genetically diverse, compact, and complex ecosystems that can be comprehensively monitored.¹ A well managed zoo represents a closed community with controlled animal movements.¹ The variety of species represented in a large zoological collection run the gamut of major Orders observed in numerous and diverse habitats. The variety of Taxa gathered in one place provide a unique opportunity to study the effects of environmental agents on a great number of species. A uniform effect in several species, many of which have short life cycles, would have considerable implications for the study of these agents.

Methods

Serum samples taken from apparently healthy mammals, birds, and reptiles during annual physical exams are stored at -70 °C. Volumes of samples are varied, but generally consist of a minimum

volume of 0.25 ml. Frozen samples collected from 1997 to date are currently being analyzed. Each sample is tested for the presence of endocrine disrupting chemicals.

Serum samples from various species at the Audubon Zoo have been assayed utilizing a strain of *Sacharomyces cerevisiae* yeast that, in turn, have been transformed to express wild type human estrogen receptor (hER) and a reporter plasmid with two estrogen response elements (ERE) linked to a lacZ gene. Incubation of the transformed yeast with a positive control, 17 β -Estradiol and appropriate β -galactosidase substrate (ONPG) has resulted in a chromogenic change that is quantified via spectrophotometry at A420. This system has been utilized to detect changes in lacZ reporter activity by incubating the transformed yeast with 17 β -Estradiol in the presence of the zoo serum.

Results

Preliminary results suggest that measurable estrogenic activity in the YES system was decreased due to competition by serum proteins for available ligand (estradiol) in the system. Incubation of estradiol in the presence of zoo animal serum variably reduced the amount of estrogen available to bind to the estrogen receptors. There also appears to be a correlation between the diets consumed by these species and the degree of decrease in reporter activity in this system. These data revealed decreases in lacZ reporter activity anywhere from 5-60% of normal activity levels. Further studies are required to more clearly elucidate the degree and pattern of these changes.

Discussion

The study of endocrine disrupting chemicals is a relatively recent but rapidly growing field. For many years the subject of environmental health has been focused on the health of human beings. More and more we are finding that environmental agents with adverse effects on humans also have similar adverse effects in a variety of other species. Endocrine disrupting chemicals are environmental agents that work by mimicking or blocking the hormone signals within the endocrine system. Recent publications provide extensive reviews on the matter.⁵

Experiments in the laboratory, and in both the field and clinic, have shown that numerous compounds of diverse chemical structure can function as estrogens or antiestrogens.⁵ The molecule estradiol-17 β is the natural steroidal estrogen secreted by the ovaries of most vertebrates studied, and interacts with a conserved protein called the estrogen receptor (ER). In addition, plants such as soybeans also produce estrogenic molecules or isoflavones, which can interact with the vertebrate estrogen receptor and induce an estrogenic response in wildlife, livestock and humans.⁵ Another class of estrogenic environmental chemicals of natural origin are aflatoxins secreted by *Fusarium* sp. These aflatoxins are responsible for the "moldy corn syndrome," or hyperestrogenization of pigs. There are, therefore, at least three natural sources of estrogenic chemicals: ovarian steroids, phytoestrogens, and mycotoxin estrogens.⁵

Other sources of estrogenic environmental chemicals include industrial pollutants such as bis-phenol A, polychlorinated biphenyls (PCBs), and dichlorodiphenyldichloroethylene (DDT); a plasticizer, a coolant, and a pesticide, respectively. These ubiquitous and high volume contaminants are found in most ecosystems and have been measured in various tissues of humans,⁵ seals,⁵ and even alligator eggs^{2,4} throughout the world. Thus, most species exist in contact with a variety of estrogenic sources in addition to their own.

In laboratory experiments, fish, bird and reptile eggs have been treated with estrogenic chemicals with the resultant appearance of sex reversal in genetically male individuals.⁴ Likewise, laboratory rodents have been feminized by in utero treatment with estrogenic environmental chemicals.⁵ In acute studies in adult animals, experiments detect direct estrogenic effects in a variety of animals such as uterine growth in mammals and vitellogenin production in male nonmammalian vertebrates. Thus, the entire vertebrate kingdom responds to a number of estrogens in the environment with both acute and long-term effects.⁵

At another level of organization, reproductive anomalies associated with possible environmental estrogen exposure have been reported in many wildlife species including cases of intersexed fish,⁶ malformations of external genitalia in alligators,^{2,3} reproductive impairment in fish-eating birds,⁶ reproductive failure in seals,⁶ and pseudohermaphroditism in polar bears.⁷ Because many environmental estrogens enter the environment through agricultural runoff and industrial effluent, it is not surprising that most examples of affected wildlife either live in the water or eat aquatic organisms.^{5,6}

A critical missing link between experiments with laboratory species and observations in the wild is the ability to pose hypotheses regarding the environment and reproduction in multiple species of wildlife in relatively controlled conditions. That missing link can be found, in part, in the experimental zoo. It is here that the academic laboratory/field work can be applied in conditions that permit replication and sampling that are not possible or relevant in the lab or field. For example, species differences in processing phytoestrogens are likely to be related to the dietary exposure to such compounds in a natural setting; carnivores are least likely and herbivores most likely to have evolved physiologic mechanisms for dealing with hormonally active compounds in plants.² If so, exposure to these estrogens may result in different, yet predictable outcomes among such classes of animals. This would be a topic for which the experimental zoo would be an important resource.

Conclusion

Zoos can and should provide an extremely biologically diverse, well managed, and well monitored study population for the effects of endocrine disrupting chemicals. Long-term studies of zoo animals may provide clues and answers to the overall health of an environment, and the effect of endocrine disruptors on local, regional, and migratory wildlife. Extrapolation of relevant data may eventually influence similar studies on the human population of the region.

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HEALTH ASSESSMENT OF PINNIPEDS POPULATIONS FOUND IN THE GALAPÀGOS ISLANDS

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Abstract

Pinnipeds play an important role in coastal regions around the world; they are high metabolism predator species with high consumption of food, associated to up-welling zones with high productivity.⁵ The study of adult females would reflect local environmental conditions because they remain most of the year near the reproductive areas, with long longevity. The raising of offspring can extend up to 1 yr or more, and thus a good body condition of the female would reflect in general, a good health condition of the pups.⁴ The health study of pups offers a practical alternative to indirectly evaluate the condition of the mothers and the general condition of the colony.¹¹

The populations of the two species of pinnipeds found in the Galapagos islands, (Galapagos fur seal, *Arctocephalus galapagoensis*, and Galapagos sea lion, *Zalophus wollebaeki*) are relatively small, approximately 20,000-30,000 individuals and highly vulnerable to climatic changes, such as El Niño Southern Oscillation events.^{9,12,16,17} This had caused high mortality in pups in 1997 with a 99% of mortality.¹ These pinnipeds, like other species of the archipelago, have a high risk of being affected by the introduction of exotic species (dogs, cats, and rats), and other problems, such as the oil spill that occurred on San Cristobal Island in January 2001, or a distemper outbreak in dogs reported in 2001 (Sandie Salazar, personal communication). In addition, the Galapagos sea lion is one of the main ecotourism highlights of the islands, due to the accessibility of the colonies. This increases their contact with international tourists, local residents and the interaction with solid waste, contaminated water and exotic species.

Long-term monitoring of health parameters of these populations would help to document changes in the prevalence of infectious agents and exposure to toxics. These data, together with general population dynamics and ecology, would provide essential information that enables the interpretation of the state of health of both of these pinniped populations and the ecosystem, with an interdisciplinary integration of knowledge.¹⁵

Since 2002, a field study has been carried out on pups during two reproductive seasons. Eleven and three rookeries have been monitored for the Galapagos sea lion and fur seal, respectively. Morphometric measurements, body weight, hair, blood, samples for microbiologic cultures from

rectum and external lesions, and parasites were obtained under general isoflurane anesthesia.^{13, 14}

Serum samples are being tested for selected infectious pathogens known to occur in marine mammals. Testing for *Brucella* spp. included serology (ELISA), PCR and bacteriologic culture from blood, was negative for all cases. Serology was performed for leptospirosis using the microagglutination test and positive titers were found in Galapagos sea lions to pathogenic serovars autumnalis, bataviae, canicola, celledoni, copenhageni, grippotyphosa, hardjo-pratjino, pomona, and wolffi. Overall, seroprevalence was 71%. Copenhageni was the most represented serovar (55%). Highest antibody titers (1:200) were detected against serovars canicola and hardjo-pratjino, which were the only serovars found in Galapagos fur seals.

Leptospirosis is a zoonotic disease which occurs worldwide and is identified in humans, domestic livestock, terrestrial and marine mammals. The same pathogenic *Leptospira* serovar can infect humans and marine mammals.^{6,7} Populations of sea lions have experienced outbreaks of leptospirosis that cause significant mortality.⁸ Because the Galapagos Islands are a tourist destination for water-related activities, such as scuba diving and snorkeling, and as a destination for international scientists, there is a potential risk for susceptible individuals to come into contact with *Leptospira*. An infected host may eliminate up to 105 leptospires/ml urine during the first weeks of infection, and it has been reported that sea lions eliminate leptospires in the urine for up to 154 days after infection.² Maintenance of leptospirosis in sea lions could be enhanced by crowding in rookeries and contact with urine.²

In addition, there is some concern with regards to animal welfare as perceived by tourists. For example, in the Galapagos sea lion colonies there is a high prevalence of purulent conjunctivitis mostly in pups that is frequently noted by the visitors. We identified this problem to be related with an ocular fluke, a digenetic trematode, in the genus *Philophthalmus*. The members of this genus are normally found in the eyes of birds. In some colonies, the prevalence of the parasite is as high as 92% of the pups examined. The presence of the eye parasites correlated with leukocytosis with a left shift. It is important to identify the potential carriers of this parasite in the Galapagos Islands, not only for the sea lions, but also as a public health hazard for tourists practicing water-related activities, because this parasite has been reported to infect humans.¹⁰

In order to better interpret the health status of both populations of pinnipeds, the results of this health survey are being compared to those obtained from a similar work done with California sea lions (*Zalophus californianus*) from the Gulf of Baja California, México.^{4,7,11,13} An analysis of the information is being performed using a simulation model to assess the effect of diseases and other environmental factors like El Niño year on the population (Fig. 1). This will enable us to predict potential problems and identify missing information that could have a major impact in the population, and guide us in future research. The dynamics of infectious diseases and their ecological impacts in marine ecosystems are poorly understood. They can affect species of ecological value, and may cause mass mortalities or negatively affect the abundance and reproduction rates of host populations.² However, these diseases can also be present in populations not in decline, depending on how they contribute to population dynamics.

The development of an integrated regional monitoring program for a sentinel vertebrate species as an indicator of marine ecosystem health is making health and ecological professionals understand the interconnections of species and the complexity of ecological health problems.^{3,15}

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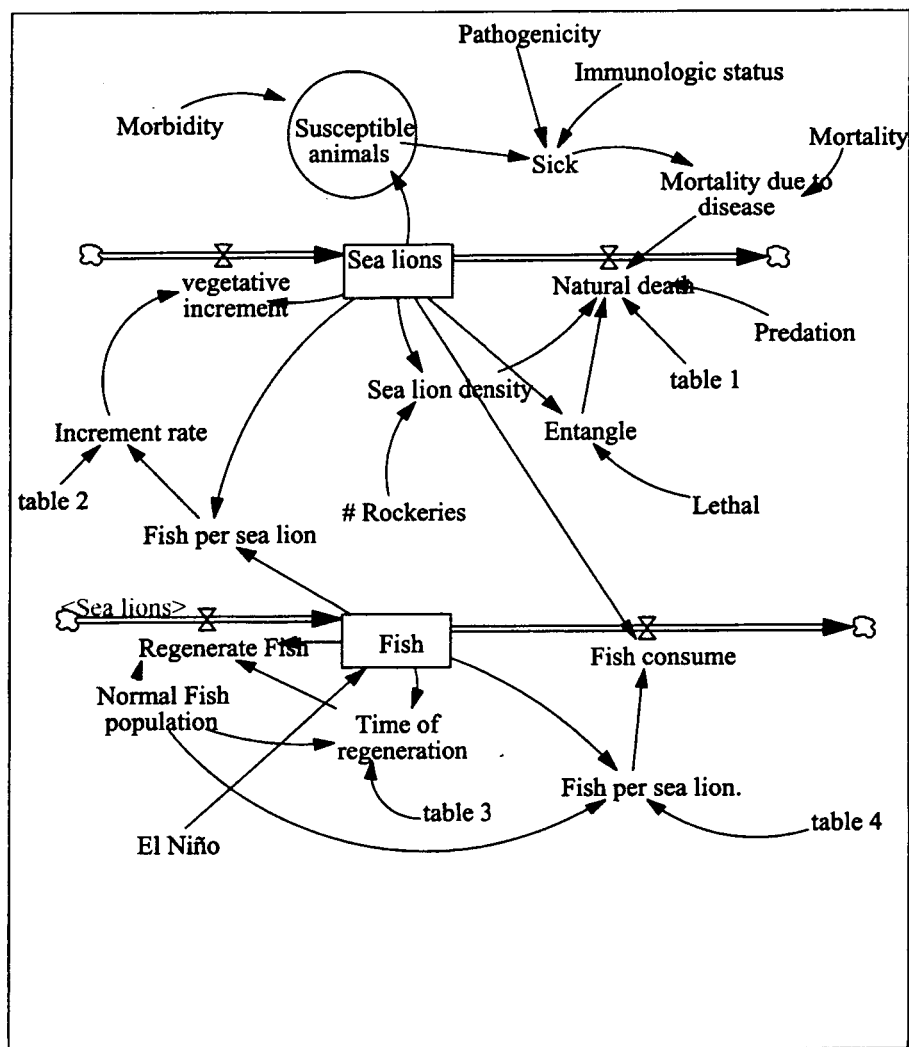


Figure 1. Simulation model for Galapagos sea lion (*Zalophus wollebaeki*).

HUMAN-INDUCED CONTAMINANT IMPACTS ON MIGRATORY BIRDS: LESSONS FROM THE NORTH AMERICAN EARED GREBE (*Podiceps nigricollis*)

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Abstract

The effects of aquatic contaminants generated by soda ash mining processes on the North American eared grebe (*Podiceps nigricollis*) population illustrates important issues associated with human-induced habitat degradation on the health of a migratory species. Eared grebes have been extensively studied in their staging and breeding habitats, but little is known about their 2- to 3-day migratory periods.^{2,3,5} During migration, few bodies of water are available to the birds for refuge between freshwater breeding areas in Canada and hypersaline lakes (e.g., Great Salt Lake in Utah or Mono Lake in California) to which they migrate. One geographic refuge area includes a series of “tailings” ponds associated with soda ash mining operations in southwestern Wyoming. The ponds range from 100-1200 acres, with water containing high concentrations of sodium decahydrate ($\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$). At cool temperatures (generally $< 40^\circ\text{F}$) sodium decahydrate precipitates out of the water and crystallizes on solid objects in the ponds or on the water surface. Bird mortality on these ponds has been recognized since the early 1970's, and the mining companies have developed hazing strategies and rehabilitation programs in order to minimize mortality. In order to determine causes of grebe mortality and devise strategies to reduce mortality, a field epidemiologic investigation was developed with the following objectives: 1) to determine whether eared grebes have quantifiable physiologic abnormalities associated with exposure to soda ash mine pond water; 2) to evaluate physical effects of sodium decahydrate crystallization on grebe survival; 3) to establish cause of death based on necropsy of deceased grebes; 4) to determine long-term survivability of eared grebes after exposure to the pond water; and 5) to evaluate water quality and determine whether aquatic invertebrates are present in the ponds as a possible food source.

This is an ongoing study, with objectives still in progress. Preliminary data presented here include serum biochemical parameters from 27 adult, eared grebes of both sexes, and brain biochemical concentrations from five adult and juvenile, eared grebes collected in August 2001 and August 2002. Grebe “controls” refer to grebe data collected from birds captured or shot on Mono Lake ($n = 8$ for serum biochemical data; $n = 2$ for brain biochemical data). Blood samples were opportunistically collected from grebes caught on the ponds, and our sampling protocol was incorporated into the

standard rehabilitation procedures employed by the mines. We requested that all birds captured be maintained on freshwater for 24 hr in order to collect a pre- and postcapture blood sample for comparison of serum biochemical changes over time. Water samples from several different ponds were analyzed in August 2000.

Eared grebes captured on soda ash mine pond water had significantly higher mean ($\pm$ SD) serum sodium concentrations prerehabilitation (155.8 ± 5.2 mg/dl) compared to both postrehabilitation (149.2 ± 3.6 mg/dl) and control grebes (152.3 ± 2.9 mg/dl). Recently deceased eared grebes removed from the surface of the pond water had elevated brain sodium concentrations (4041.6 ± 1895.3 ppm) compared to control grebes from Mono Lake (1429.8 ± 142.3 ppm). All grebes collected at the soda ash ponds had brain sodium concentrations greater than 2000 ppm, while controls had brain sodium concentrations below 2000 ppm (Table 1). Gross and histopathologic lesions from the deceased Wyoming grebes were consistent with drowning. Samples of pond water contained sodium carbonate decahydrate ($\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$), with hydrated sodium sulfate ($\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$) and NaCl. The water was highly alkaline ($\text{pH} > 10.2$; range 10.25-10.66), salinities were in excess of 174 ppt (range 174-240 ppt), and total dissolved solids ranged between 80,000-150,000 ppm. There was a complete absence of zooplankton in the ponds, however, phytoplankton was relatively dense, with flagellated chlorophytes being most prevalent.

Our preliminary data indicate that eared grebes landing on soda ash mine tailings ponds may be experiencing mortality due to the effects high brain sodium concentrations, with drowning being the ultimate cause of death.^{1,5} The toxic range for sodium in domestic species is considered to be greater than 2000 ppm in the brain, and the diagnosis of salt toxicosis in wildlife species has been extrapolated from this information.^{1,5} However, we currently have limited control data, and cannot attribute cause of death with confidence. It is possible that the normal brain sodium concentrations of eared grebes is high compared to other bird or mammal species, and that this species has evolved physiologic compensatory mechanisms. Alternative explanations for causes of mortality include; weight of sodium decahydrate crystal encrustation directly leading to drowning, and hypothermia. Due to the natural history and behavior of the eared grebe, susceptibility to water-borne contaminants may be particularly important. Eared grebes are diving birds, which, when hazed, will retreat underneath the water surface. This defensive behavior keeps them immersed in the water, allowing for more rapid accumulation of sodium decahydrate crystals. In addition, when preening and/or attempting to remove encrusted sodium decahydrate from feathers, eared grebes will ingest feathers and sodium decahydrate crystals, thereby increasing the likelihood of systemic absorption. Unfortunately, grebes are not the only species affected on these ponds, with total mortality of all bird species approximating 1000-1500 annually. The mining companies are attempting to develop methods for reducing pond size by either recycling the tailings water or promoting water evaporation. Ultimately, these techniques may successfully reduce bird mortality, but as long as the pond water is present, aquatic birds will continue be impacted.

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Table 1. Individual brain sodium concentrations in parts-per-million (ppm) on five eared grebes found dead on soda ash mine ponds in southwestern Wyoming, and two "control" eared grebes collected at Mono Lake, California.

Bird Number	Age/Sex	Brain Sodium Concentration
1 (4782-1)	Adult Male	4022.82
2 (4782-2)	Adult Female	4363.6
3 (4782-3)	Juvenile Male	2288.03
4 (4782-4)	Juvenile Female	7018.31
5 (4782-5)	Juvenile Female	2515.38
Control Grebe 1	Unknown	1530.45
Control Grebe 2	Unknown	1329.23

ENDOPARASITES, FOREST FRAGMENTS AND HOWLER CONSERVATION

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Abstract

Parasites and their hosts maintain an evolutionary relationship that keeps a dynamic equilibrium between their populations.^{1,3,12} However, during the process of fragmentation, as available habitat decreases, organisms get clumped into the remaining fragments, with the consequence that transmission of infectious diseases and parasites is facilitated.^{8,10,16,17} In fact, the incidence of parasitism is much higher near edges than in forest interiors and in more fragmented than in less fragmented forests.¹⁸ It is already known, for example, that fragmentation promotes higher infestation indexes due, probably, to the limited space available which forces visitation to the same trees and increases population densities.^{13,6} This condition may contribute to population declines and even to the total extinction of a population.

Loss of habitat is considered as the main reason for biodiversity decline and it is clearly causing a loss of species from tropical forests.^{2,4} In particular, the destruction of habitat is the principal factor affecting the conservation of primate species in Mexico.^{5,14} Like most rainforest ranges around the world, the one of Sierra de Santa Marta, Veracruz, Mexico, has been subject of important transformation, therefore howler monkeys (*Alouatta palliata mexicana*) inhabiting this landscape have been isolated for many years due to deforestation. Several studies at Santa Marta have already suggested connecting fragments of forest to increase chances of long term survival of these primates. However, there is concern that corridors may allow the movement of undesirable species, ie: exotic species and parasites, along with the target species.^{9,11} Thus, increased movement of the host individuals among fragments may result in higher prevalence, higher parasitic loads and increased rate of spread of contagious diseases and parasites.⁷

At Santa Marta, the risk of transmission of endoparasites between howler monkey populations inhabiting forest fragments was assessed at two different scales: first, veterinary techniques allowed the determination of the diversity and endoparasite burden in the howler groups. Finally, we evaluated potential scenarios at a landscape scale, in the event connections between patches were implemented in the future. Since parasite diversity and parasite burden are related to the area and the extent of fragmentation, parasitology studies may formulate important recommendations to conservation and management programs of species such as howler monkeys.

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EVALUATION OF THE HEMATOLOGY AND BIOCHEMISTRY OF SCARLET MACAWS BEFORE AND AFTER REINTRODUCTION IN COSTA RICA

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Abstract

As part of a rehabilitation and reintroduction program of scarlet macaws (*Ara macao*), hematologic and biochemistry values were determined during captivity, at the release site, and once they were released back in to the wild. A group of 21 scarlet macaws (*Ara macao*), 13 females and 8 males were confiscated during 1993, 1994 and 1996. Their average age was estimated to be 20 yr. They were rehabilitated and reintroduced in 1998 and 1999 in the central Pacific area of Costa Rica. This species had disappeared from this area approximately 40 yr ago due to hunting and illegal capture for sale as pets. A soft release program was used to reintroduce the individuals. A special cage was developed to condition the animals to natural feeding behaviors. Two cages were constructed. One was hung inside the release cage while the other one was hung outside. The exterior cage functioned as a feeding trap device in order to feed the animals and recapture them for future analysis. Several artificial nests were built. Two were hung inside the release cage and eight were hung throughout the release area in order to encourage nesting in the area. An environmental education program was developed and executed. Farming communities and primary schools were visited several days each week to be educated about the reintroduction program as well as the multiple benefits that a program such as this would bring their communities. These benefits lead to the same ending—improving quality of life by introducing a commercial element because the project contributes to tourism, which will give them (the communities) another source of money for their own use. In addition, there is a spiritual aspect because future generations will see free-flying scarlet macaws (*Ara macao*).

Changes (before release vs. after release) in the hematology and blood chemistry values of released macaws were used to evaluate adaptation of the released animals. All of the birds had identical housing, diet and health status during captivity. The results of all analytes during captivity were within the range reported for this species. There were 19 individuals in the final release group. One individual died with of severe obstructive uropathy due to a renal parasite, while the second one developed bilateral ocular dysfunction related to the presence of cataracts. Two sets of blood samples (July, 1998 and September, 1998) were taken during captivity and two more (May 1999 and June, 1999) were taken once the animals were released. Data obtained both in captivity and after release were compared using nonparametric statistics to verify significant changes. All of the hematologic values, except lymphocyte, monocyte and eosinophil counts tended to increase over time. However, only seven of the 10 variables measured were statistically different before and after release. (Ht, Hgb, MCHC, heterophils, lymphocytes, monocytes and unopette cell count). Out of the nine biochemistry values (TP, glucose, uric acid, Ca^{2+} , P, ALKP, CPK, ALT, AST) measured,

six tended to increase (TP, Ca, P, CPK, uric acid, BUN, AST) and three to decrease (ALT, ALKP, glucose) but only five changes were statistically significant. (TP, Ca, P, CPK, ALT). According to the results, variables tended to increase 1 mo after the release date and then to decrease 1 mo after. Hematologic and biochemistry variables measured in this study, after the release, were not that different from the data published for captive individuals for the same species. Minor differences could be due to diet, environmental conditions, gender, laboratory procedures, or sample handling. It is reported therefore, that based on observations and the results after the release, the individuals were apparently healthy and that the changes found, were due to adaptation to several factors such as stress, hypoxia and exercise.

HEALTH EVALUATION OF THE FLAMINGO POPULATIONS OF THE *Phoenicoparrus* GENUS IN NORTHERN CHILE

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Abstract

The *Phoenicoparrus* genus is composed of two species: the Andean flamingo (*P. andinus*) and the James flamingo (*P. jamesi*). The abundance of the populations has been declining since 1986.^{2,3} Sudden death episodes have been described in those species; however, the cause, or a link to human activities has not been reported.¹

The objectives of our study were to assess the health status of free-ranging flamingo populations and to improve the capture methods in terms of safety and effectiveness.

The first sampling campaign was performed in November 2002 in the Salar de Atacama, II district, Chile (23° 18' 31'' S, 68° 08' 25'' W, at 2,330 m above sea level). Thirty-two flamingos (11 Andean, 21 James) were captured with foot traps ($n = 8$) or during night captures with nets ($n = 24$). The second sampling campaign was performed in March 2002, in Surire I district (18° 50' 42'' S, 68° 59' 21'' W, at 4,283 m above sea level). Nineteen flamingos (7 Andean, 12 James) were captured with foot traps ($n = 1$) or during night captures with nets ($n = 18$).

The animals were weighed and then physically examined and tagged. Swabs were obtained from the cloaca and oral cavity for culture. Blood samples were collected for complete blood cell counts, biochemical analysis and serology. Fresh fecal samples were obtained for parasitology.

The Andean flamingos weighed an average of 2.86 ± 0.34 kg, while the James flamingos weighed 2.32 ± 0.32 kg. The average of white cell count was $8,084.62 \pm 2,584.52$ cells/mm³ for the Andean flamingos, and $5,909.58 \pm 1,615.87$ cells/mm³ for the James flamingo. Percent cell volume averaged $44.06 \pm 8.03\%$ for Andean flamingos, and $45.13 \pm 5.57\%$ for James flamingos. The most frequent bacteria isolated from the cloaca and choana was *Bacillus* spp. In addition, *Penicillium* and *Aspergillus* were identified in both species. Antibodies were present for influenza virus ($n = 4$), Newcastle disease virus ($n = 11$), and Eastern and Western equine encephalitis diseases virus ($n = 2$). Fecal samples were negative. At necropsy, one Andean flamingo was found heavily parasitized with *Flamingolepis* spp. Two hundred *Flamingolepis* tapeworms were recovered from a James flamingo. This parasite has not previously been reported in this species.

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VETERINARY INVOLVEMENT IN THE KAKAPO RECOVERY PROJECT

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Abstract

The kakapo (*Strigops habroptilus*) is a large (1.5-3.0 kg) nocturnal, flightless, lek-breeding parrot endemic to New Zealand. Its distribution was once widespread, but the arrival of humans 600-800 yr ago impacted the kakapo population. Reduction of numbers was caused by hunting, habitat destruction and the introduction of predators such as kiore (Polynesian rats) and dogs by the Maori and other species of rats, cats and mustelids by Europeans in the 1800 & 1900's.

In the late 1960's only a few males were found in remote areas of the Fiordland Mountains. In 1981 the first female kakapo were identified in a re-discovered population of 200 birds on Stewart Island, a large island to the south of the mainland. The relief was short lived because feral cats were decimating the population and cat control measures were ineffective at halting the decline. Kakapo were transferred to smaller islands, which were either predator free or had only kiore present. Kiore were not suspected to be a problem for kakapo until almost 10 yr later. Monitoring nests discovered kiore preying upon newly hatched chicks when the females left the nest to feed at night. Kakapo and other native avian species were temporarily removed from island preserves and sodium monofluoroacetate pellets (commonly called "1080") was air-dropped to eliminate mammalian predators.

Over the next 15 yr there was no significant increase in the population (50 individuals) under a hands-off management system. In 1995, a review of the Kakapo Recovery Program led to an intensive population management approach. A multidisciplinary management team was organized to direct the recovery program composed of technical officer, team leader, scientist, and an aviculturist and in 2000 a veterinary position was added. Techniques were introduced to increase productivity. Radio transmitters were placed on all birds to facilitate finding nests. An infrared camera was used to monitor nest activity and linked to a video recorder, which continually taped activity inside the nest. It was also linked to a video screen, which two staff members watch each night. The radio transmitters permit close monitoring of adult kakapo to minimize morbidity and mortality. Specialized radio receivers with data logger and memory storage record the movements of birds within a 10-m radius. It is useful at breeding times to monitor which females visit the track and bowl areas of males. When placed next to a feeding station, it is linked to a set of electronic scales to record which birds are feeding at which stations and their body weights.

Supplementary feeding of nuts, fruit and vegetables was initially used in an attempt to increase the

frequency of breeding in the early 1990's but the results were equivocal and may have lead to obesity related problems. Natural food items consumed by kakapo were identified. A palatable balanced organic kakapo pellet was developed as a feeding supplement.

Annual health assessments are completed for every bird. Plasma chemistry panels, CBC and bacterial/fungal swabs are collected to establish baseline normal values and allow detection of alterations. During the 2002 breeding season, blood and microbiology samples were collected at least twice from all 24 chicks to establish baseline normal values. *Candida famata*, was cultured from the crops of slow growing chicks and was not found in the crops of chicks with steady body weight increases. The significance of this novel yeast is still under investigation; is it a newly emerging primary pathogen or is it secondary to altered crop motility? Electroejaculation techniques were developed for semen collection from free-ranging kakapo to investigate problems with perceived male infertility. There are no previously published accounts of electroejaculation techniques used to study free-ranging psittacines. In a preliminary study at the end of the 2002 breeding season, sperm was collected and examined from 12/13 anesthetized sexually mature male kakapo. In January 2003, electroejaculation was the first indicator that no male birds were producing sperm and that a breeding season for 2003 was unlikely.

In conclusion, the change in management and a multidisciplinary approach has had a significant impact on the success of the Kakapo Recovery Program. Thirty-six kakapo chicks have successfully fledged, representing a 72% increase in the population (Fig. 1). These successes demonstrate the effectiveness of intensive population management with veterinary assistance.

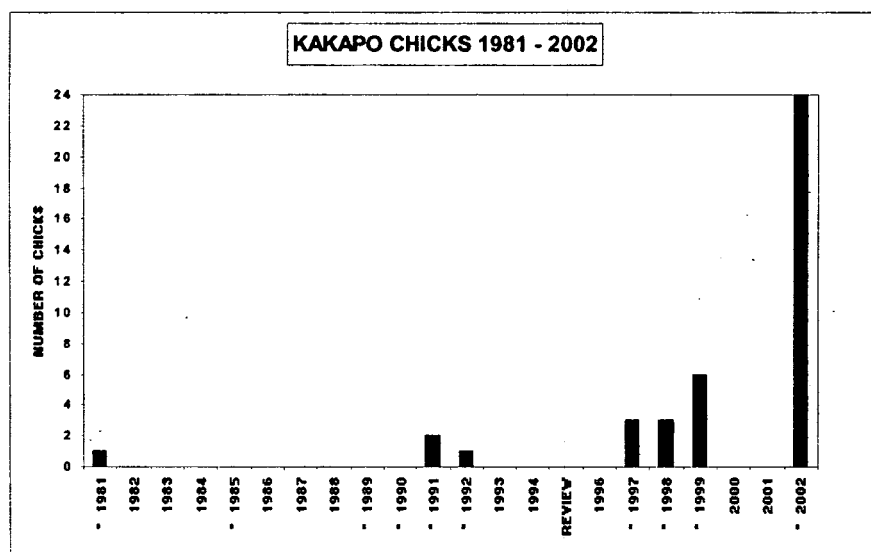


Figure 1. Bar graph demonstrating the annual production from 1981-2002 of chicks ($n = 36$) from free-ranging Kakapo.

DIAGNOSIS AND TREATMENT OF *Lawsonia intracellularis* PROLIFERATIVE ENTERITIS IN A YOUNG JAPANESE MACAQUE (*Macaca fuscata*)

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Abstract

Lawsonia intracellularis is an obligate intracellular bacterium causing proliferative enteritis in a broad range of species.¹⁻⁵ A 2.5-yr-old Japanese macaque (*Macaca fuscata*) was presented for vomiting and mild depression. Radiographs indicated metallic foreign body ingestion. A thickened ileum was found during exploratory laparotomy. Biopsies of the thickened ileum revealed severe chronic pyogranulomatous and lymphocytic enteritis, and presence of spindle shaped organisms on silver stain (Warthin Starry). Immunohistochemistry and PCR of the ileal biopsy were positive for *Lawsonia intracellularis*. The animal developed severe hypoproteinemia and pitting edema. It recovered 42 days after presentation with a treatment regime including azithromycin and hetastarch. Enteropathies are common in nonhuman primates and difficult to diagnose. *L. intracellularis* should be included in the differential list. The zoonotic potential of this bacterium should be considered.

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AN UPDATE ON SERUM GESTATIONAL HORMONAL LEVELS IN A BORNEAN ORANGUTAN (*Pongo pygmaeus pygmaeus*)

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Abstract

Urinary estrogen, pregnanediol, estrone, estradiol, and androsterone values during pregnancy and menstrual cycles of orangutans have been studied by several investigators.^{4-7,9} Serum values for estradiol, progesterone, testosterone and luteinizing hormone (LH) have been evaluated during the menstrual cycles of orangutans and macaques.^{10,12}

In this ongoing study, serum progesterone, β -human chorionic gonadotropin (β -HCG) and estradiol-17 β were serially monitored before, during and after three successive pregnancies in an adult female Bornean orangutan (*Pongo pygmaeus pygmaeus*). Results were compared to a nonpregnant 33 yr-old female Bornean orangutan in the same facility, and human reference normals. Blood samples were obtained during weekly to biweekly conditioning programs without sedatives or tranquilizers. Trans-abdominal ultrasound images were obtained during the later stages of the first pregnancy, and throughout gestation in subsequent pregnancies. Differences in β -HCG and progesterone values resulting in a stillborn infant were retrospectively observed to differ from two subsequent successful births.

Serum progesterone (4-prenen-3,20-dione), β -HCG, and estradiol [1,3,5 (10)-estratrien-3,17 β -diol] values were determined using microparticle enzyme immunoassays (AxSYM®, Abbott Laboratories, Abbott Park, Illinois 60064 USA) performed on samples obtained from the orangutan through a stationary conditioning program. These samples were frozen at -86° C and analyzed at a later date.

Values for serum progesterone, β -HCG, and estradiol are presented in Fig. 1-3. Results of pregestational, gestational and postgestational values closely parallel human normals.^{2,13,14} However, significant differences were noted in serum progesterone and β -HCG values of the first pregnancy compared to two subsequent successful pregnancies. In humans progesterone is produced primarily by the corpus luteum of the ovary.¹ Progesterone prepares the uterus for implantation and maintains the uterus during pregnancy. During the menstrual cycle in humans, serum progesterone values elevate to 10-20 ng/ml 5-7 days after ovulation. During the sixth week of pregnancy, the placenta becomes the major source of progesterone.⁸ Values during this time elevate from 10-50 ng/ml during the first trimester to 50-2,809 ng/ml during the third trimester.^{2,13} In all three pregnancies, progesterone levels were between 10-15 ng/ml during the first trimester, and elevated to 30-40 ng/ml during the second trimester. However, progesterone levels decreased to 10-15 ng/ml during the third

trimester of the first pregnancy (Fig. 1). Serum levels during this pregnancy began to elevate to previous values but never fully recovered. Reasons for this are unknown, but we speculate that these values indicated the potential for gestational complications. Although this pregnancy proceeded to term, the infant was stillborn. An underlying cause was not determined.

Beta-HCG appears to maintain the corpus luteum during early pregnancy in humans, allowing continued secretion of progesterone, which supports the endometrium. Values for serum β -HCG in humans demonstrate a maximal peak in the late first trimester, with a decline to a fairly constant level by mid gestation.² In our study, serum β -HCG values during the first pregnancy elevated beyond the values for the subsequent pregnancies and remained elevated for an extended period when compared to the two subsequent gestations. We speculate that this value maintained elevated levels due to fetal complications of excreting progesterone to maintain a healthy pregnancy (Fig. 2).

Estradiol levels in human studies demonstrate a constant rise until parturition.^{11,14} Levels of 100-500 pg/ml occur during the first trimester, 5,000-15,000 during the second trimester, and 10,000-40,000 pg/ml during the third trimester.¹⁴ A similar curve was observed in this group of data for all three pregnancies (Fig. 3).

In this orangutan, values for serum progesterone and β -HCG may have predicted gestational complications resulting in a stillborn infant. Two subsequent pregnancy evaluations demonstrated parallel values and both resulted in uncomplicated births. These values may provide a basis for evaluating serum reproductive hormone levels in Bornean orangutans during pregnancy.

ACKNOWLEDGMENTS

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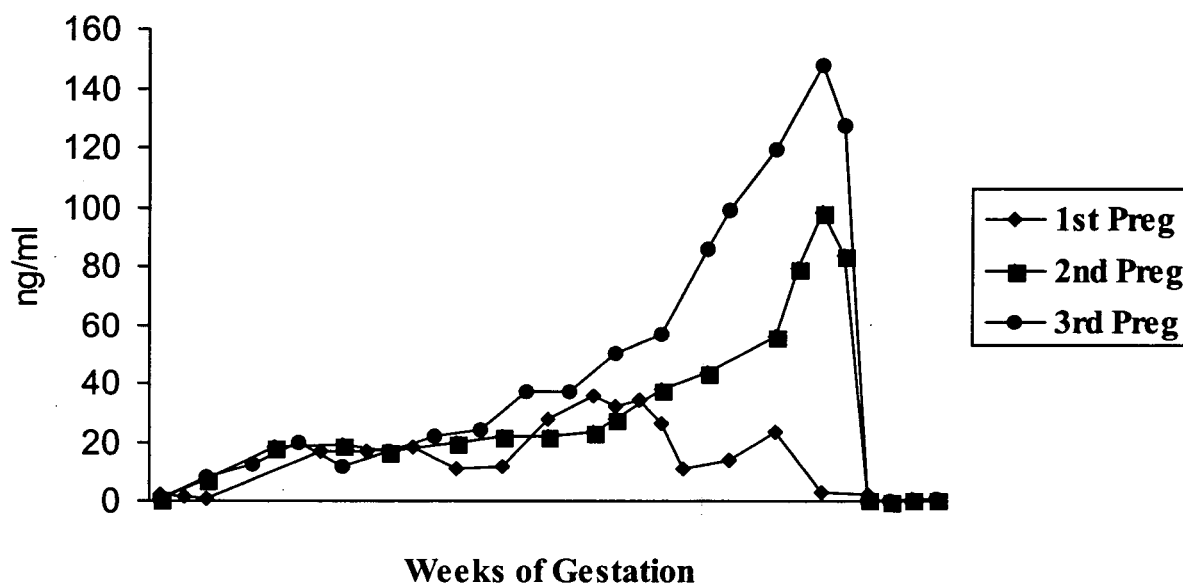


Figure 1. Serum progesterone values (ng/ml).

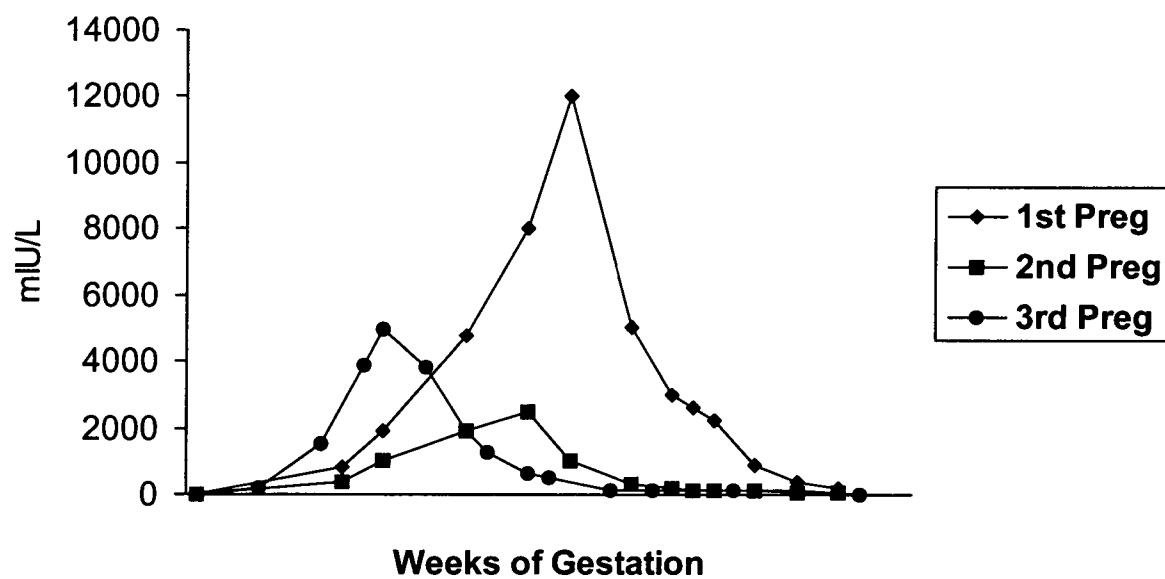


Figure 2. Serum β -HCG values (mIU/L).

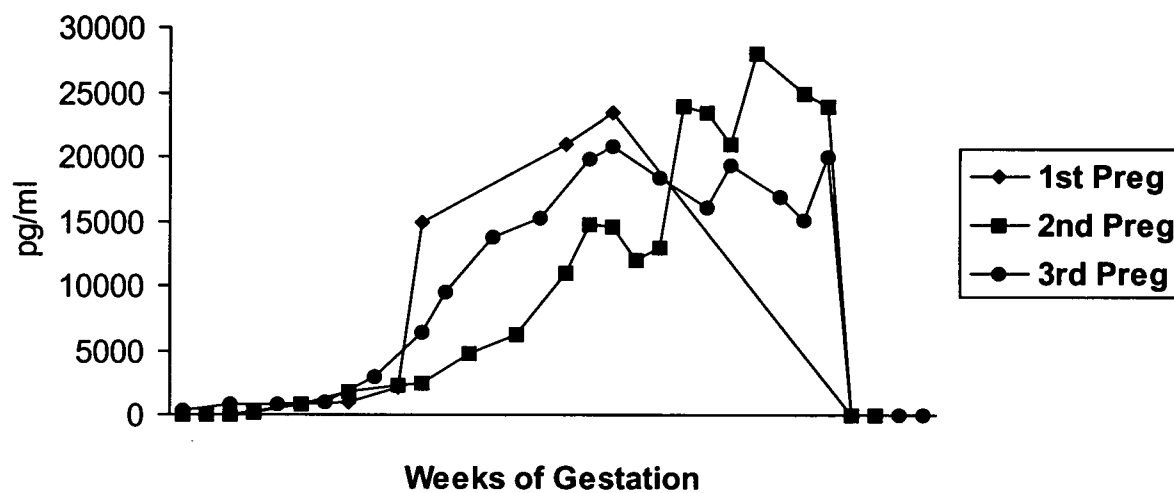


Figure 3. Serum estradiol values (pg/ml).

CONTROL AND TREATMENT OF *Gongylonema* sp. INFECTIONS IN CALLITRICHIDS

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Abstract

Gongylonema sp. are spirurid parasites that infect the upper digestive and respiratory tracts of a variety of mammals and birds. In most mammals, gongylonemiasis, is considered an incidental finding, associated with few clinical signs and minimal pathologic changes.^{1,5} There have been multiple reports of the parasite infecting a wide range of primate species.²⁻⁶ Recent reports of lingual gongylonemiasis (*G. pulchrum*) in Goeldi's monkeys (*Callimico goeldii*) and common marmosets (*Callithrix jacchus*) are the first cases to describe pathologic changes associated with infection in neo-tropical New World primates.^{2,4} In these cases, pruritis of the perioral tissues, inflammation of the tongue and lips, and chronic pytalism were the primary clinical signs.

Numerous *Gongylonema* sp. infections with associated pathologic lesions and clinical signs have been identified in Goeldi's monkeys at Brookfield Zoo over the course of several years. An increase in the incidence of clinical signs in Goeldi's monkeys, as well as other callitrichid species, prompted a study to attempt to determine the prevalence of infection within the collection and evaluate treatment and control protocols.

There have been no studies that we are aware of specifically investigating the treatment of *Gongylonema* sp. in callitrichids. Treatment with a variety of antihelmintics in these species has been primarily empirical.^{4,6} The lack of definitive information about the prepatent period of *Gongylonema* sp. in primates, the efficacy of anthelmintic drugs, appropriate dosages, and length of treatment have made control of this parasite challenging. Diligent insect control and routine prophylactic deworming have been suggested as the primary method to control infections within a collection.^{4,6}

Twenty-one callitrichids, representing four species, housed in the same room were included in this study. Repeated cytologic evaluations of scrapings taken from the mucosa of the tongue were performed to determine if individual animals were infected with parasite. The callitrichids were divided into two random groups and received treatment with either ivermectin or mebendazole. Numerous animals displayed clinical signs throughout the study, however, *Gongylonema* sp. infections were only confirmed by tongue scrapings in two animals. Although the low number of positive tongue scrapings precluded comparative statistical evaluation of the efficacy of the anthelmintic treatment protocols, clinical signs consistent with *Gongylonema* sp. and the number

of confirmed cases have decreased since the treatment study.

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A PRELIMINARY REPORT ON INTRA-ABDOMINAL ABSCESES IN CAPTIVE WESTERN LOWLAND GORILLAS (*Gorilla gorilla gorilla*)

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Abstract

Captive western lowland gorillas (*Gorilla gorilla gorilla*) have a high prevalence of intra-abdominal abscesses when compared to other reported diseases in this species. A database was created from medical records obtained from North American zoos to investigate possible etiologies. The goals were to 1) evaluate methods used to diagnose abscesses, 2) establish prognostic and risk factors, 3) determine etiologies, and 4) evaluate treatment strategies. This report presents the initial results of the study.

Introduction

While it is not surprising that gorillas develop intra-abdominal abscesses, there appears to be an abnormally high prevalence in captive animals with considerable morbidity and mortality in an unpublished examination of captive gorilla records. Reviews of gorilla diseases have not reported any cases of intra-abdominal abscesses.^{4-8,12-13} Gastrointestinal disease is often cited as one of the top three medical problems in gorillas and includes peritonitis, ulcerative colitis, and parasitism, however, severity of disease is rarely mentioned and abscess formation is not reported.

Intra-abdominal abscesses have been reported in both exotic and domestic species including a chimpanzee.^{1,3,6,9,15} The frequent occurrence of abdominal abscesses in humans is well documented in the literature. Common causes include a ruptured appendix, intestinal parasitism (*Entamoeba histolytica*), perforating ulcers, pelvic inflammatory disease, and sequelae to abdominal surgery.^{11,16}

A differential list of diagnoses for intra-abdominal abscesses in gorillas includes: 1) organ perforation due to a) foreign body trauma, b) parasitic, bacterial, viral, and toxic enteritis, c) reproductive tract infection; 2) iatrogenic causes: infection or foreign body introduction secondary to surgery; and 3) nutritional deficiencies leading to poor intestinal mucosal integrity (e.g., low fiber content of diet).¹⁰

Methods

Medical and necropsy records were requested for normal and clinically ill animals from American Zoo and Aquarium Association (AZA) accredited zoos in North America. Animal identification was based on the studbook number. Animals without a studbook number were assigned a unique number,

which would not conflict with legitimate studbook numbers. A database was created using a relational database (Microsoft Access; Microsoft Corporation, One Microsoft Way Redmond, WA 98052-6399 USA) that can accommodate complex medical record information. An 'event' table was created to record major occurrences in an animal's record, for example, severe dental tartar would be a 'disease' event. Additional tables were used to record details specific to different types of events, including: anesthesia, physical examination, disease diagnosis, parturition, vaccination, parasitology, bloodwork (CBC, serum chemistry, viral titers), urinalysis, surgery, microbiologic culture, pathology, treatment, and other diagnostics. An animal was classified as having an intra-abdominal abscess if there was gross or histologic diagnosis of purulent material and inflammation of tissue in the abdominal cavity.

Results

Prevalence

The 2000 North American gorilla studbook lists 1,462 animals (live and dead) with assigned studbook numbers. Of these, 712 (49%) could be tracked and potentially had a medical record. Three hundred and seven (43%) partial or complete files were collected from 35 participating institutions for this project. Records were collected from August 1969 to early 2002. Gender distribution was 152 females (49.5%) female, 153 (49.8%) males, and 2 (006%) unknown.

Twenty-six animals had suspected intra-abdominal abscesses. Of these, 21 records were available to the author. All 21 were subsequently confirmed to have abscesses. The population of animals confirmed to have abscesses was predominantly female (90%), typically 17-36 yr of age, obese (72-140 kg) and reproductively inactive.

Clinical History

Clinical signs in all cases included lethargy and anorexia. Recto-genital discharge, constipation, abdominal distension, and vomiting were occasionally reported. Less frequently noted were diarrhea, trembling, and weakness. Clinical symptoms were often present weeks to months prior to confirming a diagnosis of intra-abdominal abscess. Several animals had reproductive exams involving an invasive procedure (laparoscopy or uterine flush with contrast) prior to abscess formation.

Diagnostics

Diagnostics most often performed were: bloodwork, abdominal radiography (survey and contrast), abdominal ultrasound, fluoroscopy, gallium scan, computed tomography (CT scan), ultrasound-guided percutaneous fine needle aspiration, culture of the abscess, and surgical exploration. Abdominal palpation was not a good indicator of the presence of an abscess. Challenges to making a diagnosis included concurrent disease, early stage of abscess formation, and poor quality of imaging studies. In every case human physicians were consulted.

Initial Results

Abscess origin was frequently difficult to ascertain due to severe adhesion formation. The colon and reproductive organs were often incorporated within a fibrous mat, which occasionally involved the urinary tract organs. Fistulas were a common occurrence and often drained perirectally or perivaginally. Intestinal perforations, including the appendix in two cases, were documented, although in many circumstances perforations were suspected but could not be confirmed due to adhesions. Specific muscles (rectus abdominus, coccygeal muscles, and the psoas muscles) were occasionally associated with abscesses, but this was not typically documented in the medical record. Plant material foreign bodies were an additional finding and in some instances, were posited as the causative agent. Several animals had purulent material free in the coelomic cavity.

Bacteria cultured from abscesses included: *Escherichia coli*, *Morganella morganii*, *Fusobacterium necrophorum*, *Bacteroides* sp., hemolytic *Streptococcus* and *Staphylococcus* spp., *Enterococcus* sp., *Hemophilus* sp., *Actinomyces* sp., *Proteus* sp. and *Klebsiella pneumoniae*. The protozoan *Balantidium coli*. was found in fecal exams and biopsies of intestinal mucosa in many animals with abscesses. While this organism can cause pathology in gorillas,¹⁸ its relevance in abscess formation is unclear.

Treatment

Treatment included multi-modal long-term antibacterial therapy and analgesics in all cases. Additional treatments included antifungal therapy, gastric protective drugs (H2 blockers, proton pump inhibitors), peritoneal lavage, surgical debridement, surgical resection of portions of the intestinal or reproductive tract, and antibiotic impregnated methylmethacrylate bead placement where resection was not possible. When surgery was elected, massive amounts of fibrous adhesions within the abdomen were universally encountered.

Challenges to treatment included poor compliance with drug administration, adhesion formation impairing surgical access and identification of organs, manipulation/self-mutilation of surgical wounds on recovery, recrudescence of disease, concurrent disease, and misdiagnosis. Adverse sequelae to therapy included severe adhesion formation, intestinal torsion, peritonitis, dehiscence of incisions, inability to control additional abscess formation, and sepsis.

Outcome

Seven of 21 animals succumbed to sequelae of intra-abdominal abscessation. Two animals died of unrelated causes with no evidence of abscesses noted on necropsy. Ten animals survived the abscesses; of these, one is currently under treatment while the others appear resolved. Outcomes for two animals are unknown because of incomplete records. Two additional animals were diagnosed with abscesses in the latter part of 2002 and early 2003. To the author's knowledge these animals are currently stable.

Discussion

Preliminary findings suggest that intra-abdominal abscesses are an important problem in captive western lowland gorillas and are most likely to occur in older, obese, females. Affected females are usually reproductively quiescent, although a cause/effect relationship cannot be made at this time. One report on four senescent female gorillas describes cause of infertility as secondary to fibrous peritoneal adhesions attributed to endometriosis, possible pelvic inflammatory disease and nonspecific trauma.¹³

Because strong similarities exist between great ape and human diseases,^{14,17, 19} advanced human diagnostic and treatment regimes can be explored for veterinary application. As additional normal animals added to the database for comparison of clinical signs, valuable guidelines can be suggested for identifying animals with possible abscesses. It appears that early diagnosis and intervention can result in a positive outcome. Further analysis is needed to determine the most successful treatments.

Limitations of this study include incomplete records, a lack of dietary information in records provided, an inability to compare diseased animals to the total captive animal population due to a low number of records, as well as an inability to compare the information with data from wild populations.

As data are compiled and completed, this ongoing project should continue to give pertinent information for identification and medical management of intra-abdominal abscesses in gorillas. Further, this database will serve as a useful tool for examining other disease issues within the population entered into the database.

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ANTE-MORTEM DIAGNOSIS AND MANAGEMENT OPTIONS FOR RENAL DISEASE IN A PYGMY MARMOSET (*Callithrix pygmaea*)

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Abstract

A captive, 8-yr-old intact male pygmy marmoset (*Callithrix pygmaea*) was evaluated during an annual exam. While no prior health issues had been appreciated, the animal was thin and blood tests demonstrated azotemia. Subsequent testing showed azotemia with isosthenuria and proteinuria leading to a diagnosis of renal disease, tentatively involving the glomerulus and tubules. Diagnosis of renal disease in callitrichids is possible utilizing standard techniques, however patient size makes it difficult to run all urine tests at one time. Routine behavioral training protocols were essential in managing this case. A target station provided a physical platform for regular urine collection and training sessions allowed routine weight evaluation and individualized medication administration. Routine monitoring for proteinuria may be utilized as a screening tool for renal disease.

Introduction

The presence of azotemia in association with isosthenuria is highly suggestive of renal insufficiency.⁵ Tubular and glomerular disease may exist concurrently, and glomerular disease will frequently progress to severe tubular damage.⁵ Significant functional loss occurs prior to elevations in blood chemistry values.⁵ In glomerular disease such as glomerulonephritis, the urine protein:creatinine ratio (UPC) may be utilized to detect problems before azotemia develops.⁵ Urine protein:creatinine ratio values greater than 1.0 are indicative of renal disease, including some tubular disease, with higher values being associated with glomerular disease. Urine protein:creatinine ratio needs to be evaluated in combination with urine sediment as significant levels of red or white blood cells will increase the ratio. In addition, cases should be evaluated for extrarenal sources of protein.^{4,5} Definitive ante-mortem diagnosis of renal disease requires kidney biopsy.⁵

A syndrome linking glomerulonephritis, presumed hypertension, heart failure and death has been described in callitrichids.⁸ While postmortem diagnosis of immune complex deposition and renal failure in callitrichid species exists in the literature, ante-mortem description of renal disease is lacking.^{1,2,8,11} Early diagnosis of renal disease can improve management options for animals and lead to better understanding of disease progression.^{4-7,9,10}

Case Report

An 8-yr-old, male pygmy marmoset was evaluated during a routine exam. The animal was housed

with one other adult male pygmy marmoset in the Amazon Exhibit of the John G. Shedd Aquarium. Keeper staff did not identify health concerns prior to sedation but body condition appeared thin on physical exam. Isoflurane (Iso-thesia, Abbot Laboratories, North Chicago, IL 60064 USA, 1-5%) in oxygen administered in a chamber then via facemask was utilized for anesthesia. Serum chemistry analysis showed azotemia (Table 1). Whole body radiographs were considered normal. A voided urine sample collected 3 days later was isosthenuric, with proteinuria and negative sediment (Table 2). A second voided sample collected on day 6 showed isosthenuria and proteinuria. A tentative diagnosis of renal disease, possibly glomerulonephritis, was made based on azotemia, isosthenuria, and proteinuria with inactive sediment. A urine protein:creatinine ratio run on day 12 was 13.6.

On day 15, the animal was re-sedated in an attempt to collect blood and urine for evaluation on the same day. Bloodwork showed anemia, azotemia, and hypoalbuminemia. Voided urine collected on the same day was isosthenuric with inactive sediment but no protein was present. A drop of urine expressed from the bladder was cultured and yielded scant growth of three organisms, considered to be contaminants. Ultrasound showed small, hyperechoic kidneys. Measurements were not taken due to concerns over patient stability.

Enalapril maleate suspension (Martin Avenue Pharmacy, Naperville, IL 60540 USA, 0.5 mg/kg p.o. every 12 hr) was used to treat for renal failure related to possible glomerulonephritis as it has been associated with increased longevity in some cases^{3,5} Derm® Caps ES (DVM Pharmaceuticals, Inc., Miami, FL USA, 1 drop p.o. every 25 hr) was used to supply omega 3 fatty acids, which may be renoprotective.³ Supplemental feeding with Ensure® liquid (Abbott Laboratories, North Chicago, IL 60064 USA) was begun to increase caloric and fluid intake.

Subsequent monitoring included sedation with sevoflurane in oxygen via chamber and facemask (SevoFlo, Abboth Laboratories, North Chicago, IL 60064 USA, 3-7%) on day 43 for a repeat examination. Bloodwork demonstrated progressive azotemia and continued anemia. Blood pressure was measured noninvasively using a modified digit cuff (Pedisphyg, CAS Medical Systems, Inc., Branford, CT 06405 USA). A systolic value of 100 mm Hg was obtained. Ultrasound of the heart demonstrated adequate contractility although measurements were not obtained due to concerns over patient stability. Three subsequent urine samples were collected opportunistically for UPC (4.7-18.7) and two fecals were negative for occult blood.

Seventy-two days after initial evaluation cimetidine hydrochloride (Cimetidine Hydrochloride Injection 150 mg/ml, Endo Pharmaceuticals Inc., Chadds Ford, PA 19317 USA, 4 mg/kg p.o. every 8 hr) and metoclopramide (Metoclopramide Injection 5 mg/ml, Endo Pharmaceuticals, Inc., Chadds Ford, PA 19317 USA, 0.1 mg/kg p.o. every 6 hr) were started to alleviate possible nausea. The animal died 97 days after initial exam.

Necropsy and histopathology were completed. Histopathology showed diffuse, severe interstitial fibrosis and tubular loss with marked tubular and glomerular ectasia ("end stage" renal disease).¹⁰ Mild, multifocal lymphoplasmacytic interstitial nephritis was also noted. Moderate, multifocal arteriosclerosis was present in the renal blood vessels and left ventricular hypertrophy with mild,

multifocal interstitial fibrosis was evident grossly. The cardiac changes were attributed to possible hypertension secondary to renal disease.

Discussion

Ideally, blood and urine tests are run from samples collected at the same time. Urinalysis with sediment exam, culture, and UPC are run on the same clean free catch, catheterized, or cystocentesis sample. Due to small sample size in this patient, however, it was difficult to monitor all urine parameters at one time. Attempts to overcome this were made by running tests multiple times and running different tests in a short time period. Overall, six of seven urine tests for protein (UPC and dipstick combined) demonstrated significant protein elevation. A simple method for collecting samples from an awake animal was developed. Urine samples were collected opportunistically from a plastic platform hung in the cage during daily training sessions. Samples could be obtained reliably and did not involve altering feeding or drinking schedules. Platforms were cleaned then washed with copious amounts of water after each session. Urine samples obtained in this manner from a healthy conspecific had UPCs consistently <1.0.

The animal in this report had severe interstitial disease and glomerular ectasia. Glomerular disease, such as glomerulonephritis, in other species is associated with UPCs well over 1.0,^{4,5} as seen in this case. The lack of obvious glomerular membrane change is interesting in this individual. One possible explanation is the presence of minimal change glomerulonephritis in which glomerular damage without obvious membrane change is present.³ Electron microscopy and immunohistochemical staining of kidney samples are needed to evaluate the full extent of glomerular lesions. Extraglomerular sources of urine protein and contamination^{3,4} were not believed to be contributing to urine protein value measurements but cannot be completely ruled out. Urine obtained in the same manner from a normal conspecific consistently had UPC <1.0.

The renal vascular and cardiac changes seen at necropsy and on histopathology were supportive of possible hypertension.⁸ Hypertension is commonly seen with renal disease but is defined in antemortem testing.¹⁰ There is value to measuring and recording blood pressure in individual animals using a standard site-specific protocol. Evaluating pressure trends over time contributes to better monitoring of disease development as well as response to treatment. In this case, we only obtained one value so were unable to evaluate a trend.

Renal biopsy is needed to definitively diagnose renal disease ante-mortem, however obtaining a renal biopsy in small patients can be associated with severe hemorrhage and ischemic injury to the kidney and would be challenging in this species.⁴ Investigation of microsurgical or laparoscopy techniques may be warranted for this procedure.

Behavioral training was essential in case management. Weights could be obtained frequently and daily training sessions facilitated urine collection and medication administration.

Conclusion

Given the reported prevalence of renal disease in this species, regular monitoring for proteinuria is warranted. Urine can be collected reliably from awake animals with training. At the John G. Shedd Aquarium, UPC evaluation is currently performed monthly. Consistent elevations in protein warrant closer evaluation for renal disease. Early diagnosis may be helpful in improving quality of life, prolonging life, and better defining disease syndromes in this species.^{4,5}

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Table 1. Selected CBC and serum chemistry values for a pygmy marmoset (*Callithrix pygmaea*).

Date	Hct ^a (%)	Creatine (mg/dl)	BUN ^b (mg/dl)	Albumin (g/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)
3 April 2002	44.0	0.8	72	3.6	6.8	5.6
18 April 2002	24.7	0.9	81	1.5	6.6	5.1
16 May 2002	26.0	2.6	100	3.7	8.2	9.1
Normal range ^c	39.4-49.4	0.2-0.6	10-22	3.3-5.3	8.2-10.4	2.8-8.8

^aHematocrit.

^bBlood urea nitrogen.

^cInternational Species Information System, Apple Valley, Minnesota, 55124 USA; *Callithrix pygmaea*; physiologic reference ranges for males >2 yr old, March 2002.

Table 2. Selected urine values for a pygmy marmoset (*Callithrix pygmaea*).

Date	USG ^a	Protein (mg/dl)	Leukocytes	Blood	Sediment	pH	UPC
6 April 2002	1.010	500	neg ^b	neg	No cells seen	7	---
9 April 2002	1.015	500	neg	---	---	6	---
15 April 2002	---	---	---	---	---	---	13.6
16 April 2002	1.013	---	---	neg	0-2 WBC/hpf ^c	7	---
18 April 2002	1.012	neg	---	neg	0-2 WBC/hpf 0-2 RBC/hpf ^d	7	---
15 May 2002	---	---	---	---	---	---	7.1
18 May 2002	1.012	---	---	---	---	---	4.7
18 June 2002	1.015	---	---	neg	---	---	18.7

^aUrine specific gravity.

^bNegative.

^cWhite blood cells/high power field.

^dRed blood cells/high power field.

EVALUATION OF BLOOD LIPID PROFILES AS PART OF A COMPLETE CARDIAC ASSESSMENT IN WESTERN LOWLAND GORILLAS (*Gorilla gorilla gorilla*)

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Abstract

As much as 41% of mortality in captive adult lowland gorillas has been attributed to cardiovascular disease.¹² Hypercholesterolemia has been considered a likely etiologic factor in the development of heart disease in gorillas,^{8,15} but the definition of what constitutes hypercholesterolemia in this species is uncertain.^{1,6,11} In humans, treatment is recommended for cholesterol levels greater than 200 mg/dl.¹³ The average cholesterol level in gorillas, however, is over 280 mg/dl.^{5,11} In human patients, hypercholesterolemia is an important factor in the development of atherosclerosis and subsequent coronary heart disease, with the most common clinical sequelae being myocardial and cerebral infarction.^{3,9,14} Atherosclerosis in gorillas has been reported with some frequency,^{4,8,15,17} but has only rarely been directly associated with mortality.^{4,15} Furthermore, the incidence of atherosclerosis in gorillas has not previously been correlated with hypercholesterolemia.¹ This study describes lipid profile results and proposes a possible correlation between specific lipid levels and risk of atherosclerosis in gorillas.

Fifteen gorillas (five males and ten females), ranging in age from 3-40 yr, were evaluated by blood lipid profile and transesophageal echocardiography. The lipid profile included total cholesterol, high-density lipoprotein (HDL), cholesterol/HDL (Chol/HDL) ratio, low-density lipoprotein (LDL), apolipoprotein B (Apo B), apolipoprotein A1 (Apo A1), apolipoprotein B/A1 ratio, and triglyceride. Transesophageal echocardiography (Hewlett-Packard Sonos 1000, 5 MHz transesophageal probe, Hewlett-Packard, Melville, NY 11747 USA) was performed only on animals greater than 5 yr, including two males (30 and 40 yr old) and eight females (9-36 yr old).

Transesophageal echocardiography demonstrated five animals with atherosclerotic plaques in the descending aorta. One 40-yr-old male was found to have a grade II atheroma, and four females, aged 10, 26, 27, and 36 yr, had grade I atheromas (based on published grading system).⁷ Animals were identified as "affected" or "unaffected" based on presence of plaques. For the purposes of statistical analysis, the five youngest animals that were not examined with echocardiography are included in the "unaffected" group. It is extremely unlikely that these juveniles would have

atheromas.

Average total cholesterol for this population was 261.9 ± 64.5 ($n = 15$). This is consistent with previously reported cholesterol values for gorillas (284 ± 83 , $n = 863$).⁵ For all animals, there was a negative correlation of HDL levels with age (Pearson product moment correlation, -0.72 , $P = 0.002$). There was a positive correlation of both Chol/HDL (0.72 , $P = 0.002$) and Apo B/A1 (0.69 , $P = 0.004$) with age. There were significant differences in age (unpaired t-test, $P = 0.007$), Chol/HDL ratios (Mann-Whitney rank sum test, $P = .023$), and Apo B/A1 ratios (Mann-Whitney rank sum test, $P = 0.027$) between affected and unaffected groups. No significant correlations were found for LDL or triglycerides with age nor were there significant differences between affected and unaffected groups for total cholesterol or any other individually measured lipid (significance defined at $P < 0.05$).

In human patients, evaluations of lipid profiles are useful in predicting risk of coronary heart disease.^{2,10,13,18} Thus, it might follow that lipid profiles are also useful in predicting risk of disease in gorillas. In this study, using atherosclerosis as an indicator of the presence of coronary heart disease, elevated ratios of Apo B/A1 and Chol/HDL, were the most significant. This is consistent with what is reported in human literature.^{2,10,18} HDL is considered protective against atherogenesis.¹³ HDL transports cholesterol out of cells and may also inhibit oxidation and accumulation of LDL, which transports lipids into cells and promotes atherogenesis and thrombus formation. Indeed, low HDL is itself a strong independent predictor of coronary heart disease in humans.^{2,13} In this group of gorillas, as age increased, HDL decreased without significant concomitant change of LDL. The resultant increase of Chol/HDL ratio appears to contribute to development of atherosclerosis. Apolipoproteins are components of the lipoproteins, with Apo A1 associated with HDL and Apo B associated with LDL. As their relationships suggest, Apo A1 is considered protective and Apo B is considered to contribute to risk.

This study suggests that evaluation of complete lipid profiles as part of a comprehensive cardiac evaluation in gorillas may be valuable. It is misleading to evaluate total cholesterol alone as an indicator of coronary health. There was no significant difference in total cholesterol between the affected and unaffected groups. The findings reported here may imply a relationship between specific lipid ratios and possible correlation to risk of coronary disease. As more data becomes available from a greater number of animals these conclusions may be challenged. It is the hope of the authors that this study be used as a starting point to increase the database of measured lipid profiles and to increase understanding of hypercholesterolemia in gorillas.

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UNEXPECTED HYPOTHERMIA INDUCED DEATHS IN GUINEA BABOONS (*Papio hamadryas papio*)

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Abstract

In November 2003 one female and four male Guinea baboons (*Papio hamadryas papio*) died over the course of 6 days at Brookfield Zoo. The animals were in good nutritional condition with no significant previous medical history. Their ages ranged from 13-23 yr and they were housed with 40 other baboons in a single species exhibit. Their habitat consisted of an island where they had access to warmed outcroppings on a gunite hill and a large heated multi-room holding building providing food, water and shelter. The animals are fed inside the shelter to allow animal care staff to observe the baboons on a daily basis. Despite having access to a heated shelter at all times, several baboons in subordinate groups spend more time outdoors due to their social grouping than other more dominant animals. The animals that died belonged to two small subgroups in this population of baboons. The female and one male made up one subgroup and three males made up another.

Animal keepers observed very brief periods of lethargy, ataxia and abnormal vocalizations prior to death in several animals. Review of the pathology reports from 1972-2002 for adult baboons (> 6 yr) in this colony showed that the mean and median ages at the time of death were 16.9 yr and 17.5 yr respectively. Four of the five dead baboons were at least 2 yr older than the historic median age of death for this species at the Brookfield Zoo. All five baboons that died had some age related lesions at necropsy but no specific findings to confirm the cause of death. During the gross necropsy it was noted that stomach contents of four of the baboons contained orange food material two of which had a strong citrus or alcohol-like odor. Postmortem testing ruled out alcohol intoxication from possible fermenting pumpkins and fruit offered as enrichment items. Concerns of carbon monoxide poisoning from a furnace heating the island exhibit were ruled out with postmortem hematologic carboxyhemoglobin, methemoglobin and oxygen saturation testing. Gas chromatography/mass spectroscopy for toxicologic analysis was also negative. Histologically, all five baboons had pancreatic islet amyloidosis and postmortem serum biochemical analysis on two animals revealed hyperglycemia (716 mg/dl, 484 mg/dl within 3 hr postmortem) suggestive of diabetes.²

The average temperature for the month of November in 2002 was 37.3 °F.⁷ All baboon deaths occurred on days where the mean temperature was below average for the month (Table 1). A

mortality review of this species at Brookfield Zoo identified a previous incident of acute deaths in January 1995 during which the average temperature was 11.5 °F. Interestingly, prior to both mortality events, there was a 10 °F temperature drop in the average daily temperature over 2 days prior to the baboon deaths. This was the largest drop in average temperature in November 2002 and January 1995.⁷ Furthermore, solar radiation, which can provide significant warmth, was below average on the days during which baboons died in both 2003 and 1995.⁷ Animals and people can tolerate very cold weather if the change is gradual and there is sufficient time to acclimate. However sudden decreases in temperature, even if temperatures are still above freezing, can result in hypothermia. It is because of this that hypothermia cases are particularly common during the late fall and early winter months.

Predisposing factors for hypothermia in humans include underlying disease, alcoholism, liver damage, gender (male > female), age and lesions limiting a person's mobility.^{3,4,6} In animals thermoregulatory responses may be inadequate in neonates, geriatric animals, hypothyroid and anesthetized animals.^{1,5} The animals in this report likely had a combination of predisposing factors that contributed to their deaths. All five of the baboons were geriatric, two may have been diabetic and it appears probable that sociologic factors may have contributed as well.

Diagnosis of hypothermia in living patients can be made through measuring core-body temperature, pathognomonic ECG abnormalities (lengthening of PR, QRS, and QT intervals) and clinical signs.^{1,5} Diagnosis of hypothermia postmortem is much more difficult and is based on exclusion of other causes of death taking into account the animal's history and its environment.^{3,4,6} Lack of gross necropsy, histopathologic, and toxicologic findings combined with compatible temperature and weather data lead us to believe that hypothermia was a plausible cause of death in these baboons. The management practices and exhibit of this aging baboon population are being closely evaluated in an effort to prevent similar incidents in the future.

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Table 1. Temperature data in relationship to baboon deaths.

Date	Animal deaths (sex, age)	High Temp (°F)	Low Temp (°F)	Mean Temp (°F)
16 Nov 2002	(F, 19 yr) (M, 22 yr)	36	31	34
18 Nov 2002	(M, 13 yr)	45	24	35
22 Nov 2002	(M, 23 yr) (M, 21 yr)	37	24	31

WEST NILE VIRUS ENCEPHALITIS IN A BARBARY MACAQUE (*Macaca sylvanus*)

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Abstract

Acute neurologic disease occurred in a 25-yr-old male Barbary macaque (*Macaca sylvanus*) at the Toronto Zoo, on August 17th 2002. The animal was housed with 10 other Barbary macaques, in an outdoor exhibit with access to indoor overnight housing. Clinical signs included severe ataxia, unresponsiveness to surroundings and bilateral nystagmus. Due to unrelated chronic intercurrent disease and the severe neurologic signs the animal was euthanatized. Gross lesions consisted of moderate gingivitis, generalized severe muscle atrophy, and severe chronic bilateral femorotibial osteoarthritis. Microscopically there was severe nonsuppurative meningoencephalitis, characterized by generalized gliosis, scattered glial nodules, and perivascular lymphoplasmacytic cuffs. Immunohistochemistry for West Nile virus (WNV) was positive, and the diagnosis was confirmed by reverse transcriptase-PCR amplification of viral genome and by isolation of WNV from the brain.

The Barbary macaque was the first clinical case of WNV infection recognized at the Toronto Zoo. To our knowledge this is also the first case of naturally occurring WNV infection in a primate in North America. The animal was infected in mid-August, at which time the prevalence of WNV was high in southern Ontario. All other zoo species affected were birds, and included a black-billed magpie (*Pica pica*), a red-tailed hawk (*Buteo jamaicensis*), two green-naped lorikeets (*Trichoglossus haematodus haematodus*), two American kestrels (*Falco sparverius*), five loggerhead shrikes (*Lanius ludovicianus*) and an American flamingo (*Phoenicopterus ruber ruber*). They subsequently presented with WNV-associated neurologic signs or sudden death

PHARMACOKINETICS OF MARBOFLOXACIN IN THE BLUE AND GOLD MACAW (*Ara ararauna*)

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Abstract

Because there is a lack of approved pharmaceutical agents in the United States for pet birds there has been an increased interest in the clinical pharmacology of pet bird species in recent years to insure proper dosing and treatment of these animals. Marbofloxacin is a synthetic antimicrobial agent of the fluoroquinolone class. This drug is safe and efficacious, and is approved for the treatment of skin and soft tissue infections in dogs and cats, and urinary tract infections in dogs. There have been no pharmacokinetic studies on marbofloxacin in pet birds. Although the pharmacokinetics of marbofloxacin in broiler chickens and Eurasian buzzards (*Buteo buteo*) have been determined, there have been no pharmacokinetic studies of marbofloxacin in pet birds.

Because antimicrobial management of bacterial infections is one of the most challenging aspects of therapy encountered in avian medicine, a study was designed to determine the pharmacokinetics of marbofloxacin in blue and gold macaws (*Ara ararauna*), a species commonly kept both as a companion animal and as a display animal in zoological collections. Marbofloxacin (2.5 mg/kg) was administered orally via crop gavage to five birds and intravenously to five birds, and blood samples obtained at 0, 0.5, 1, 3, 6, 12, 24, 48, 72, and 96 hr post-marbofloxacin administration. Following a 4-wk wash-out period, the study was repeated, with the first five birds receiving the intravenous dose and the second five birds receiving the oral dose. The data derived from this study was used to calculate therapeutic dosage regimes for treating infectious disease.

ACKNOWLEDGMENTS

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THE ABSORPTION AND DISTRIBUTION OF CEFTIOFUR IN ASIAN ELEPHANTS (*Elephas maximus*)

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Abstract

Captive elephants are prone to infections of the feet, lungs and skin as evidenced by clinical and necropsy reports.³ Antimicrobials are commonly used to treat these infections with or without culture and sensitivity information. Often treatment regimens are established with no pharmacokinetic data on the agents being used in these species. Many times antimicrobial dosage regimens are extrapolated from equine doses or are allometrically scaled from other domestic species dosage regimens. There are only a few antimicrobials that have been studied for pharmacokinetic information in the elephant. These include trimethoprim-sulfadiazine, oxytetracycline, amikacin, amoxicillin, penicillin, and ampicillin.^{1,2,5,7,8} When treating animals without the benefit of pharmacokinetic information, there is risk of either overdosing or underdosing the medication to the animal resulting in adverse effects or ineffective dosing and the possibility of developing antimicrobial resistant infections. More treatment options are needed that are based on scientific information in order to treat these appropriately.

A pharmacokinetic study using ceftiofur sodium (Naxcel, Pharmacia & Upjohn Company, Kalamazoo, MI 49001 USA) was performed in our captive Asian elephants (*Elephas maximus*) at Busch Gardens in Tampa, Florida. Health status was determined by physical condition, complete blood counts, and serum chemistry parameters.

Elephants were randomly assigned to either an intravenous (i.v.) or an intramuscular (i.m.) administration group for the first administration. At the end of this phase the animals were not used again for a minimum of 3 wk to ensure that the ceftiofur had cleared their system. Then the groups were switched so that the group receiving the i.v. injections on the first set of trials received the i.m. injections the second time around and vice versa. The dosage of ceftiofur administered by either route was 1.1 mg/kg based on the recommended dosage ranges for cattle and horses.⁶

Blood samples were collected just prior to drug administration and at 0.33, 0.67, 1, 1.5, 2, 4, 8, 12, 24, 48, and 72 hr postadministration. Samples were collected in lithium heparin tubes, centrifuged within 10 min after collection, the plasma separated into cryovials and these aliquots frozen at -80°C until assayed. Ceftiofur analysis was performed using a validated liquid chromatography/mass spectrophotometry assay.

Analysis showed a steady decline in plasma levels for both i.v. and i.m. administrations through the first 12 hr. The i.v. dosing samples showed further decline through 24 hr. Levels at 48 and 72 hr were below detectable limits for this assay.

The study overall shows a similar pattern in decline of plasma levels between i.m. and i.v. administration of ceftiofur sodium in these elephants. Plasma concentrations for the i.m. samples were lower than expected. This likely could be adjusted by administering ceftiofur at a higher dosage rate of 2.2 mg/kg. This would result in an extremely large injection volume and necessitate increasing the number of injection sites. Efficacy of this drug at this dose is also dependent on the MIC of the target organism.

Results indicate that ceftiofur used at 1.1 mg/kg i.m. could be useful in elephants when given 2-3 times per day depending upon the type and location of infection.

ACKNOWLEDGMENTS

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FAMCICLOVIR PHARMACOKINETICS IN YOUNG ASIAN ELEPHANTS (*Elephas maximus*)

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Abstract

Asian elephants (*Elephas maximus*) are susceptible to a unique infection caused by elephant endotheliotropic herpesvirus (EEHV).^{3,4} Worldwide, between the years 1983 and 2000, there have been 26 confirmed deaths from this virus in Asian elephants.² Although most cases have been fatal, treatment with famciclovir (Famvir, SmithKline Beecham Pharmaceuticals, Philadelphia, PA 19101 USA) has been associated with survival in three of six cases of EEHV infection proven by PCR.^{2,5,6} Dose selections for surviving elephants (5.5-8.0 mg/kg, p.o. every 8 hr) were made without the benefit of elephant pharmacokinetics, and were a direct extrapolation from recommended human dosages (7 mg/kg, p.o. every 8 hr).^{5,6} In this study, famciclovir was administered both orally and rectally in healthy young Asian elephants. The doses tested in this study were 5 mg/kg orally, 5 mg/kg rectally, and 15 mg/kg rectally. Blood samples were analyzed for famciclovir and penciclovir using a validated LC/MS assay. Famciclovir was absorbed well by both routes and underwent rapid biotransformation to the active compound penciclovir. None of the plasma samples had detectable famciclovir. Pharmacokinetic parameters for penciclovir were determined using noncompartmental analysis. After an oral dose of 5 mg/kg the C_{max} was 1.3 $\mu\text{g/ml}$ with a T_{max} at 1.1 hr. After a rectal dose of 5 mg/kg the C_{max} was 1.2 $\mu\text{g/ml}$ with a T_{max} at 0.34 hr. After a rectal dose of 15 mg the $t_{1/2}$ was 2.6 h, with a C_{max} of 3.6 $\mu\text{g/ml}$ at T_{max} 0.66 hr. These results were similar to those reported in humans where an oral dose of 500 mg (7 mg/kg) had a $t_{1/2}$ of about 2 hr with a C_{max} of 3.3 $\mu\text{g/ml}$.¹ A dose range of 8-15 mg/kg given orally or rectally every 8 hr should produce penciclovir concentrations in Asian elephants that are considered therapeutic in humans.

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PHARMACOKINETICS OF ORALLY ADMINISTERED IBUPROFEN IN ELEPHANTS

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Abstract

Musculoskeletal disorders (e.g., trauma, arthritis) occur commonly in captive elephants, affecting 73% of the animals studied in 69 zoos in North America.¹ To treat these and other conditions, nonsteroidal anti-inflammatory agents (e.g., ibuprofen and phenylbutazone) are used strictly on an empirical basis in elephants. There is some indication that species differences in drug metabolism exist between African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants, although this has not been substantiated.² Determination of safe and therapeutic dosing regimens for ibuprofen and phenylbutazone will improve medical management of captive elephants by providing efficacious dosage regimens, improved control of pain, and prevention of potential toxic side effects resulting from improper drug administration.

The purpose of this study was to: 1) determine the pharmacokinetic parameters of ibuprofen administered per os in elephants, and 2) establish therapeutic dosage regimens for African (*Loxodonta africana*) and Asian (*Elephas maximus*) male and female elephants. Twenty healthy elephants (five males and five females of each species) housed in zoos throughout North America were used in this study. Pilot studies were conducted at the Oregon Zoo with Asian elephants using empirically derived dosing regimens and preceded each set of clinical trials to ensure that proper ranges for dosage and dosing frequency determinations would be utilized. Therapeutic dosage requirements were determined using 4, 5 and 6 mg/kg dosages in each animal, and blood samples were collected at -5, 15, 30, 45, 60 min, 1.5, 2, 4, 10, 12, 24 and 48 hr post-oral administration from superficial ear veins. Optimal dosing frequency was then determined by conducting 12- and 24-hr dosing interval trials, with blood samples collected hourly for 4 hr after each of three administrations, then every 6 hr plus 1 hr prior to the next administration. Washout periods between all trials were 3 wk in duration and allowed for complete elimination of residual drug metabolites.

Following administration of 4 mg/kg ibuprofen and a rapid absorption phase, mean ibuprofen serum concentrations peaked in African and Asian elephants at 4 hr at 16.75 ± 6.79 $\mu\text{g/ml}$ (mean $\pm$ SD). Five mg/kg dosages of ibuprofen resulted in peak serum concentrations of 17.20 ± 7.78 $\mu\text{g/ml}$, and with 6 mg/kg dosages, serum concentrations increased to 22.42 ± 12.30 $\mu\text{g/ml}$. Ibuprofen was eliminated with first-order kinetics characteristic of a single-compartment model with a half-life of 4-4.5 hr. The volume of distribution (V_d/F) was estimated to be 200.8 ± 101.17 ml/kg for African and 164.4 ± 34.60 ml/kg for Asian elephants. The doses used in this study with elephants resulted in serum concentrations at or above therapeutic concentrations for humans (15-30 mg/L) for up to 12 hr. Serum ibuprofen concentrations decreased to below 5 $\mu\text{g/ml}$ 24 hr postadministration in all

elephants. There were no statistically significant pharmacokinetic parameter differences between males and females of either species, and differences between African and Asian elephants existed but were not significant ($P < 0.12$). The mean AUC and $t_{1/2}$ life values for Asian elephants were higher as compared to African elephants, and the mean clearance and elimination rate constant were lower in Asian elephants as compared to African elephants. Ibuprofen administered at 6 mg/kg/12 hr for Asian elephants and at 7 mg/kg/12 hr for African elephants resulted in therapeutic serum concentrations of this anti-inflammatory agent.

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IMPORTATION OF UNAPPROVED DRUGS: HOW TO ORDER AZAPERONE AND CONCENTRATED MEDETOMIDINE

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Abstract

Azaperone is a tranquilizer with multiple applications in zoo medicine.¹ It is used as a part of sedative combinations in elephants, giraffe and rhinoceros. It has also been used to decrease aggression during introductions in a number of species. Concentrated (10 mg/ml) medetomidine facilitates immobilization of large carnivores and great apes.² The medetomidine available in the United States comes as 1 mg/ml. This more dilute formula means that large volumes of drug must be delivered if it is to be used on large animals. This is frequently not practical when anesthetic agents must be delivered via dart or hand injection.

These drugs are not manufactured in the United States and permits are required to import them. There is variable information available on how to apply for and receive permits. The purpose of this presentation is to clarify and streamline the process.

Stresnil (azaperone 50 mg/ml) is currently manufactured by Midwest Veterinary Distribution, 1600 Inkster Blvd., Winnipeg, Manitoba, Canada (800-356-4799).

The product Zalpoine (medetomidine 10 mg/ml) is manufactured by Orion Pharmaceuticals, PO Box 425, Fin-20101 Turku, Finland. They can be contacted via www.orionpharma.com.

The permit required is a letter allowing "Medically Necessary Personal Veterinary Imports". It is issued by the Department of Health and Human Services, Food and Drug Administration, Office of Surveillance and Compliance. The permit application is submitted to:

Toni Wooten
Division of Compliance, HFV-236
Center for Veterinary
Food and Drug Administration
Metro Park North
7500 Standish Place
Rockville, Maryland 20855
FAX 301-594-1812
Phone 301-827-1168

The supervisory person in the office is Kim Young. He can be contacted at:
301-827-3353

The following information must be submitted to apply for the permit:

1. Veterinarian's name, address and phone number
2. Clinic name and address
3. Client's name and address
4. Patient name and nonfood species
5. Name of drug
6. Drug family or class
7. Name and address of drug supplier
8. Legal status of the drug in the foreign country
9. Amount of drug to be imported – must be small noncommercial quantities
10. Disease condition to be treated
11. Reason why an approved human or animal drug will not treat the disease condition
12. A statement that:
 - a. You will notify the animal owner that the drug is not approved
 - b. That the drug will not be used in any food animal
 - c. And that you agree to notify the FDA if there are any adverse reactions
13. How did you learn of the existence of this drug?
 - a. The FDA wants to verify that foreign drugs are not actively promoted in U.S. markets.
14. The veterinarian must sign at the end of the submitted information.

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DYSTOCIA IN AN AFRICAN ELEPHANT (*Loxodonta africana*)

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Abstract

A 24-yr-old African elephant (*Loxodonta africana*) was inseminated on 2 December 2000 and again on 4 December 2000. Pregnancy was confirmed on 21 January 2001 with an ultrasound evaluation, and elevated progesterone levels of over a 12- to 16-wk period supported the diagnosis. The pregnancy progressed without complication. Ultrasounds confirmed calf growth and movement throughout the pregnancy.

On 5 October 2002, 673 days postinsemination, the cow passed the mucous plug in the evening. The chorio-allantois ruptured shortly after that. There were a few modest contractions over a period of about 3 hr, which then ceased. Fetal front feet could be palpated in the birth canal, just into the cranial pelvis. Fetal circulation was confirmed using color doppler ultrasound of the fetal extremities. Oxytocin was administered on 6 October 2002. The first two doses resulted in a few good contractions. Fetal feet entered further into the pelvis, and fetal viability was confirmed again with color doppler ultrasound. Oxytocin was administered three more times resulting in a few small contractions and some stretching. One dose of 110 IU oxytocin resulted in one large contraction, subsequent doses of oxytocin did not yield any appreciable additional efforts. That evening while walking the elephant, the amniotic sac broke releasing several liters of fluid. Blood was drawn and serum chemistries were within normal range. On 7 October 2002 the uterus did not respond to oxytocin. Color doppler ultrasound of the fetal legs revealed no discernable fetal circulation. Antibiotics and supportive care were initiated. For the next week the feet were easily palpated within the pelvis. For the several days the elephant was stiff and moderately depressed. Her appetite was good and she was drinking water. The elephant was given another dose of oxytocin on 15 October 2002, which resulted in a few moderate contractions, but no progress was made in delivering the calf. The next day the elephant seemed more depressed and uncomfortable. Her appetite and water intake were markedly reduced and within 2 days she exhibited what appeared to be abdominal pain. The elephant was treated aggressively with antibiotics and fluids for several weeks. Severe peritonitis was confirmed during a laparoscopic procedure and the elephant was euthanatized. The postmortem examination revealed a tear in the uterus and a normal-sized fetus in normal presentation in the birth canal. The fetus however was abnormal and had arthrogryposis affecting all four limbs to varying degrees. Both rear limbs of the fetus were particularly affected and the joints of those limbs were bent in an abnormal way, anchoring the fetus inside the dam. The arthrogryposis of the calf was likely the cause of the dystocia, and was likely the contributing factor to the tear in the uterus.

FETOTOMY IN THE ELEPHANT

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Abstract

There are several reports about dystocia and its treatment in elephants maintained in captivity in western zoos and safari parks.¹⁻¹⁰ In the last 2 yr there has been a dramatic accumulation of reports about birth-associated problems (Table 1). Causes for this development are probably the intensified captive elephant breeding programs and the involvement of more older nulliparous cows.

Over the last 100 yr, there were more dystocia cases in captive Asian elephants (*Elephas maximus*) than in African elephants (*Loxodonta africana*).⁷ However, this difference seems to be abolished with the increasing number of pregnancies in African elephants to date. Increased percentage of dystocia cases in both species can have two consequences for captive management. First, older nulliparous cows will be strictly excluded from breeding in the future or second, management of the birth process will be improved.

Three of 10 cows died from the consequences of dystocia in western zoological institutions over the last 2 yr (Table 1). In total, there were eleven cases of fatal dystocia listed in the literature since 1972 (Table 2).⁵⁻⁷ Seven cesarians performed (Table 2) as the ultimate intervention to treat dystocia in elephants all ended with euthanasia or death of the females.

This paper describes a potential new way of birth management in the elephant, the fetotomy. The authors believe that the fetotomy is prospective tool that could be used to save the life of a dam with severe dystocia. This method is recommended by the authors as an ultimate tool besides all the other important preparations in pregnant cows for an upcoming birth. Most important classic preparations are the following: 1) intensive physical exercise, 2) optimization of body weight with a pregnancy-associated diet, and 3) training for safe handling in case of necessary intervention in free or protected contact. The fetotomy was never applied before in elephants because of the limited exploration field and the high degree of skeleton calcification in comparison to domestic and exotic hoofstock.

In this particular case it was first tried to remove the dead fetus by episiotomy.^{2-4,7,9,10} However, the episiotomy attempt failed due to he inability to extract a dead oversized or malpositioned fetus. From the two options to leave the dead fetus inside the uterus despite the surgically opened genital tract or to cut out parts of the fetus it was decided to perform a fetotomy. The initial hesitation to

perform such drastic and complicated procedure led to the complication of an emphysemic fetus and progressive peritonitis in the female even before the procedure started. The fetus was successfully removed in six parts using an extra long and durable carthorse embryotom (Thygesen's type). Unfortunately, the patient died 3 days later because of the treatment resistant peritonitis.

In conclusion, it was demonstrated that fetotomy can successfully be applied in elephants. The authors suggested in order to achieve a positive outcome for the dam to decide on such ultimate intervention earlier in the dystocia process. Active elephant birth management should always include preparation for dystocia treatment ranging from: 1) the administration of oxytocin, 2) rectal massage of the caudal birth channel, 3) episiotomy as the last option, and 4) fetotomy to save the life of the mother. Based on the complex preparation for an elephant birth and the knowledge about the huge range of potential complications it is advised to consult veterinary elephant specialists well in advance to ensure maximum expertise and experience.

ACKNOWLEDGMENTS

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Table 1. Dystocia cases in captive elephants in the last 2 yr.

Dam Year of Birth Reproductive Status	Cont.# Stud.#	Institution	Date of Birth/ Dystocia	Body Mass (Sex) Remarks
1983 Nulliparous	EM ^a 1 258	Burnet Park Zoo, NY USA	06 June 2001	Data not accessible. ^b Calf was stillborn with several health problems; dam survived.
1982 Nulliparous	EM 2 8209	Whipsnade, UK	01 March 2002	Data not available. ^c After intensive labor, calf was not expelled; treated dam survived.
1967 Multiparous	EM 3 80	Fort Worth Zoological Park, TX USA	13 March 2002	Data not accessible. ^b Treated dystocia, calf was stillborn; dam survived.
1982 Nulliparous	EM 4 8216	Whipsnade, UK	22 June 2002	120 kg (male) Episiotomy with fetotomy; treated dam died 12 days later.
1974 Nulliparous	EM 5 335	Riddle's Elephant Breeding Farm & Wildlife Sanctuary, AR USA	29 Jan 2003	170 kg (Gender data not accessible.) After intensive labor calf was not expelled; treated dam died 18 days later.
1968 Nulliparous	LA ^d 1 65	Kansas City Zoological Gardens, MO USA	19 Sept 2001	Data not available. ^c Calf was not expelled; treated dam survived.
1981 Nulliparous	LA 2 210	Toledo Zoological Gardens, OH USA	27 July 2002	Data not accessible. ^b Stuck in the caudal part of the vestibule; dam survived.
1978 Nulliparous	LA 3 371	Six Flagg, CA USA	01 Oct 2002	118 kg (female) Calf was not expelled; treated dam was euthanatized 56 days later.
1983 Nulliparous	LA 4 389	Six Flagg, CA USA	21 March 2003	127 kg (Gender data not accessible.) Calf was stillborn; dam survived.
1970 Nulliparous	LA 5 92	Disney's Animal Kingdom, OR USA	06 April 2003	Data not available. ^c Calf was not expelled.

^a*Elephas maximus*.^bData were not accessible for the authors when abstract was submitted.^cData not available because of the intrauterine fetus.^d*Loxodonta africana*.

Table 2. Known dystocia cases with a fatal outcome for the dam.

Dam Year of Birth Reproductive Status	Cont.# Stud.#	Institution	Date of Birth/ Dystocia	Body Mass (Sex) Remarks
1939 ^a Pluriparous	EM ^b I	Mysore Zoo, India	1972	Data not accessible. ^c Cesarian; dam died few hours later.
1963 ^a Nulliparous	EM II	Charkow Zoo, Ukraine, GUS	29 April 1979	Data not accessible. ^c Dam died during birth because macerated placenta and septic endometritis.
1970 ^a Nulliparous	EM III	San Diego, CA USA	20 Nov 1989	159 kg (male) Cesarian; dam died 11 days later.
1957 ^a Nulliparous	EM IV	Burnet Park Zoo, NY USA	23 May 1990	120 kg (male) Episiotomy continued with cesarian; dam died 19 days later.
1964 ^a Nulliparous	EM V	Dickerson Park Zoo, Springfield, MO USA	07 Sept 1992	95 kg (male) Episiotomy continued with cesarian; dam euthanatized after surgery.
1968 ^a Nulliparous	EM VI	Port Lympne, UK	11 June 1993	103 kg (male) First classic dystocia treatment; 10 days later a cesarian was performed; dam died 13 days after surgery.
1966 ^a Pluriparous	EM VII	Metro Zoo Miami (Ringling-Station- Williston), FL USA	May 1996	Data not accessible. ^c Episiotomy continued with cesarian; placental strangulation; euthanasia of the dam after surgery
1969 Nulliparous	EM VIII	Phoenix Zoo, AR USA	Nov 1998	120 kg (female) Episiotomy unsuccessful; cesarian performed 10 days later; dam euthanatized during surgery.
1982 Nulliparous	EM 4 8216	Whipsnade, UK	22 June 2002	120 kg (male) Episiotomy with fetotomy treated; dam died 12 days later.
1974 Nulliparous	EM 5 335	Riddle's Elephant Breeding Farm & Wildlife Sanctuary, AR USA	29 Jan 2003	170 kg (Data on gender not accessible.) After intensive labor calf was not expelled; treated dam died 18 days later.
1978 ^a Nulliparous	LA ^d 3 371	Six Flagg, CA USA	01 Oct 2002	118 kg (female) Calf was not expelled; treated dam was euthanatized 56 days later.

^aApproximate year of birth.^b*Elephas maximus*.^cData were not accessible for the authors when abstract was submitted.^d*Loxodonta africana*.

MANAGING DENTAL DISEASE IN AARDVARKS (*Orycteropus afer*)

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Abstract

The aardvark (*Orycteropus afer*) is the only living representative in the order Tubulidentata, family Orycteropodidae.² Tubulidentata means, “tube teeth” and refers to the unique tubules found within the teeth. The genus name means, “burrowing foot” another prominent feature of the aardvark. Aardvarks are completely nocturnal in the wild and feed primarily on ants and termites with their long sticky tongue. This hardy animal is currently found throughout much of sub-Saharan Africa wherever there is suitable habitat but once had a much larger geographic range reaching as far north as Europe.⁶

The aardvark is under CITES Appendix II yet many authors have reported that aardvarks have decreased in numbers.^{6,7} Their specialized diet may render them more vulnerable to habitat change caused by expanding human populations.⁸ In addition, they are also hunted for their meat, hide and claws.⁶ Today’s North American population consists of 26 animals held at 10 institutions. Although aardvarks in zoos are living longer, reproduction remains challenging as evidenced by a captive population, which has been decreasing since 1994. Due to the dwindling numbers of captive aardvarks, preventative medicine and veterinary care are even more important for this small North American population.

Brookfield Zoo’s (BFZ) first aardvark arrived in 1949. Since that time 23 aardvarks have been cared for at the zoo; 4 were wild caught and 15 were captive born (11 at Brookfield). In captivity their diet has primarily consisted of a gruel made from ground raw meat, eggs, milk, cereals, vitamin and mineral supplements. It is our experience at Brookfield that most of the aardvarks fed this diet go on to develop significant dental disease which has included caries formation, periodontal disease, and osteomyelitis. Attempts to approximate the animal’s natural diet using dried locusts, flies, ant pupae, crickets and mealworms have been unsuccessful.^{2,3}

The aardvark’s skull is elongated with a very small oral opening just large enough to allow its 30 cm tongue in and out. This unique anatomic feature makes routine dental examination and treatment especially difficult. The dental formula is I 0/0, C 0/0, P 2/2, M 3/3; additional vestigial premolars are sometimes present. In the adult the teeth are located only in the posterior part of the jaw. The deciduous teeth are numerous, traversed by several parallel vertical canals, and do not erupt. Aardvark’s teeth do not erupt simultaneously those nearest the front of the jaw develop first and often fall out as the animal ages. The cheek teeth are quite unusual, as they grow throughout life

and are composed of hexagonal prisms of dentin surrounding a tubular pulp cavity. This pattern of growth gives the order its name, Tubulidentata (tubule-toothed).^{1,6} The aardvark's teeth lack enamel, which may make them more susceptible to decay, and dental disease.

Previous reports discussing dental disease in aardvarks are few.³⁻⁵ At Brookfield Zoo dental disease seems more prevalent and difficult to treat in aardvarks compared to numerous other species. All three of the current adult aardvarks (two male, one female) at the zoo have been diagnosed and treated for dental disease. Both of the adult males required many months of treatment to resolve chronic mandibular osteomyelitis caused by complications associated with infected cheek teeth.

Aardvarks are darted with ketamine (Ketaset[®], Fort Dodge Animal Health, Fort Dodge, Iowa 50501 USA; 3 mg/kg) and medetomidine (Domitor[®], Pfizer Animal Health, Exton, Pennsylvania 19341 USA; 0.03 mg/kg) i.m., transported to the hospital, maintained on oxygen and supplemental isoflurane (Isoflo[®], Abbot Laboratories, North Chicago, Illinois, 60064 USA) and intubated (size 10 long endotracheal tube) as necessary for oral surgery. Initial evaluation includes routine physical examination, oral examination (prior to intubation), hematologic and biochemical evaluation, as well as skull radiographs.

Aardvarks with dental disease have occasionally shown symptoms including difficulty chewing, ptyalism, anorexia and pain. Due to their small mouth opening and the caudal location of the cheek teeth, oblique skull radiographs appear to be the most sensitive method to detect dental disease in this species. Early signs of tooth abscessation include subtle pocket formation between the molars and the jaw. Significant erosive lesions may develop and teeth can even be completely detached but are not able to fall out because the mouth does not open far enough. Both maxillary and mandibular cheek teeth are affected, however mandibular teeth are more commonly associated with osteomyelitis. The first clinical sign of osteomyelitis has been a slow growing, firm, ventral, mandibular mass with or without a draining tract. Radiographs at this site may reveal abscessed teeth, bony lysis, periosteal proliferation, cyst formation, bony sequestra, fractures and retained tooth roots. Loose teeth can occasionally be removed with extra long forceps (before endotracheal tube placement). More frequently, diseased teeth are sclerosed to surrounding bone and a buccal approach is required for extraction. Removal of retained tooth roots, bony sequestra, cyst ablation and debridement of proliferative lesions have required a ventral approach to the mandible. Curettage and debridement of mandibular lesions is accomplished with ronguers and a round bur on a high-speed dental hand piece. Placement of ticarcillin impregnated methylmethacrylate beads within the mandibular lesions appears to significantly aid in treatment of osteomyelitis.

Purulent malodorous material is routinely associated with the roots of affected teeth and bacteria isolated include: *Arcanobacterium* sp., *Bacteroides* sp., *Streptococcus* sp., *Staphylococcus* sp., *Enterococcus* sp., *Aerococcus* sp. *Arcanobacterium pyogenes* associated infections have been the most difficult to resolve and required long term antibiotic therapy, dental extraction, and multiple surgeries to remove bony sequestra. Antibiotics used to treat aardvarks with dental disease include: amoxicillin ($\pm$ clavulanic acid), enrofloxacin, clindamycin, ceftiofur, metronidazole and tetracycline. Possible complications encountered during the treatment of dental disease in aardvarks include

lingual swelling, hypoglossal nerve damage with subsequent tongue tip trauma necessitating amputation (secondary to attempts at retrograde pharyngeal intubation), and cheek wound dehiscence. The tongue begins to swell if it remains outside the oral cavity for even short periods of time and should be maintained within the mouth during dental procedures. Retrograde laryngeal intubation is not necessary as tracheal intubation can be done blindly with the animal in lateral recumbency with its head extended if at an appropriate depth of anesthesia. Auscult respirations and advance the endotracheal tube into the larynx at inspiration. Secure endotracheal tube to the mandible since the animals appear to be obligate nasal breathers.

Recently the aardvark diet has been changed at BFZ to eliminate the raw ground meat, which got caught in between teeth and likely contained high levels of bacteria. The diet now consists of ground dry cat food and leaf eater monkey biscuit mixed with water. It is our hope that the change in the aardvark's diet at Brookfield Zoo will help decrease the incidence of dental disease in this species.

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CALCIUM OXALATE UROLITHIASIS IN FOUR CAPTIVE MACROPODS

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Abstract

Urolithiasis, although a relatively common clinical entity in humans and domestic carnivores, has been infrequently reported in native Australian marsupials. Calcium oxalate urolithiasis has not previously been reported in macropods. We report the occurrence of predominantly calcium oxalate urolithiasis in four captive macropods in a large urban zoo. Two of the cases presented with obstructive urethropathy and, in the other two cases, uroliths were discovered incidentally at necropsy. The approach to the two clinical cases was based on the first principles of management of obstructive uropathy in domestic animals modified to accommodate some of the unique aspects of macropods. Potential causes of calcium oxalate urolithiasis and strategies for risk reduction in macropods are discussed.

Case Studies

Case 1

A 49.5-kg, 7-yr-old, castrated male red kangaroo (*Macropus rufus*) presented with sudden onset inappetence and restlessness. Dribbling of urine and frequent cloacal grooming were observed. The animal was anesthetized with 100 µg/kg i.m. medetomidine (Domitor, Novartis Animal Health Australasia Pty Ltd; NSW, Australia) and 3 mg/kg i.m. ketamine (Ketamil, Ilium Veterinary Products; NSW, Australia) and maintained on isoflurane (Forthane, Abbott Australasia; NSW, Australia) and oxygen delivered by mask.

A blood sample was taken and intravenous fluid therapy was initiated. Abdominal radiography revealed abundant intra-intestinal gas. Although the animal's conformation precluded bladder palpation, abdominal ultrasonography revealed bladder distension. A dorsoventral pelvic radiograph with the beam directed obliquely from cranial to caudal revealed an irregular radiodense mass just caudal to the ischial arch slightly to the left of the midline. A gritty mass was palpated within the pelvic urethra via a digital rectal examination just anterior to the level of the anus. A diagnosis of obstructive urethral urolithiasis was made. Serosanguinous urine (1.8 L) was aspirated from the bladder by cystocentesis and an attempt was made to pass a urinary catheter. The catheter could not be passed beyond a point approximately 4 cm proximal to the external urethral orifice. This point was several cm distal to the level of the palpable urethral obstruction. Urohydropropulsion was unsuccessful in dislodging the urolith.

Abdominocentesis revealed free fluid in the abdominal cavity and a decision was made to euthanize

the animal. Hematology was unremarkable, the white cell count was $12 \times 10^9/L$. A PCV of 42% was assessed to be slightly elevated reflecting dehydration. Serum biochemistry and electrolyte concentrations were within normal ranges. Urinalysis revealed pyuria, hematuria, and amorphous phosphates and USG was 1.033. Uroperitoneum and an associated diffuse peritonitis were confirmed during postmortem examination. A single, irregular urolith was retrieved from the urethra. The bladder was markedly distended and there was significant segmental transmural necrosis. A urinary catheter could not be advanced beyond the penile urethra at necropsy and dissection of the structure revealed that the catheter was consistently lodging in a dorsal diverticulum of the urethra, 4 cm proximal to the external urethral orifice. No additional uroliths were found. Analysis of the urolith by semi-quantitative methods revealed it to be 55% calcium, 10% oxalate, 5% phosphate, 2% magnesium and 28% organic constituents.

Case 2

A captive, castrated male, 32.2-kg, 9-yr-old western grey kangaroo (*Macropus fuliginosus*) was noted to be depressed, weak and ataxic. There was clinical dehydration and a pain response to abdominal palpation. The animal was premedicated with 15 mg diazepam i.m. (Pamlin Injection, Parnell Laboratories Aust Pty Ltd; NSW, Australia) and anesthesia was induced and maintained with isoflurane and oxygen administered by mask.

A blood sample was taken and intravenous fluid therapy with 0.9 % sodium chloride initiated. Antibiotic therapy was also initiated with 350 mg amoxicillin/clavulanic acid i.m. (Clavulox Injectable, Pfizer Animal Health; NSW Australia). A hard obstruction was palpable in the penile urethra 8 cm proximal to the external urethral orifice just caudal to the ischial arch. A urinary catheter could not be passed beyond a point 4 cm proximal to the external urethral orifice. Ultrasonography revealed an apparently intact bladder, partially filled with urine. The animal was moderately azotemic (BUN 35 mmol/L), dehydrated (PCV 56%, TPP 81 g/L) and had elevated serum concentrations of CK (3,178 mmol/L). A smooth yellow/grey bean shaped calculus was removed from the urethra surgically. The penis was extended caudally and a longitudinal midline approach made from the ventral aspect over the urolith. The surgical wound was left open to granulate. The animal was not observed to pass urine over the ensuing 24 hr and the animal subsequently died.

Postmortem examination of the animal revealed marked distension and necrosis of the bladder wall, hydronephrosis of the left kidney and multiple red foci within the renal cortices. There was a small volume of serosanguinous urine within the abdominal cavity and a diffuse peritonitis. There was marked distension and necrosis of the bladder, which contained sanguinous urine. Crystals were not evident upon microscopic examination of urine sediment. Attempts to pass a urinary catheter at necropsy demonstrated the presence of a similar urethral diverticulum as described in the animal described above. Histopathology revealed pyelonephritis and an acute necrotizing enteritis. Bacterial culture of several organs identified the presence of *E. coli* and *Aeromonas hydrophila* supporting a diagnosis of terminal septicemia. The calculus was composed of 75% calcium, 10% oxalate, 5% magnesium, 5% phosphate and 5% organic material.

Case 3

A female red-necked wallaby (*Macropus rufogriseus banksianus*) was euthanatized due to poor body condition and progression of mandibular osteomyelitis despite an intensive 5-wk course of antibiotic therapy. Repeated hematologic and serum biochemistry analyses had revealed no abnormal findings. In addition to right mandibular osteomyelitis, postmortem examination revealed bilateral nephropathy. Both kidneys had undulating capsular surfaces with multiple small white, fibrous foci. On cut section, the renal medullae were thickened and white, surrounding a series of irregularly shaped stones. The ureters were patent and no stones were evident within the lower urinary tract. Histologic examination of the renal parenchyma revealed multifocally extensive renal interstitial fibrosis, multifocal glomerular sclerosis, and mild nonsuppurative interstitial nephritis. Analysis of the calculus by semi-quantitative methods revealed it to be 40% calcium, 5% magnesium, and 20% oxalate.

Case 4

A female eastern grey kangaroo (*Macropus giganteus giganteus*) was euthanatized due to failure to respond to a 16-wk regimen to treat a necrotizing soft tissue injury to the right antebrachium and secondary ulceration of the left plantar tarsal footpad. The animal's wounds were debrided and topically treated during a series of weekly general anesthetics. The kangaroo had also received several courses of parenteral and oral antibiotic therapy. In addition to the wounds noted above, necropsy revealed unilateral irregularity of the capsular surface of the left kidney, pallor and fibrosis of the left renal cortex, bilateral renal medullary fibrosis, and bilateral distension of the renal pelvises with large, firm, tan irregularly shaped concretions. Serum removed from blood collected during the necropsy contained concentrations of urea, creatinine and phosphorus within normal ranges.

Discussion

Urinary calculi form as a result of the precipitation and concretion of salts within the urinary tract. Generally, an individual is predisposed to the precipitation of specific salts where they are present in relative excess, exceeding the capacity of urine to maintain them in a state of supersaturation. Additional risk factors may also be involved in the pathogenesis of urolithiasis.

The pathogenesis of calcium oxalate urolithiasis has been most extensively studied in humans and domestic dogs. In humans, hypercalciuria and/or hyperoxaluria are significant predisposing factors whereas affected dogs are more likely to have hypercalciuria with normoxaluria.^{5,7}

Generally, hyperoxaluria may occur secondary to:

- Increased production of metabolic oxalate. Heritable disorders of glycine metabolism in humans have been described that result in hyperoxaluria.¹
- Increased intake of oxalate containing foodstuffs (e.g., oxalate-associated disease in ruminants

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- is usually associated with the ingestion of oxalate-rich herbage⁶)
 - Inappropriately increased intestinal uptake of dietary oxalate as occurs secondary to many enteric diseases in humans.⁷
 - Inappropriately increased renal tubular excretion of oxalate. A cytoplasmic membrane defect in humans that causes abnormal transport of oxalate across renal tubular cells resulting in a persistent mild hyperoxaluria has been described.⁴

Although hypercalciuria occurs secondary to diseases characterized by hypercalcemia, canine hypercalciuria is more commonly associated with normocalcemia. In dogs, normocalcemic hypercalciuria is usually due to intestinal hyperabsorption of calcium ('absorption hypercalciuria') wherein hypercalciuria is only present postprandially.⁵ Less commonly, canine normocalcemic hypercalciuria is associated with a failure of appropriate renal reabsorption of calcium in the distal tubule ('renal leak hypercalciuria') wherein hypercalciuria is consistently present.⁵

Although it is not currently known whether macropods are susceptible to hypercalciuria or hyperoxaluria by means of the mechanisms outlined above, it is likely that a urinary excess of one or both of these salts was involved in the development of urolithiasis in the cases described here. Captive macropods are often fed rations relatively high in calcium and vitamin D which may predispose them to absorption hypercalciuria and the subsequent development of calcium oxalate urolithiasis. Additional known predisposing factors for human calcium oxalate urolithiasis include reduced urine volume, hyperuricosuria, low urinary pH and low urinary levels of various crystallization inhibitors including citrate and magnesium.⁵ Desquamated epithelial cells are common nidi for the formation of uroliths and conditions that result in increased epithelial desquamation (e.g., hypovitaminosis A) may also increase the likelihood of urolithiasis where other predisposing conditions are present. The presence of greater than normal levels of urinary mucoprotein, as may occur in animals on a high concentrate/low roughage ration, may also favor the development of urolithiasis in predisposed individuals. It is suspected that some of these factors may also involve in the development of calcium oxalate urolithiasis in captive macropods.

The clinical consequences of urolithiasis are due to urinary tract mucosal damage and bacterial infection. More serious consequences arise when uroliths obstruct urine outflow resulting in postrenal azotemia. Unrelieved total obstruction to urine outflow results in acute renal failure and death. The male urethra is longer, more tortuous and narrower than the female and, in domestic animals, obstructive urethral urolithiasis occurs almost exclusively in males. Prepubescent castration retards urethral development and increases the risk of obstruction in predisposed individuals. Captive male macropods, particularly the larger species, are commonly castrated as sub-adults for management reasons and would consequently be expected to be more susceptible to obstructive urolithiasis.

Captive female macropods are susceptible to the formation of uroliths, yet obstruction of the lower urinary tract has not been observed in affected female animals. Although the two macropods with uroliths in the renal pelvises did not suffer from obstructive uropathy, the presence of uroliths was associated with morphologic lesions in the renal parenchyma, most notably interstitial fibrosis. The

presence of uroliths within the renal pelvis has the potential to result in hydronephrosis, or predispose the animal to pyelonephritis.

Diagnosis of uncomplicated urolithiasis in domestic animals is based on observation of clinical signs of urinary tract infection (UTI), confirmation of UTI by urinalysis and demonstration of the presence of uroliths by plain and/or contrast radiography. Crystalluria may be observed upon microscopic examination of urine sediment. Signs of UTI were not observed in animals 1 and 2 prior to obstruction, which may reflect the difficulties inherent in observing the micturition habits of captive macropods. The onset of urethral obstruction in both these animals was characterized by anuria, bladder distension and abdominal discomfort, all signs typical of urinary outflow obstruction in domestic animals. Conventional abdominal radiographic views were of no value in making a diagnosis in these animals due to the tissue mass surrounding the hips and pelvis in macropods of this size. A ventrodorsal pelvic radiographic view directed obliquely from cranial to caudal permitted visualisation of the urolith in animal 1. The uroliths in animals 1 and 2 were palpable per rectum allowing a definitive diagnosis to be made ante-mortem. It is interesting to note that hematologic and serum biochemical parameters belied the severity of the pathologic changes in these animals. This is contrary to the situation in domestic animals where total urinary obstruction is characterized by rapid and marked elevations in serum concentrations of blood urea nitrogen, creatinine and potassium. Azotemia was not present in animal 1 despite the severity of the clinical presentation and advanced pathology observed at necropsy. The severe renal pathology present in animal 2 was accompanied by only a moderate azotemia. Hyperkalemia was not a feature of either case.

General principles of treatment of obstructive urolithiasis are rapid decompression of the bladder to restore renal function and fluid therapy to address hydration, electrolyte and acid base derangements. Ideally bladder decompression is achieved by restoration of urethral patency by removal of the urolith, either by passage of urinary catheter or hydropropulsion.³ Where urethral patency cannot be restored by these means, the bladder is emptied by cystocentesis and the patient prepared for surgical removal of the uroliths. Attempts to pass a urinary catheter in normal male macropods are seldom successful. Although the difficulty has been attributed to the presence of a sigmoid flexure in the fibroelastic male macropod penis, in the cases described here catheterization was prevented when the catheter consistently passed into a dorsal diverticulum of the urethra, located 4 cm from the external urethral orifice.

In animals 1 and 2, urethral obstruction occurred at the level of the ischial arch. In dogs this would be an indication for perineal urethrotomy.² The macropod penis is positioned caudal to the scrotum within the cloaca relatively close to the pelvic symphysis. Consequently, a surgical approach via the penis is necessary for obstructions at this level in macropods. The postoperative death of the animal precluded the opportunity to assess the technique employed in case 2 for urethrotomy.

Conclusions

Although this paper documents the occurrence of obstructive and incidental calcium oxalate

urolithiasis in macropods it is unknown whether these uroliths were formed as a result of hyperoxaluria, hypercalciuria or other factors.

Until further studies define specific predisposing factors for calcium oxalate urolithiasis in captive macropods it would seem prudent to address those aspects of husbandry that are likely to result in crystalluria and the subsequent concretion of urine crystals into uroliths. Oxalate-containing foodstuffs should be avoided and food items that are high in vitamin D and calcium should be limited. Fresh herbage or vegetables should be made available to ensure all animals have an adequate vitamin A intake. Care should be taken to ensure that all animals in an enclosure have access to water ad. lib in order to maintain an optimum urine output. Urinalysis should be performed as part of the routine macropod veterinary examination for the early detection of crystalluria.

Urethral obstruction in macropods is investigated and managed according to the principles applied to domestic animals. Ultrasonography is useful for demonstration of bladder distension as the conformation of large macropods makes bladder palpation difficult. Similarly, radiography of the pelvic cavity may be problematic due to the large tissue mass. Oblique radiographic views may be more useful in these animals for the demonstration of uroliths in the pelvic urethra. The penile and pelvic urethra should be palpated as far proximally as possible to exclude the possibility of uroliths that are not readily visible on radiographic examination.

Catheterization is unlikely to be successful in restoring urethral patency in macropods and cystocentesis will usually be necessary for bladder decompression. Biochemical parameters such as bun, creatinine and serum potassium may not be as sensitive indicators of the extent and duration of renal failure in macropods with acute urine outflow obstruction as in domestic species. Urethrotomy is likely to be necessary for re-establishment of urethral patency in affected macropods and surgery should be undertaken with consideration of the genital conformation of these animals.

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UTAH'S HOGLE ZOO: PREPARATION FOR ONE OF AMERICA'S LARGEST PYROTECHNIC DISPLAYS, THE CLOSING CEREMONY FOR THE 2002 OLYMPIC WINTER GAMES

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Abstract

With huge pyrotechnic displays increasing in popularity and availability, zoos may be challenged with the noise that these shows can produce and the stress that they may have on their collections. Utah's Hogle Zoo, in Salt Lake City, Utah, was challenged with the most intense fireworks display to date on American soil. This paper describes the teamwork involved, the precautionary measures and the outcome from the pyrotechnic display for the closing ceremony at the 2002 Olympic Winter Games with regards to the animals at Utah's Hogle Zoo.

Salt Lake City won their bid for the 2002 Olympic Winter Games in June 1995. As preparations began to host the world in February of 2002, plans were made for fireworks displays during opening and closing ceremonies. The ceremonies would be held at Rice Eccles Stadium at the University of Utah just about 1 km from the zoo. The fireworks for the opening ceremony would be at the stadium but the fireworks for the closing ceremony would be spread throughout the Salt Lake valley in eleven locations and use shells up to 24 inches in diameter which are larger than any that had been fired off in Utah to date. These shells explode at 2000 feet and burst to a size of one-third to one-half of their height. This show also contained more explosions per minute than any other show previously done in America.

The zoo became aware of the plans of the Salt Lake Olympic Committee (SLOC) when applications for permits were submitted to the Fire Department. Of the initial thirteen sites proposed, two sites were located on either side of the zoo. One site was on the south side of the zoo at the Bonneville Golf Course and was within 850 feet and the other site, which was scheduled to fire the 24-inch shells, was less than 2000 feet north of the zoo at This Is The Place Heritage Park.

Zoo staff, consisting of the Director, Assistant Director, Veterinarian and Animal Care Supervisors began attending meetings with the Pyrotechnic Coordinator of SLOC to learn about the fireworks being proposed and the firing sites. It was decided to do a test of some of the smaller and medium fireworks at the two locations closest to the zoo. The largest fireworks (24 inch shells) had not arrived yet but would not be tested due to their high cost.

For the testing, zoo staff was placed on alert and communication was via radio. Animals that would be inside the night of the show were moved inside for the testing. Staff members were placed near enclosures where the animals would be outside and in "sensitive" areas throughout the zoo. A

sensitive area was considered as an area containing animals that might be sensitive to loud/sudden noises or an enclosure that might be difficult to navigate in if an animal were in a flight response. SLOC personnel were partnered with zoo personnel so all could be in contact via radio, as SLOC was also in radio contact with those firing the fireworks. The test was started with single reports from 3-inch shells and the animals' reactions were broadcast over the radio before the next shot was fired. If everything was ok, the test was continued. This test, however, was stopped short of completion due to the negative reaction from animals close to the Bonneville Golf Course site which included startle responses, running, and risk of hitting barriers.

With the results of this test, the golf course site was deemed to be too close, as the reaction to the noise from this site by giraffe and Desert Bighorn sheep was greater than the zoo was comfortable with. SLOC was asked to abandon this site as part of the program and they complied.

Arrangements were then made to contact other zoos to see if they had any experience with large fireworks displays close to or on zoo property. Disney has a fireworks display every evening but it is over 3 km from the Animal Kingdom Collection (M. Miller, personal communication, 2001). The Henry Doorly Zoo in Omaha, Nebraska (L. Simmons, personal communication, 2001) and Calgary Zoo in Canada (D. Whiteside, personal communication, 2001), have had experience with large displays and have found success with firing low tone sonic bursts leading up to the show, which seemed to help warn the animals and allow them to prepare. We also felt that if the show built up over the first few minutes, instead of starting off with a huge bang, then it might not be so startling to the animals.

Manned with this information, the zoo went back to the meeting room with SLOC to see if these concepts could be incorporated as part of the show. It was decided to do another test, without the golf course site, and incorporate a repeater or multi-shot device that discharges 100 small caliber shots. The zoo staff was stationed again and partnered with SLOC for communication purposes. The test was completed successfully. There was little or no reaction to the low-level blasts and the animals tolerated the multiple reports with minimal stress or running.

In the after-test meeting, it was thought that even though the test went well, it was probably not enough to determine the reaction of the animals for a 10-min show, throughout the sky and with 24-inch shells. Discussions were then started on what more the zoo could do to protect the animals.

It was decided that the Desert Bighorn sheep and Bactrian camels would have their enclosures lit by a light tower, which consisted of a bank of four, 1000 watt, metal halide bulbs, which had been used to light parking lots for the Olympics. This would help them see their fence lines and minimize the view of flashing lights from above. Boom box radios would also be placed in areas determined to be sensitive throughout the zoo: Savannah barn, which houses Grevy's zebra and common waterbuck; elephant and giraffe buildings. The interior lights were to be left on, with the radios playing loudly, but not full volume, when the zookeepers left for the day so the animals would not be startled by people coming into their area that evening and turning on the radio.

Since the show was to be televised, the zoo had been informed as to the approximate start time but SLOC personnel were at the zoo once again for radio communication and confirmation of start time. Approximately 30 min before the start of the show, zoo personnel were positioned and the boom boxes were turned up further, all lights were turned on, heaters or any other common noise that the animals were accustomed to were activated. A minute or so before the start of the show, the radios were turned to maximum. Any untoward animal activity was to be reported via radio although there was the understanding that once the show started, there would be little SLOC could do if there was a problem.

The show was amazingly loud, vibrations shook buildings and windows, even with the lights on in buildings, the flashes of light were obvious. The animals, however, did well throughout the show. The Desert Bighorn sheep, which had previously run the fence line during the two tests, did not get up from their resting positions. One of the giraffe paced but the other two did nothing. The elephants and rhinoceroses did not respond. The show was amazing; the valley was ablaze with color and sound. It was a dramatic finale to an amazing Olympic experience.

Conclusion

In conclusion, zoo managers should consider that the organizers of fireworks shows will not always think to see if there is an animal facility in the area, leaving the facility vulnerable to the effects the show might cause. In this case, however, once it was brought to the attention of the Olympic Committee, they were very good about working with the zoo to protect the animals. Without the efforts of SLOC and the zoo staff, the intensity of this particular show probably would have been devastating to the zoo, but with all of the precautions taken, we had no injuries and the stress to the animals was minimized.

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DETECTION AND MANAGEMENT OF CHRONIC WASTING DISEASE IN WISCONSIN WHITE-TAILED DEER (*Odocoileus virginianus*)

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Abstract

Chronic wasting disease (CWD) is a progressively degenerative and ultimately fatal disease of deer (*Odocoileus* spp.) and elk (*Cervus elaphus*) associated with transmissible protease resistant prion proteins.³ From the recognition of CWD as a transmissible spongiform encephalopathy in 1978 until 2000, the range of CWD in wild cervids was thought to be limited to Colorado, Wyoming and Nebraska. In the last 3 yr, CWD has also been detected in wild deer and elk in Illinois, New Mexico, Saskatchewan, South Dakota, Utah, and Wisconsin. CWD has been documented in captive cervids in Alberta, Colorado, Kansas, Minnesota, Montana, Nebraska, Oklahoma, Ontario, South Dakota, Saskatchewan, Wisconsin, and Wyoming.

The detection of CWD in Wisconsin was the first report of CWD in wild cervids east of the Mississippi river. Between 1999 and 2001, the Wisconsin Department of Natural Resources (DNR) had tested 1100 white-tailed deer for the presence of CWD. In 2001, three males, 2.5-3.5 yr of age and shot within 3 miles of each other, were found to have CWD on immunohistochemical analysis of brainstem samples. After subsequent sampling of 476 deer within an 11 mile radius of the three index cases revealed 15 additional CWD-affected deer, Wisconsin initiated a CWD management program with the goal of controlling spread of the disease and, if possible, eliminating it.¹

Wisconsin's CWD management to date has included intensive statewide surveillance, significant deer population reduction in the known affected area, the passage of legislation to ban deer feeding and baiting for hunting, and the implementation of an import moratorium and CWD monitoring program for farmed cervids. Since the beginning of 2002, lymph nodes from approximately 40,000 wild white-tailed deer harvested by hunters across the state have been screened by immunohistochemistry for evidence of CWD. Over 200 CWD affected deer have been detected, all within 35 miles of the index cases in south-western Wisconsin.² Using liberal hunting seasons and targeted harvesting by agency staff, the deer population in the affected area has been reduced by 25%. Mandatory testing of all farmed cervids transferred live or slaughtered has resulted in the detection of three CWD affected farms (one elk farm, two white-tailed deer farms).

The Wisconsin CWD outbreak is part of a significant expansion of the known range of this disease, and evokes particular concern as very high deer densities in southern Wisconsin may facilitate rapid transmission of the agent and may have significant negative impacts on this important wildlife resource.

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EXOTIC NEWCASTLE DISEASE OUTBREAK IN SOUTHERN CALIFORNIA: BIOSECURITY MEASURES FOR PREVENTION IN ZOO COLLECTIONS

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Abstract

Exotic Newcastle disease (END) is classified as an Office International des Epizooties (OIE) List A disease and is a foreign animal disease in the United States.¹ It is caused by a highly contagious and pathogenic paramyxovirus strain that affects many species of birds. In domestic poultry, the mortality rate is high, though there is variable mortality in other species of birds. A carrier state may exist in psittacine and some wild birds. Southern California had an extensive outbreak in commercial poultry in the early 1970's that lasted 2 yr and resulted in 12 million birds being destroyed.

In October 2002, END was diagnosed in southern California backyard poultry flocks that spread to commercial poultry flocks in early December 2002. On December 30, 2002, END was confirmed in a commercial egg-laying facility in San Diego County within 10 miles of the San Diego Wild Animal Park. As of April 1, 2003, 22 commercial poultry premises were affected, seven of which were in San Diego County within a 15 mile radius of the San Diego Wild Animal Park.²

The consequences would be catastrophic if our premises at the San Diego Zoo or Wild Animal Park became infected. Those consequences could include partial or complete depopulation of the avian collections, closing of all or part of the public facilities, and loss of genetic diversity for key conservation programs, including the California condor (*Gymnogyps californianus*). It was recognized early on that prevention, through stringent biosecurity measures, was essential. Close communications were established with the multi-agency END Task Force including critical protocol reviews, site visits, and outreach presentations to employees. The following is a summary of the biosecurity measures, based on recommendations for domestic poultry,³ put into place in response to the threat of END occurring in our region. Since many of these measures are generally a good practice, some will likely stay in place once the quarantine is lifted. These biosecurity measures are categorized by the potential source of infection to the avian collection.

Employees

- 1) Employees working in bird areas were required to wear footwear that did not leave the workplace and was cleaned and disinfected before entering to or exiting from bird areas.
- 2) Employees entering bird areas were required to wear uniforms that were supplied and laundered. Uniforms were not permitted to go home with the employee.
- 3) All employees working in bird areas were required to disinfect their shoes when first coming to

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- work.
- 4) Informational signage was placed at the entrance to all bird areas identifying them as a site for END biosecurity precautions.
 - 5) Employees were instructed not to come to work and to call their supervisors if they have a bird at home that was sick or died.
 - 6) Employee education efforts included the following measures:
 - a) General employee biosecurity briefings were held numerous times.
 - b) Members of the END Task Force provided outreach presentations.
 - c) An "Open Forum" summary publication was provided to all employees.
 - d) Periodic (three to five times weekly) email updates were sent to all employees with latest update on END situation.
 - e) A document listing biosecurity measures was written and distributed to employees at both facilities through their supervisors.
 - f) An information letter was sent to all employees describing the disease outbreak and precautions that they could take as individuals.
 - g) Employees were discouraged from visiting other bird or bird product facilities (e.g., pet stores, feed stores, etc.).
 - 7) A protocol was written for employees leaving grounds and returning same day.
 - 8) A protocol was developed for proper footbath preparation, maintenance, and disposal techniques.

Collection Birds

- 1) Movement of birds into and out of the collections, including transfers between facilities, was discontinued. Exceptions occurred only in accord with the multi-agency END Task Force.
- 2) All free-flight bird programs were discontinued.
- 3) Laboratory samples from collection birds could not be sent outside the quarantine area without permission from the receiving state veterinary office and with a permit granted from the END Task Force.
- 4) Transport cages, feed trays, and any equipment used with birds was required to be cleaned and disinfected after each use and where possible was restricted for use within a specific group of birds.
- 5) Birds that die are placed in closed, leak-proof containers and transported to the necropsy room through an outside entrance. Dead birds from the Wild Animal Park were transported to the San Diego Zoo for necropsy.
- 6) If five or more birds died in 1 day at the Wild Animal Park, suspicion of END would be assessed. If indicated, necropsies would be performed in a biosecure location at the Wild Animal Park.
- 7) Collection birds that escape and are recaptured were quarantined under certain circumstances.

Public Guests and Visitors

- 1) Direct public contact with collection birds was discontinued.

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- 2) Collection birds were no longer allowed to go off premises for any reason.
 - 3) Walk-through aviaries were closed to the public.
 - 4) The chick hatching display at the Children's Zoo was closed.
 - 5) Behind-the-scenes tours of animal forage warehouses and areas housing birds were discontinued.
 - 6) Neighbors were notified of the disease outbreak and were given educational materials to encourage the use of biosecurity measures in poultry husbandry.⁴

Foods and Feeds

- 1) The use of poultry as an animal food item was discontinued (except for commercially processed and disinfected eggs).
- 2) Bulk poultry feed, though never used, was formally banned from entering facilities.
- 3) Fresh or frozen poultry used for human consumption at catering events was obtained only from vendors outside the quarantine area.
- 4) Bird toys used for enrichment were disinfected before being given to birds.
- 5) Feed sources were scrutinized for possible exposure to poultry (e.g., cricket and rabbit vendors).
- 6) General biosecurity was reviewed at animal food warehouses.

Vendors, Contractors, Volunteers, and Consultants

- 1) All noncompany vehicles had tires and wheel-wells sprayed with a disinfectant when entering the service entrances. Drivers were queried regarding their recent bird contact and required to step in footbaths.
- 2) Those needing to go into bird areas were provided shoe disinfection and protective outerwear.

Wild and Feral Birds

- 1) Protocols were updated and revised for handling sick and injured native birds brought to our facilities or found within our facilities.
- 2) Protocols were developed for short-term treatment of sick and injured native bird species before release to rehabilitation facilities.
- 3) Free-roaming birds (e.g., peafowl and guinea fowl) were confined or removed where feasible.
- 4) Surveillance of wild birds for END in the vicinity of our facilities was performed by the END Task Force.

Other Measures

- 1) Members of the END Task Force made site visits to both facilities to critically evaluate biosecurity measures in place and to familiarize themselves with the facilities.

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A VETERINARIAN'S ROLE IN A DISASTER: GETTING INVOLVED AT A NATIONAL LEVEL

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Abstract

The role of the veterinary community in disaster response is critical to national security. Naturally occurring emergent diseases, agri-terrorism, and zoonotic biologic assaults depend heavily on the private sector for veterinary functions. Full-time federal veterinary employees are grossly inadequate in numbers and equipment for the task. The role of the veterinarian within the National Disaster Medical System (NDMS) requires a preestablished relationship with the federal government. The Veterinary Medical Assistance Teams (VMATs) are the only disaster responders sponsored by the AVMA/AVMF and funded through the NDMS. Zoological and wildlife veterinarians possess unique skills highly valuable to VMATs. Only four teams exist today, but the NDMS may be creating six more under the Department of Homeland Security. Zoological and wildlife veterinarians are strongly encouraged to become involved as VMAT disaster responders.

Zoological veterinarians may be on the front lines of disease diagnosis as exemplified by the role of the Wildlife Conservation Society (WCS) in West Nile virus identification. Zoological institutions are not exempt from involvement in disasters themselves. Recently 4.7 million poultry were euthanatized in the eradication of avian influenza just 100 miles from the Center for Research and Conservation (CRC) in Front Royal, Virginia. The VMAT was there. The endangered status of reservoir species in a zoological collection would be no exemption from immediate disposal in a federal eradication program.

Natural disasters are indiscriminate in their destruction and may include conservation institutions. Miami Zoo can attest to the indiscriminate devastation of hurricane Andrew. The VMAT was there. Even in the World Trade Center disaster response, veterinarians played a unique yet key role. The VMAT was there. Wildlife and zoological veterinarians may join a VMAT Disaster Response Team by using the information to follow. They may be involved in confronting biologic threats, both emerging diseases and intentional terrorist events.^{1,2}

What's a VMAT? Veterinary Medical Assistance Teams were developed through the National Disaster Medical System, now under Department of Homeland Security (DHS) and remain the only veterinary disaster response forces under the federal disaster response plan. Sponsored by the American Veterinary Medical Association (AVMA) and American Veterinary Medical Foundation (AVMF), the VMATs are the only veterinary disaster response team they are willing to put their

name on and support.^{2,3}

The VMATs are composed of multi-discipline veterinarians, veterinary technicians, and animal care professionals in a public/private partnership. Currently the VMATs are sorely lacking wildlife and zoological veterinarians. Zoological and wildlife vets are strongly encouraged to become involved. More are needed to expand the teams to the projected numbers. Several team leaders will be sought for the new VMATs to be formed in the near future. Members of AAZV could be prime candidates for leadership positions.

The VMATs are voluntary, intermittent, full-time paid federal employees deployed on an as-needed basis for emerging disease eradication, natural disasters, and veterinary mission support within the United States and its territories. Pay grades are based on credentials and duty station. Typical deployments are 14 days for 12 hr each day. Requests are made directly from the NDMS for a defined number of deployable team members. Any team member may decline or accept any deployment for any reason that need not be divulged. Upon request by the NDMS, the team member may choose to repeat 14-day deployments if the mission is ongoing.

Team members are covered by employment protection within the Department of Homeland Security. This makes it difficult for employers to legally refuse to allow time off for deployments. No legal reprisals may be lodged against an individual serving their country as a VMAT member. Critical protection is provided to volunteers who are not self-employed.

Transportation is arranged by NDMS for the team members deployed to the mission site. Personal protective equipment, veterinary equipment and logistic needs are already available upon arrival. Biosecurity and safety equipment to perform the job is also provided upon request. Needs lists are created by the VMATs as soon as the inventory can be checked and the list can be submitted. Equipment is rapidly delivered from NDMS to achieve the mission.

Each team maintains medical and equipment caches including large tents and field accommodations. The largest medical caches are strategically placed by the NDMS for immediate delivery to the site. More caches will be created as more teams are assembled.

Hurricane Andrew devastated south Florida in 1992. The need for a plan for animals in disasters was clearly evident. Not only were live displaced animals a concern, animal carcasses posed a significant human health risk as well. The veterinary community was overwhelmed with requests. No comprehensive animal disaster plan existed for Florida until after the lessons of Hurricane Andrew.¹

How does a deployment happen? As local resources are overwhelmed, help is requested from state resources. When state resources are found inadequate, the governor must request disaster declaration. Only the U.S. President may declare a National Disaster. Once the President declares a disaster, funds are “released” to address the situation. As always, cash is the key. Federal resources are made available for VMATs. The nomenclature has changed since that time. Now VMATs and NDMS are under the DHS. However the chain of disaster response remains relatively unchanged.

In addition to providing biosecurity and surveillance for animal disease outbreaks such as avian influenza, VMAT members have been deployed to the Houston flood, Hurricane Floyd, and the New Mexico fires as part of the MST. At the Democratic National Convention, Presidential Inauguration, 56th United Nations General Assembly, Special Olympics, and Winter Olympics VMATs provided security support. Search and Rescue veterinary support was provided for the Alaska air crash and New York City World Trade Center by VMATs as well.

The World Trade Center disaster was a unique and tragic experience for VMAT members. Many of the early responders are still haunted by the repulsive human and architectural remains. The VMATs provided intensive 24 hr support for over 300 Search and Rescue dogs from around the world. The mission rapidly digressed from a search and rescue mission to a search and body recovery mission. It became clearly evident that victim survival was unrealistic.

On some difficult deployments situations, VMATs may be required to be self sufficient for the first 3 days. Each member maintains a “jump pack” that will provide essential personal equipment upon arrival. NDMS has stated intentions to create 10 teams. The current VMAT status includes four teams. Team size has also been raised from 20-30, to a team size of 45-60 members each. Dedicated veterinarians are needed today. Zoological veterinarians are in particularly short supply throughout the nation.

The current teams include VMAT 1-North East, VMAT 2-Mid Atlantic, VMAT 3-Southeast, and VMAT 4-Southwest. Members for the newly created VMAT 5 in the Midwest are still needed. Wildlife and zoological veterinarians are urged to become members of these teams and assist in creation of new teams as the program grows under the auspices of NDMS and AVMA.

Large and small animal rescue is frequently involved in a disaster situation. Wildlife become displaced and disoriented and require capture, treatment, and translocation. Remote immobilization may be required in any species. In the midst of a hurricane, an Arab stallion may act more like a zebra stallion. Wildlife and zoological veterinarians are key players in a disaster.

Field training exercises are provided and financially subsidized to familiarize team members with emerging or exotic diseases, chemical and biologic weapons, treatment, decontamination, and agri-terrorism. The focus of training has shifted to intensive study of emerging and foreign animal diseases as bio-weapons from a historic focus on natural disasters. Unfortunately, chemo- and bio-terrorism planning and preparation are critical.

Online training programs are available through the NDMS Office of Emergency Response (OER) and the University of Maryland. Topics include wildlife restraint, wildlife immobilization, animal decontamination and over 100 more disease, medical and disaster topics and information. Several more topics are seeking authors to complete them.

Field hospitals are established at training exercises and deployment sites. Several large veterinary caches of medical equipment are maintained in at least two sites on both the East and West coasts

by the NDMS for immediate release and delivery to a deployment site with in 24 hr of activation.

Individual teams have equipment and supplies including portable fencing panels for large animals, remote immobilization equipment, surgical and hospital/housing tents. Some teams have created Remote Response Back Packs, set up in pairs, one large animal pack and one small. A two-person team for fieldwork has all the bases covered for remote treatment and rescue.

Biosecurity and disease surveillance are critical to the epizootiology and eradication of emerging or foreign animal diseases. The VMATs are on the front lines of disease surveillance and play a critical role as a strike force for elimination of emerging diseases.

Chemo-terrorism and hazardous materials are present dangers of "modern civilization." The VMATs prepare for response to both. Chemical and biologic warfare may affect any institution including zoological institutions. Entertainment centers are considered terrorist targets.

Biosecurity to minimize disease transmission within an institution plays the key role in keeping biologic agents at the entrance gate. Biosecurity requires a lot of work to do properly. Even the addition of a simple footbath requires a commitment to using it appropriately. In an emergent disease outbreak, biosecurity is assured by VMAT's as animals are tested, evaluated, treated or humanely euthanatized and removed from contaminated premises. When foreign animal or emergent diseases strike, millions of animals may need to be disposed of in a rapid and secure manner. Veterinarians are trained to perform tasks using bio-secure methods.⁴

Routine bio-secure husbandry practices set the stage to minimize spread of disease from outside an institution. Zoological institutions are not exempt from bio-terrorist or naturally occurring emerging diseases. The obvious visual barrier of the perimeter fence is blurry at best to disease busters hot on the heels of emergent disease eradication. Avian influenza came dangerously close to Front Royal, VA. In an outbreak of agricultural and/or financial significance, perimeter fences will be of little significance.

Neither the disease nor the depopulation teams consider the conservation status heavily before exacting their toll on captive or wild animals. The VMAT Avian Influenza surveillance teams came within a hundred miles of endangered species in breeding and research programs. One confirmed positive in 50,000 and the entire group will be depopulation within 24 hr.

A zoological institution may be considered a potential reservoir of the emerging or foreign animal disease. In fact, zoological institutions full of exotic animals are frequently prime suspects as point sources of exotic diseases. Good records and an aggressive surveillance sampling record are the best defense for zoological collections.

Municipal water supplies are considered likely terrorist targets. Municipal water supply contamination, intentional or accidental, can affect any captive animal facility as well. Alternative emergency water sources for long-term needs of a zoological institution are almost nonexistent.

Even short-term water storage capabilities of zoos are limited.

The 2002 avian influenza (AI) outbreak devastated the poultry industry and agriculture of the Shenandoah Valley of western Virginia. Almost 5 million birds were euthanatized over 6+ mo. Wildlife and zoological veterinarians were a small part of the response. Although a wildlife surveillance section was listed on the USDA Incident Action Plan, when asked about the plan for wildlife, they decided not to test wild birds because they didn't know what to do if they found AI in the wild population. More wildlife and zoological veterinarians are urgently needed on every team in the country. As of summer 2002, the Virginia AI outbreak had been suppressed, although it reappeared in the spring of 2003 in New England.

Exotic Newcastle disease (END) gripped much of southern California and was already identified in the Arizona and the Midwest at the time of this manuscript submission. The likelihood of a long-term eradication program for END will require involvement of the private and zoological veterinary community to be successful.

A skilled wildlife or zoo veterinarian would be a valuable asset to every team. Both domestic and wild animals revert to instinctive behaviors if displaced and disoriented in a disaster. The key clinical skills and the resourcefulness of zoological and wildlife veterinarians would be invaluable in a biologic or natural disaster.

Individuals interested in veterinary response to national disasters including emerging disease outbreaks and natural disasters should contact the AVMA or an individual team of interest. The contact at AVMA is Dr Cindy Lovern (clovern@avma.org). Alternatively, you may examine the website www.VMAT1.org with hyperlinks to the AVMA, NDMS and other VMAT Internet sites. The USDA APHIS website provides more information on foreign animal diseases, disease outbreaks, and emerging diseases (aphis.usda.gov/vs/ep/fad_training/bibpage.htm).

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RABBIT HEMORRHAGIC DISEASE OUTBREAK IN DOMESTIC RABBITS (*Oryctolagus cuniculus*) IN A ZOOLOGICAL PARK

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Abstract

Rabbit hemorrhagic disease (RHD), also known as rabbit calicivirus disease (RCVD), is caused by a calicivirus in the genus *Lagovirus*. RHD was first reported in captive domestic rabbits (*Oryctolagus cuniculus*) in China in 1984, and subsequently spread throughout Asia, Africa, and Europe.¹⁻³ Although primarily reported in captive rabbits, it has also occurred in wild rabbits in Europe⁷ and feral rabbits in Australia and New Zealand.^{1,3} RHD was first reported in the Western Hemisphere in Mexico in 1988, where disease was introduced by importation of infected frozen rabbit carcasses from China.^{1,2} There have been several reported outbreaks in the United States, the first in Iowa in April 2000^{1,4} and a second in Utah in August 2001 (with shipment of rabbits from the Utah facility to Illinois resulting in cases in that state).^{5,6} The origin of infection in the Iowa and Utah outbreaks was not determined.

The disease is not zoonotic and only affects domestic and wild European rabbits (*Oryctolagus cuniculus*) and not the North American cottontail rabbit (*Sylvilagus floridanus*), black-tailed jackrabbit (*Lepus californicus*), or volcano rabbit (*Romerolagus diazzi*).¹⁻³ Transmission is by direct contact with contaminated feces, secretions, fomites (i.e., cages, clothing) that have been in contact with contaminated rabbits, or by aerosol transmission. The disease causes high mortality with death shortly after infection. Animals of all ages are susceptible to infection, however serious disease resulting in death generally affects animals older than 5-7 wk of age. Sudden death with no premonitory clinical signs is often the case. Clinical signs in acute cases are generally nonspecific but can include anorexia, depression, constipation, and diarrhea. CNS signs such as paddling, ataxia, convulsions or opisthotonos may be seen and are due to cerebral microinfarctions. Gross necropsy lesions may be subtle but usually include hepatic necrosis, splenomegaly, pulmonary congestion, tracheal serous to blood-tinged transudate and sometimes a catarrhal enteritis. The most marked histologic lesion is diffuse, acute, peripheral lobular hepatic necrosis and occasional visceral microthrombi and infarction, particularly in the kidneys. Rabbits that survive infection may shed virus for 4 wk or more post infection.¹⁻³

Between 1 and 9 December 2001 three domestic rabbits housed at the Queens Zoo in Queens, New York died with little or no premonitory clinical signs (index cases). Based upon gross and histologic

lesions, RHD infection was suspected and samples were sent to the Foreign Animal Disease Diagnostic Laboratory (FADDL), Plum Island, New York, where RHD infection was confirmed. The United States Department of Agriculture (USDA) classifies RHD as a foreign animal disease. Therefore, depopulation of the remaining five rabbits (contacts) at the park was required by the USDA and conducted on 10 December 2001. The contacts were anesthetized, bled, and humanely euthanatized with a concentrated pentobarbital sodium solution (Beuthanasia-D Special, Schering-Plough Animal Health, Union, NJ 07083 USA).

The index cases had very subtle gross lesions. Lesions included serosanguinous nasal discharge, pulmonary or tracheal edema, congestion or hemorrhage, and the livers were light brown/red and mildly friable. Nephritis, gastritis, splenomegaly, or rectal hemorrhage were also seen, but were mild and not consistent between cases. The contacts had only incidental findings not consistent with RHD. Significant histologic lesions referable to RHD were found in the livers of all index cases. These included severe, diffuse, acute, massive peracute hepatic necrosis with chromatolysis, chromatin margination, dissociation of hepatic cords, and few intranuclear inclusion bodies. The spleen showed moderate diffuse periarteriolar lymphoid necrosis with chromatin margination and few intranuclear inclusion bodies. Mild multifocal congestion and hemorrhage were seen in the lungs. None of the contacts demonstrated lesions consistent with RHD.

Upon diagnosis of RHD, the USDA mandated quarantine and euthanasia of the remaining rabbits and enclosure decontamination per USDA guidelines. Areas where the rabbits had been housed or transported, and potentially contaminated objects, were cleaned with one of the following: 0.5% sodium hypochlorite solution (10% solution of household bleach), 2% phenolic disinfectant (1 Stroke Environ, Calgon Vestal Laboratories, Inc., St. Louis, MO 63110 USA), or steam sterilization. Substrate and enclosures were cleaned again 24 hr later. Bedding was disposed of by incineration and materials used for euthanasia were disposed of by standard biomedical waste procedures. Staff cleaning potentially contaminated areas wore disposable coveralls. An investigation performed by the USDA was unsuccessful in identifying the origin of RHD. All collection rabbits originated from public donations of unknown source. Frozen rabbit carcasses are regularly purchased from a commercial source and are fed to carnivores at the zoo. The specific origin of rabbit carcasses purchased and fed prior to the outbreak could not be determined. However, the supplier's inventory included rabbits imported from China. Testing of remaining frozen rabbit carcasses from the zoo, and purchased from this vendor, were negative for RHD. A carrier collection rabbit, an infected frozen rabbit carcass that resulted in cross contamination or fomite transmission, or a visitor to the zoo may have introduced the infection. There is no commercially available RHD diagnostic test and no licensed RHD vaccine available in the United States.¹ Although RHD is considered exotic to the United States, it may be under diagnosed. Suspected cases should be reported to state or federal animal health authorities.

ACKNOWLEDGMENTS

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DIAGNOSTIC INVESTIGATION AND EPIDEMIOLOGY OF TUBERCULOSIS AT A BRITISH ZOO: A PRELIMINARY OVERVIEW OF A MULTISPECIES INFECTION WITH A NEW FORM OF MYCOBACTERIUM

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Abstract

Tuberculosis (TB) infection was diagnosed at a United Kingdom zoo in 1996, in two wild caught fur seals (*Arctocephalus australis*) and a captive born tapir (*Tapirus terrestris*). Retrospective DNA testing in 2001 found that these three cases were caused by the same mycobacterium species (spoligotype 46). In 2000, a pudu (*Pudu puda*) died with suspected mycobacterium infection and splenic mycobacterium infection was an incidental postmortem finding in a gorilla (*Gorilla gorilla gorilla*) which died of other causes. Tuberculosis testing of the entire mammal collection ensued using intradermal tuberculin testing, thoracic radiography and examination of bronchoalveolar lavages. Biosecurity measures across the zoo site were implemented. The in vivo testing indicated tuberculosis in another gorilla, and TB of the same spoligotype was confirmed. Further tuberculosis cases in a llama (*Lama glama*) and a tapir were diagnosed using in vivo testing and confirmed at postmortem examination. DNA typing techniques confirmed the same strain of mycobacterium to be involved in these cases; a new form of mycobacterium *Mycobacterium tuberculosis* subsp. *pinnipediae* has been proposed.¹

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EMERGENCE OF A *Perkinsus*-LIKE AGENT IN ANURAN LIVER DURING DIE-OFFS OF LOCAL POPULATIONS: PCR DETECTION AND PHYLOGENETIC CHARACTERIZATION

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Abstract

Multiple un-explained mass mortality events in free-living larval anurans of the genus *Rana* have occurred between September 1999 and March 2002. Mortality rates in many instances exceeded 90% of tadpoles, resulting in negligible recruitment that year. However, adult frogs appeared unaffected and successful breeding occurred in subsequent years. A seasonal appearance of the organism was clearly associated with the presence of 1-4 mo-old tadpoles, with outbreaks beginning December to March in southern states and April to July in central and northern states. Die-offs occurred in the following states: Florida, Mississippi, Virginia, New Hampshire and Minnesota. Sporadic infections that were not known to be associated with die-offs also occurred in Alaska, Minnesota, Virginia and North Carolina. Infected ranid tadpoles included: wood frogs (*Rana sylvatica*), Southern leopard frogs (*R. sphenoccephala*), mink frogs (*R. septentrionalis*), green frogs (*R. clamitans*), Gopher frogs (*R. silvosa*) and bullfrogs (*R. catesbeiana*). Gross pathologic findings are characteristic: mild to massive enlargement of the liver, spleen, pronephroi and mesonephroi; affected organs are homogeneously white. Histopathologically and cytologically, these four organs and others have massive infiltrates of spheric, darkly basophilic organisms morphologically reminiscent of primitive protozoa of the newly erected clade, *Mesomycetozoa* (e.g., *Dermocystidium*, *Dermosporidium*, rosette agent and *Ichthyophonus*).

Methods

DNA was isolated from tadpole livers from several outbreaks. A "*Perkinsus*-like" generic PCR was developed by sequence alignment of several organisms. The PCR amplifies a 280-nucleotide region of the 18S ribosomal RNA gene. Sequences of 18S rRNA aligned were identified from the NIH GenBank, and included: *Ichthyophonus irregularis* (Accession AF232303), *Ichthyophonus hoeferi*, (Accession U25637), *Amoebidium parasiticus* (Y191555), *Pseudoperkinsus tapetus* (AF192386), *Dermocystidium salmonis* (U21337) and *Rhinosporidium seeberi* (AFA399715). Ribosomal RNA 18S sequences from *Xenopus laevis* (XO4025) and *Bufo valliceps* (M59386) were used to identify regions to be avoided in primer design. Sequence alignment was performed using PILEUP in the GCG Wisconsin Analysis Package (Oxford Medical, Oxford, UK). PCR products obtained from affected liver tissues were sub-cloned into pCR4 TOPO (Invitrogen Co, Carlsbad, CA) and

sequenced (Davis Sequencing, U.C. Davis) in both forward and reverse directions. Next, a new PCR was designed that was specific to the *Perkinsus*-like parasite under examination, which amplified a 238-nucleotide region. The NIH GenBank was searched using the BLAST (the Basic Alignment Sequence Tool) routine, using consensus sequences of PCR product obtained from twelve clones as a reference. BLAST identified closest phylogenetic relatives whose 18S rRNA sequences were truncated for use in construction of the most parsimonious relationship. Tree construction was performed using the PHYLIP software including DNAPars, Seqboot and Consense.

Results

The PCR-amplified sequence from amphibian livers was most closely related to organisms in the genus *Perkinsus*, a group of well-known marine shellfish pathogens (e.g., *P. marinus*, etc.). Blinded specific PCR testing was performed on liver samples previously characterized as positive or negative for the agent based on light microscopy. All samples from outbreaks were identified by PCR, whereas amphibian tissues containing *Ichthyophonus* (diagnosed by histology) were consistently negative.

Discussion

Our results suggest a close relationship between the larval anuran die-offs, light microscopic findings of massive systematic tissue invasion, and the presence of DNA sequences consistent with a new *Perkinsus*-like agent. Our inability to culture the agent (at this time) precludes the fulfilment of Koch's postulates. Further work is warranted to establish the geographic distribution, host range, life cycle, and the relationship (if any) of this new agent to marine invertebrate pathogens. A definitive role of this emerging new agent in periodic mass mortality events of wild anuran population declines in the United States is suspected.

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SURVEILLANCE FOR SELECTED BACTERIAL AND TOXICOLOGIC CONTAMINATES IN CARCASS MEAT FED TO CARNIVORES

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Abstract

Wildlife Safari, a zoo located in Winston, Oregon, has fed donated carcass meat as a diet to carnivores for 30 yr. Carcass meat is an alternative to commercially prepared meat, which is used by many other zoological institutions.^{4,5} Donated meat arrives at Wildlife Safari as an entire animal. The entire carcass is fed out 10% of the time, while 90% of the time carcass meat is sectioned into measured diets. Despite the absence of problems associated with carcass meat at Wildlife Safari, this study explores techniques for improved monitoring of food safety. Prior to this study, Wildlife Safari has avoided toxicants in carcass meat by relying on medical treatment reports from owners or veterinarians associated with the donated animal. Animal carcasses that have systemic diseases or deaths due to unknown causes are also not used. Animals donated for meat consist of cattle, horse, deer, bison, elk and ponies.

As a supplement to the reported record of chemical treatment, 32 meat samples were analyzed off-site at Michigan State University's Diagnostic Center for Population and Animal Health using gas chromatography/mass spectrometry screening.² These samples were collected during a 3-mo period ranging from November 2002 to January 2003. Levels of phenylbutazone, flunixin meglumine, and xylazine were monitored as potential toxicants. None of the meat samples were found to have evidence of these or any other organic toxicants, suggesting that the records coming from the animal donors are sufficiently accurate for food safety.

In order to better monitor food-borne pathogens in a cost-efficient manner, Neogen Reveal[®] immunosorbent assays (Neogen Corporation, Lansing, Michigan 48912 USA) were performed on-site.⁶ Although the taxonomic range of monitoring was limited, immunoassays may establish a baseline of general safety associated with the carcass meat while still having the power to implicate specific pathogens. Testing of *Salmonella* spp., *Listeria* spp., and *Escherichia coli* O157:H7 was performed. Meat samples were collected from the left quadriceps region of each carcass for a 4-mo period ranging from November 2002 to February 2003 ($n = 50$). Twenty-five meat samples were randomly selected from this group to be included for the bacterial detection tests. The sensitivities and specificities of the Reveal[®] assays are comparable with those listed by the USDA/FSIS and the AOAC standards.^{1,3,7} Twenty-eight percent of the meat samples were positive for *Salmonella* spp. ($n = 25$). One sample was positive for *Listeria* spp. ($n = 25$). None of the meat samples were

positive for *Escherichia coli* O157:H7 ($n = 25$). As monitored, carcass meat appears to be a potentially safe food source for carnivores.

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DIAGNOSIS AND MANAGEMENT OF ENDOCRINE PANCREATIC APLASIA IN A JUVENILE BENGAL TIGER (*Panthera tigris tigris*)

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Abstract

A 6-mo-old female intact white Bengal tiger (*Panthera tigris tigris*) started developing clinical signs consistent with exocrine pancreatic insufficiency (EPI) soon after it was weaned from a hand-raising formula to a meat diet (Dallas Crown). Voluminous, greasy stools, poor hair coat, abdominal distension, polyphagia, and poor rate of gain compared to age and sex matched conspecifics were noted. Flatulence and borborygmus were also noted. Prior to the onset of clinical signs the cub had showed a normal development and had been vaccinated routinely with a commercial feline vaccine for rhinovirus, calicivirus, and panleukopenia, (Fel-O-Vax, PCT, Fort Dodge®, Fort Dodge Laboratories, Inc, Fort Dodge, IA 50501 USA) and rabies (Rabdomun®, Pfizer Animal Health, Exton, PA 19341 USA). The cub had two negative ELISAs to feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV). A slight titer to canine distemper was found on quarantine exam but this did not persist. Empirical therapy for EPI was started and included a commercial porcine pancreolipase supplement (Pancreazyme Plus Powder®, Butler) at 1500 IU of lipase per kg of body weight. This product was chosen due to the added vitamin A, D₃, and E. Some improvement in stool quality was noted but the cub continued to have a sub optimal weight gain, was ravenous, and was generally uncomfortable. The cub allowed blood sampling via a squeeze cage by means of operant conditioning. Cobalamine (<100 ng/L) and folate (7.9 µg/L) were found to be abnormal based on domestic cats values (290-1500 ng/L and 9.7-21.6 µg/L respectively). The tiger was hand reared and leash trained which allowed a limited physical exam by age of 10 mo. The cub weighed 54 kg, had a taunt abdomen, was moderately thin with a scruffy coat and an overall stunted appearance. Abdominal palpation revealed a mid abdominal mass that was immediately painful. Standing radiographs demonstrated a soft tissue mass in the mid-abdomen. The films were not diagnostic enough to delineate which organ was involved. The cub was anesthetized with medetomidine (18 µg/kg, Domitor®, Pfizer Animal Health, Exton, PA 19341 USA) and ketamine (2.94 mg/kg, Ketaset®, Fort Dodge®, Fort Dodge Laboratories, Inc, Fort Dodge, IA 50501 USA) i.m., intubated and maintained on isoflurane for diagnostic evaluation. Abdominal palpation and ultrasound evaluation revealed mesenteric lymphadenopathy and thickened small intestinal loops. Radiographs suggested primary lymph node involvement as all other organ systems appeared normal and suggested thickened small intestinal walls as well. Exploratory laparotomy was elected. Significant finding included grossly enlarged lymph nodes associated with the mesentery and small intestine. One node at the root of the mesentery measured 10 cm across. The small intestine was

diffusely thickened and felt heavy. The most notable finding was the sparse condition of the pancreas. The pancreatic mass was greatly reduced and only small blushes of tissue were found in the mesentery in both arms of the pancreas. There was however no evidence of inflammation nor necrosis in the organ. Biopsies of the small intestine, lymph nodes, and pancreas were taken. The cub recovered uneventfully.

Histopathology revealed severe acinar cell hypoplasia/aplasia of the pancreas, mild chronic enteritis, and marked reactive lymphoid hyperplasia. Special staining for viral induced pancreatic disease, feline parvovirus and canine distemper virus, were negative. No growth was obtained on tissue culture from surgery. The pathologist gave a grave prognosis and the clinical staff concurred. The cub was managed with 4-5 feedings daily, each treated with the pancreolipase supplement. Cyanocobalamin was given every 21-28 days s.c. and monitored based on the serum levels of cobalamine and folate levels. Simethicone (Gas Relief®, Rugby Laboratories, Inc., Duluth, GA 30097 USA) 125 mg p.o. s.i.d. was also given to manage the condition. The cub began to stabilize and gain weight. Hair coat improved, attitude became much more relaxed. Appetite was still ravenous and while stool quality improved overall there was still occasional episodes of steatorrhea. Initial feeding consisted of only meat product other items such as ribs and whole prey on an occasional basis were added. One year later it was decided that the young tigress should be permanently sterilized. The tiger now weighed 104 kg and was again anesthetized with medetomidine (20 µg/kg) and ketamine (2.11 mg/kg) i.m. and prepped for ovariohysterectomy and laparotomy. The small intestine was thickened still but not nearly as severe as the original surgery. The lymph nodes were generally enlarged and edematous but again not as severe. The pancreas was however much reduced. Biopsies of all three were taken and the ovariohysterectomy performed. The tigress recovered uneventfully. Histopathology was essentially identical to the original biopsies. The tigress continues to thrive with four other conspecifics and is maintained on the feeding regime with routine blood draws to taper the cyanocobalamin therapy. Pancreolipase dosing has been increased to 1800IU lipase per kg of body weight to prevent steatorrhea.

The clinical picture in this tiger most closely resembled juvenile pancreatic hypoplasia in German shepherds³ both clinically and on the initial histopathology. This condition is believed to be an autosomal recessive trait. Given the early onset of this condition in this tiger, a congenital etiology was likely and therefore this condition is most appropriately called exocrine pancreatic aplasia. The absence of viral particles on special staining also supported this diagnosis. The likelihood of a congenital problem prompted the decision for elective sterilization. This provided an opportunity to evaluate the condition of the pancreas firsthand after 1 yr of therapy. In felids, chronic pancreatic insufficiency has been found in a pair of Indian lions.¹ The lions in this case were not able to be maintained on a commercial pancreolipase supplement and were euthanatized. The authors concluded in their case that the disease process was a degenerative one and believed that pancreatic exocrine atrophy is most appropriate term for the condition they documented. Most cats with EPI become severely cobalamine deficient and also require tocopherol and folate supplementation.² Folate and cobalamine are useful measures of small intestinal health and can be useful in the diagnosis and management of EPI like diseases.^{2,4} In this tigress, folate levels returned to normal without specific folate supplementation. Utilizing a laboratory with specific diagnostics for

domestic cats (GI Lab, Department of Small Animal Medicine and Surgery, Texas A&M University, College Station Texas, 77843-4474) proved essential for this case. Reference values for cobalamine and folate proved adequate for the management of this tiger.

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OVARIAN CONTROL AND ASSISTED REPRODUCTION IN FELIDS

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Abstract

Assisted reproductive technologies including artificial insemination (AI), in vitro fertilization (IVF) and embryo transfer have the potential to be important tools in the management of many endangered cats. Developing successful assisted reproductive techniques requires knowledge of the female reproductive cycle and precise control of ovarian activity. Control of ovulation is one key step in coordinating procedures to optimize reproductive success.

Numerous exogenous hormones are available to induce ovarian follicular growth and ovulation in mammals, with several adapted to felids. The most common hormones used to initiate folliculogenesis are porcine follicle stimulating hormone (pFSH) and equine chorionic gonadotropin (eCG). Folliculogenesis has been successfully stimulated with a 3- or 4-day course of daily pFSH injections in the domestic cat,¹ caracal,² jaguarandi,¹ tiger,³ cheetah,⁴ leopard, lion,⁴ and puma.⁴ Equine chorionic gonadotropin (eCG), which has a longer half-life, is given as a single injection and has been used in the domestic cat,⁵ leopard cat,⁶ cheetah,⁷ clouded leopard,⁷ tiger,⁸ puma,⁹ ocelot,¹⁰ and snow leopard.¹¹ Once follicles are mature, ovulation can be induced with multiple injections of luteinizing hormone (LH)^{3,12} or a single injection of human chorionic gonadotropin (hCG).^{6,13} Less commonly, gonadotropin-releasing hormone (GnRH) or GnRH agonists also can be used to induce ovulation.^{14,15}

Felids exhibit a highly variable response to exogenous gonadotropins used to stimulate follicular growth and ovulation. This has made development of assisted reproduction difficult. Over the past 15 yr, research has identified species differences in ovulation strategies and dose-responses to gonadotropic hormones that have contributed to the variability in response to ovarian stimulation. Cats are generally considered induced or reflex ovulators, and would be expected to ovulate only following cervico-vaginal stimulation during intromission. However, several species have proven to be susceptible to occasional spontaneous ovulation in the absence of mating stimuli. Species prone to spontaneous ovulation episodes include the domestic cat,¹⁶ lion,¹⁷ clouded leopard,¹⁸ margay,¹⁹ and fishing cat.²⁰ Felids are the only known taxon to exhibit such variability in ovulation mechanisms across individuals. The felids also show great diversity in sensitivity to exogenous gonadotropin administration. For example, the 9-kg ocelot requires more than twice as much eCG and hCG to induce ovulation then does the 35-kg cheetah.^{7,10} In short, there is no relatedness between species body mass and the dose of eCG/hCG required to artificially induce ovulation.

The variability between species of felids in ovarian control mechanisms is reflected in the success

of assisted reproductive technologies. In some species, such as the cheetah where ovulation induction after eCG/hCG is fairly consistent, more than 50% of females become pregnant after AI. In contrast, those species with the most variable responses to exogenous gonadotropins experience AI success rates of less than 10%.^{7,12}

This variation occurs not only among species, but among individuals within a species. Species prone to spontaneous ovulation produce intermittent corpora lutea (CL) that blunt ovarian responses to eCG/hCG therapy that, in turn, lead to reduced AI/IVF success.^{21,22} A variable female response also occurs because the exogenous gonadotropins can perturb the endocrine environment, which disrupts oocyte maturation, fertilization, embryo development and implantation. Perturbations in ovarian stimulation resulting in decreased fertility in felids include ovarian hyper-stimulation episodes that cause elevated or protracted estrogen concentrations, premature or hyper-progesterone secretion and luteal insufficiency/premature luteolysis.^{3,23-26}

Recent studies have investigated the use of ovarian inhibition prior to ovulation induction to create a more predictable response. The ability to suppress cyclic activity before gonadotropic therapy has improved assisted reproduction success in other species.²⁷⁻³⁰ A suppressed ovary is more likely to give a uniform response to exogenous gonadotropins. To this purpose, the progestin levonorgestrel (Norplant) recently was compared to the GnRH antagonist, antide, as a pretreatment to gonadotropin stimulation in the domestic cat. Pretreatment with levonorgestrel, but not antide, prior to gonadotropin stimulation more than doubled the embryo yield after IVF^{31,32} and resulted in a uniform excellent response for AI³³ compared to gonadotropin stimulation alone.

Although pretreatment with levonorgestrel implants before gonadotropin stimulation has improved ovarian response to eCG/hCG therapy in the domestic cat, results in other species have been species-specific. Preliminary trials have been conducted in the fishing cat, cheetah and clouded leopard. Results in the fishing cat and cheetah are promising. Two fishing cats given Norplant followed by eCG and hCG stimulation exhibited fresh ovulation sites with minimal numbers of unovulated follicles, a perfect response for AI (Bauer, unpublished data). Similarly, three of three cheetahs pretreated with Norplant ovulated after gonadotropin stimulation, and only one had significant numbers of unovulated follicles (Howard, unpublished data). Unlike in the domestic cat, fishing cat and cheetah, however, pretreatment with Norplant in the clouded leopard appeared to interfere with ovarian response to gonadotropin stimulation. Only three of eight (37.5%) clouded leopards given Norplant before eCG/hCG ovulated, and all females had multiple unovulated ovarian follicles (Pelican, unpublished data). Further research will be required to elucidate the species differences in ovarian response to similar gonadotropin protocols.

Exogenous gonadotropins also may be useful in treating pathologic conditions, including persistent estrus or secondary anestrus associated with ovarian follicular cysts. In any case of abnormal estrous cycling, a thorough history and physical examination should rule out nonreproductive causes of dysfunction including neoplasia, nutrition or stress-related alterations in reproductive activity. In cases of persistent estrus, a thorough ultrasound examination should be undertaken to identify the source of elevated estrogens (follicular cysts versus neoplasia) and to evaluate the condition of the

uterus. Fecal hormone analysis also can be used to verify anestrus or hyper-estrogenism.

Follicular cysts have been documented in the domestic cat,^{34,35} but the prevalence of this condition in nondomestic felid species is unknown. Even in the domestic cat, the etiology of this condition is unclear, and the recommended treatment is ovariectomy. Clearly, this is not a desirable option in most rare wild felid species. In other species, a variety of hormonal treatments have been used with variable success. Ovulation induction hormones including GnRH, LH and hCG can be administered to artificially rupture the anovulatory follicles.^{36, 37} However, the dysfunctional follicles are often insensitive to ovulatory stimuli.^{38,39} Recent research indicates that follicular cysts in cattle develop following an inadequate luteal phase with low levels of progesterone.⁴⁰ Exposure to high progesterone concentrations successfully re-sets the hypothalamo-pituitary-ovarian axis and returns the animal to normal estrous cycling.⁴⁰ In cats, ovulation induction and progesterone treatment must be undertaken with caution because prolonged estrogen exposure followed by elevations in progesterone due to treatment or ovulation could result in cystic endometrial hyperplasia and pyometra. Again, more research is required to determine the most successful treatments for ovarian follicular cysts in felids.

In conclusion, ovulation induction is important for timing assisted reproductive procedures appropriately and for treating anovulatory conditions secondary to ovarian follicular cysts. Significant progress has been made toward developing a consistent ovulation protocol for felids. Species differences are significant, and it is relatively easy to cause ovarian hyperstimulation and altered endocrine profiles that, in turn, can compromise reproduction. Fundamental studies directed at each species, however, generally have been effective at elucidating efficient ovulation induction protocols. Furthermore, recent investigations into using progestogen pretreatment to improve ovarian response to exogenous gonadotropins have yielded promising results.

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NEOPLASIA IN SUGAR GLIDERS (*Petaurus breviceps*): THIRTY-THREE CASES

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Abstract

The sugar glider, (*Petaurus breviceps*), is a small (95-160 g) arboreal marsupial native to Australia and New Guinea. Although held by no fewer than 43 North American zoos, and having become a popular pet in recent years, little concerning them has been published in the veterinary literature.^{1,5,6}

In August, 2001, at the Albuquerque Biological Park (ABP), a 7-yr-old male sugar glider was reported to have an alopecic spot on one of its legs. Physical exam found the alopecic spot to be associated with a subcutaneous mass on the left lateral thigh. A second subcutaneous mass was also found in the right axillary region. Both masses were surgically removed and submitted for histopathology. Both excised masses, one an axillary lymph node, contained neoplastic lymphocytes with a high mitotic index of 4-8 mitotic figures per hpf, consistent with a diagnosis of lymphosarcoma. A recheck examination 2 wk post surgery found two additional subcutaneous masses. Euthanasia was elected and postmortem histopathology confirmed that these additional masses were also lymphosarcoma.

A review of pathology records at ABP revealed that, between 1997 and 1998, neoplasia was diagnosed in all six members of a group of sugar gliders received as a group from another institution in 1993. An investigation into the history of this group of sugar gliders and similar groups of sugar gliders was initiated. In addition to ABP records, medical records of sugar gliders that died and had a complete necropsy examination at the Metropolitan Toronto Zoo and the Gladys Porter Zoo were reviewed, and a search of the Australian Registry of Wildlife Pathology was conducted.

The results of this preliminary investigation are listed in Table 1. Males were affected roughly twice as commonly as females. Gliders as young as 1 yr of age were affected. Many different systems are affected, but the more common types included lymphosarcoma, leukemia, and adenocarcinoma. Among the marsupials, the dasyurids are disproportionately represented in their susceptibility to neoplasia.⁴ Neoplasia has been described in nearly all tissue types in dasyurids and are well documented in the literature. In contrast, previous documented reports of neoplasia in sugar gliders are limited to a single case report³ and a smattering of cases included in a large survey of neoplasia in native Australian marsupials.² Neoplasia has been reported in other gliders, such as squirrel gliders and greater gliders.¹ The true incidence of neoplasia in the Petauridae may be common, as

in the Dasyuridae.

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Table 1. Cases of neoplasia in sugar gliders.

Case#	ID	Sex	Age	Source	Diagnosis
1	D8	M	A	Canfield et al	splenic erythroid leukemia
2	D25	M	A	Canfield et al	splenic erythroid leukemia
3	D54	M	-	Canfield et al	splenic erythroid leukemia/duodenal adenocarcinoma
4	D107	-	-	Canfield et al	subcutaneous fibroma
5	D140	-	-	Canfield et al	subcutaneous fibroma
6	-	M	-	Canfield et al	lymphoid or myeloid leukemia
7	-	M	4	Hough et al	cutaneous lymphosarcoma
8	N97035	M	12	ABP ^a	cutaneous lymphosarcoma
9	N97036	M	11	ABP	spindle cell tumor
10	N97073	M	7	ABP	pericloacal tumor
11	N97077	F	6	ABP	mammary carcinoma
12	N98034	M	8	ABP	lymphosarcoma
13	N98047	F	12	ABP	lymphosarcoma
14	N01050	M	7	ABP	lymphosarcoma
15	Z042-90	F	A	MTZ ^b	adrenal cortical carcinoma
16	Z250-91	F	4	MTZ	mammary carcinoma
17	Z010-93	M	11	MTZ	intestinal carcinoma
18	Z040-93	F	5	MTZ	pheochromocytoma
19	Z215-93	F	7	MTZ	vaginal squamous cell carcinoma
20	3297	M	11	GPZ ^c	fibroma
21	3299	M	11	GPZ	myxosarcoma
22	3302	M	11	GPZ	lymphosarcoma/fibrosarcoma
23	4065	M	4	GPZ	sebaceous epithelioma
24	4066	F	A	GPZ	adenocarcinoma
25	4067	F	7	GPZ	adenocarcinoma(lung)
26	4590	F	1	GPZ	dysgerminoma
27	7588	M	1	GPZ	fibrosarcoma(incidental)
28	D251	F	7	ARWP ^d	histiocytoma, subcutaneous
29	D366	-	-	ARWP	adenocarcinoma(chest gland)
30	D396	-	-	ARWP(NZ)	mammary adenocarcinoma
31	D399	-	-	ARWP(NZ)	adenocarcinoma-metastatic
32	D400	-	-	ARWP(NZ)	splenic erythroid leukemia
33	D1498.1	M	10	ARWP	adenocarcinoma(abdominal)

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ALUMINUM PHOSPHIDE TOXICOSIS IN TWO RED PANDAS (*Ailurus fulgens fulgens*)

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Abstract

Aluminum phosphide (AIP) is a regulated pesticide used worldwide against rodents and insects. Its main use is fumigation of grain and other agricultural products and it is also used for treatment of rodent burrows.¹ Upon contact with moisture, toxic phosphine gas (hydrogen phosphide) is liberated. The liberation of phosphine is accelerated by an acidic environment such as the hydrochloric acid of the stomach following ingestion.^{1,2}

Toxicosis associated with AIP can occur following either inhalation of phosphine gas or ingestion of the solid product leading to a subsequent release of phosphine internally. The toxic mechanism of the gas has not been fully elucidated,² however, phosphine has been shown to block cytochrome C oxidase in rat liver preparations. The resulting blockage of electron transfer and inhibition of oxidative phosphorylation lead to an energy crisis in the cells.¹ Postmortem findings in rodents and humans include cyanosis, pulmonary edema, pleural effusion and congestion of internal organs.^{1,2}

On the morning of 11 January 2003 two previously healthy adult male red pandas (*Ailurus fulgens fulgens*) were found dead on the ground of their outdoor enclosure at the Smithsonian National Zoological Park (SNZP). On the previous afternoon, 1-2 aluminum phosphide pellets weighing 3 g each (Fumitoxin, Pestcon Systems, Inc. USA) were buried in each of seven rodent holes in the enclosure by a private pest control contractor; the pellets were said to have been placed approximately 60 cm deep. The treated holes were then covered with metal plates or soil. Several other rodent holes remained open and uncovered. During the placement of the AIP pellets, both red pandas were observed in a tree in the exhibit.

Full necropsies were performed under chemical hoods on both red pandas. Both animals were found to be in excellent nutritional condition with stomachs partially filled with bamboo ingesta, and normal feces in the lower digestive tract. There was no evidence of external or internal injury or trauma and no outward or systemic signs of infectious diseases. Significant gross abnormalities noted in both animals included subcutaneous dehydration, cyanosis of mucous membranes and lungs, generalized dark venous congestion, and aspiration of fluid gastric contents into the trachea and bronchi. Peri-oral staining of bilious vomitus was present in one of the animals.

Histopathologic findings in both cases were similar and included acute pulmonary edema and emphysema, degenerative changes in the liver, including eosinophilic, diastase-resistant PAS-positive cytoplasmic inclusion bodies within hepatocytes, edema of the gallbladder and

proteinaceous casts in the kidneys. These changes are compatible with a systemic insult by a toxic agent. Similar liver inclusion bodies have occurred in association with certain chemicals toxic to the liver in humans.⁴ Mild inflammatory changes were noted in the kidneys and livers of both red pandas and a few lymphocytes in the meninges of one of the animals. These changes were considered preexistent and noncontributory. Cytologic examination of abdominal fluid and heart blood were within normal limits, and cultures from abdominal fluid, heart blood, liver and feces were negative or determined to be nonpathogenic and noncontributory to any of the histopathologic changes noted.

Using gas chromatography-mass spectrometric methods (GC-MS), stomach contents and tissue samples from both red pandas were analyzed specifically for the constituents of aluminum phosphide and generally for heavy metals, pesticides, drugs and industrial chemicals by the Michigan State University Animal Health Diagnostic Laboratory. After treatment of stomach contents from each red panda with strong acid, phosphine gas was detected at 163 and 58 ppm respectively. Aluminum levels in the stomach contents of each animal were 1030 ppm and 444 ppm. Aluminum levels in their feed (bamboo and biscuit) and feces ranged from 30-147 ppm. In addition, lung levels of aluminum in both affected red pandas were 69.3 ppm and 71.3 ppm, respectively. The aluminum level in archived frozen normal lung tissue from another SNZP red panda used as a control was 5.48 ppm. Aluminum levels in kidney and liver tissues were <1 ppm in both affected red pandas.

The high phosphine gas levels and relatively high amounts of aluminum detected in the stomach contents of both red pandas indicate the digestive tract as the portal of entry, and that the animals most likely ingested the aluminum phosphide rodenticide. The increased level of aluminum in the lungs of both red pandas relative to the low level in the control red panda's lung tissue is likely due to agonal aspiration of gastric fluid.

Red pandas are nocturnal animals and most likely descended from the tree to investigate changes in their environment the evening after their exhibit yard had been treated with the rodenticide. Physical signs of digging were not evident, and it is unlikely a red panda would be able to dig deeply enough to retrieve and ingest aluminum phosphide pellets buried 60 cm deep. However, the curious nature and chemo-sensory behavior of investigating unknown materials in their environment by tongue-tasting,³ suggests the red pandas ingested fragments of aluminum phosphide that may have escaped in the area during the rodenticide placement procedure.

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REPRODUCTIVE DISORDERS IN CAPTIVE AMUR LEOPARDS (*Panthera pardus orientalis*) IN EUROPE

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Abstract

The remaining wild population of Amur leopards (*Panthera pardus orientalis*) is estimated to be about 40 individuals. An additional 208 individuals are present within the captive Europaisches Erhaltungszucht-Programm (EEP) and Species Survival Plans (SSP).⁵ Captive breeding programs have therefore become increasingly important for the conservation of this species. In addition, the development of reliable methods for reproductive assessment is needed to optimize breeding management. Although ultrasonography and laparoscopy are widely used techniques for reproductive assessment, more recently transrectal ultrasonography has also shown great promise in non domestic felids.²⁻⁴ On behalf of the EEP an international team representing six European institutions performed an interdisciplinary project on captive Amur leopards, consisting of general health screening, interchange of husbandry and management ideas, collection of tissue samples for genetic and taxonomic work, and the assessment of reproductive soundness.¹ In Europe a total of 102 Amur leopards (53 males, 49 females) are held in captivity from which only 11 animals are classified as pure-bred. In this paper, we present the data on reproductive health assessments of the genetically most valuable individuals.

Four females (7-14 yr old) were evaluated by transrectal ultrasonography. One female was examined twice, with a 0.5-yr interval. Blood samples were collected for steroid hormone analysis. Additionally, fecal samples of three animals were available for noninvasive determination of estrogen and progesterone profiles over a time period of 3 mo. One female showed a healthy but inactive genital tract which was confirmed by the hormonal data, too. In the remaining females ($n = 3$) pathologic findings in their urogenital tracts ranged from vaginitis and cystitis to myometrial cysts, focal fibrosis in the myometrium, single or multiple myomas, luteal and follicular cysts. Although the ovarian disorders could be confirmed by the hormonal data, uterine pathology could not be diagnosed by the same methods. Fecal analysis of progesterone and estradiol of females ($n = 3$) housed without a male indicated the capability of spontaneous ovulation in Amur leopards, which is in contrast to many other felid species. The detected cycle length was 14-17 days.

Nine male Amur leopards (seven pure-bred, two hybrids) were investigated by transcutaneous (testes) and transrectal (accessory sex glands) ultrasonography. Six of them were examined twice with a 0.5-yr interval. In addition, semen collection was performed by rectal electro stimulation

in each individual. The semen was assessed for motility and morphology by light and scanning electron microscopy (SEM).

The transrectal ultrasound indicated normal development of urogenital tract in eight males. In one pure-bred male an incomplete descensus of one testis was diagnosed. This 2-yr-old male had an aspermatic ejaculate. In the remaining pure-bred males ($n = 6$) it was possible to collect semen of variable quantity and quality (volume: 250-1,500 μ l; total sperm number: $8\text{-}300 \times 10^6$; motility: 5-70%). The semen samples of both hybrid males were characterized by significantly higher sperm concentration ($> 2000 \times 10^9$ sperm/ml) and sperm motility ($> 80\%$). Morphology assessment indicated a high percentage of defect sperm cells in the pure-breds. The hybrids showed abnormal shaped acrosomes only detectable by SEM. In comparison of ejaculates obtained to different times of the year it was hypothesized that a seasonal spermatogenesis exists in the Amur leopard.

In conclusion the presented data on the reproductive status provided basic information about Amur leopards and will be of great benefit of both genome banking and future assisted breeding programs. The application of ultrasonography offered additional information to endocrine monitoring and is a reliable tool for nonsurgical assessment of reproductive soundness. This species most threatened to extinction in the wild is also characterized by a very limited number of reproductively healthy pure-bred individuals in captivity and has only a chance to survive if assisted reproduction will be applied.

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NEURODEGENERATIVE DISORDERS IN SNOW LEOPARD CUBS (*Uncia uncia*)

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Abstract

This report presents the clinical features and pathologic findings of two neurologic disorders affecting young snow leopard (*Uncia uncia*) cubs born recently in different zoological institutions in Switzerland and France. The first disorder was diagnosed in snow leopard cubs born to three breeding pairs in one Swiss and two French zoological institutions (Parc d'Auneau and Zoo de Thoiry) between 1997 and 2002. The disorder appeared in two or three consecutive litters from each breeding pair and all cubs born in the affected litters developed neurologic signs. Beginning at the age of 2-4 mo, the cubs developed locomotional disorders characterized by swaying gait, hypermetria and weakness of the hind limbs, associated with progressive muscle atrophy of the hindquarters. The interpretation of the neurologic assessment was very difficult, however the spinal reflexes of the pelvic limbs (patellar and flexor reflexes) were present. Due to the progressive nature of the symptoms most cubs were euthanatized within 1 yr of age. Necropsies were performed on five animals and did not reveal any gross lesions. Histopathologic examination revealed degenerative lesions in the cervical, thoracic and lumbosacral segments of the spinal cord. The lesions were confined to the lateral and ventral columns, with the dorso-lateral and ventro-medial peripheral aspects being most severely affected. The changes were characterized by the dilation of myelin sheaths containing preserved axons, myelinophages or axonal debris, occasional axonal swelling, and decreased density of myelin and axons associated with astrocyte proliferation and perivascular gitter cell cuffs. The severe Wallerian-type degeneration of the axons was better appreciated in the longitudinal sections.

A second neurologic syndrome was diagnosed in three of four cubs born in July 2002 at the Zoo of Doué-la-Fontaine, France. The first cub drowned at 2 wk of age during an accidental flooding of the building. At the age of 3-5 wk the three remaining cubs started to show clinical neurologic symptoms characterized by head and body tremors, swaying gait, followed by inability to stand and paresis of hind limbs. Additional clinical findings were loss of body weight and a shaggy hair coat. Due to the progression of the neurologic symptoms all three cubs were euthanatized at the age of 9-11 wk and necropsies performed. Gross examination revealed severe emaciation. The hair coat appeared shaggy due to the presence of small dystrophic, hyperkeratotic hair. No lesions were observed in the other organs. Histopathologic investigation of the nervous system revealed lesions

characterized by the presence of chromatolytic neurons in the spinal cord, predominantly located in the proprioceptive nucleus thoracicus in the proximal lumbar segments with associated microgliosis. Evidence of neuronal necrosis and neuronophagia was not evident. In one animal there was also distinct myelin sheath dilation and axonal degeneration in the corresponding thoracic and cervical spinocerebellar tracts (dorso-lateral). No changes were seen in the brain, spinal ganglions and peripheral nerves.

The cases from Switzerland, Auneau and Thoiry appear to be clinically and pathologically similar to a previously described neurologic disorder of snow leopard cubs at the Helsinki zoo¹. In Helsinki, snow leopard cubs presented with spastic paresis. Pathologic lesions from these cubs were characterized by myelin and axonal loss associated with astrocytic proliferation without inflammatory reaction, predominantly in the thoracic and lumbosacral segments of the spinal cord. These cases also resemble the degenerative myelopathy described in cheetahs, an emerging disease which severely affects the European cheetah population.^{3,4} To date, the cause of the degenerative lesions in these two species could not be determined.

The cases from Doué are clearly distinct. Not only are the age of onset, the clinical symptoms and histopathologic features distinct, but also the fact that this was an isolated litter from a breeding pair that had already produced several normal litters. This contrasts the situation in the three other institutions where afflicted litters were repeatedly produced.

Neurodegenerative diseases characterized by spinal white matter degeneration or neuronal chromatolysis have been described in human and in several animal species, including cheetahs and snow leopards, but the cause is mostly unknown.¹⁻⁴ Some of these diseases have been shown to have a hereditary basis, but nutritional deficiencies, intoxication or metabolic disorders were suggested as other etiologies. The genetic basis of captive snow leopards is narrow. Going back one to five generations, a preliminary pedigree analysis showed that all affected snow leopards from Switzerland, Auneau, Thoiry and Doué have common ancestors. This may indicate a possible genetic predisposition, but whether this is the sole etiology remains to be elucidated.

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HISTOPLASMOSIS OUTBREAK AT THE HOUSTON ZOO

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Abstract

Histoplasma is a dimorphic fungal organism found worldwide and has caused granulomatous disease in both humans and animals. The organism has a high nitrogen requirement and thus is commonly found in soils contaminated with bird or bat feces. The most common site of infection in humans and domestic animals is the respiratory system due to inhalation (rarely orally) of contaminated aerosols. Treatment in animals is efficacious if started at a timely basis.

Starting in 1998, the Houston Zoo experienced an outbreak of histoplasmosis. The disease was found in 25 mammals of six different species (Table 1), all housed at some point in their life in the zoo's small mammal house. The majority of the animals were found dead in their enclosure with no prior illness detected. Lesions containing *Histoplasma* organisms were found on gross necropsy and confirmed with histopathology. Several cases were confirmed *Histoplasma capsulatum* by culturing.

A trend of histoplasmosis cases was noted at the end of 1999. After the first group of animals succumbed to the disease at the small mammal house, a review of husbandry protocols was conducted. Changes in husbandry practices were made, although by this time, many animals in the small mammal house had possibly already been exposed to fungal spores. It is unknown how the histoplasma organism was introduced into the building but, it was suspected that the organisms were present in the mulch used as substrate for many of the exhibits.

There was a lapse of 1 yr, from November 2001 to December 2002, when no cases of histoplasma were detected. This was attributed to major changes in husbandry methods, personal protection protocols, and prophylactic treatment of all animals housed in the area. Treatment was attempted first in the Egyptian fruit bat group using ketoconazole (June 2000, approx. 30 mg/kg p.o., s.i.d. × 1 mo) mixed in the food. When additional cases of histoplasma were discovered on necropsy, oral itraconazole (June 2001, 10 mg/kg p.o. in food s.i.d. × 4 mo) was initiated for the Egyptian fruit bats. An artibeus bat, a flying fox, and the sugar glider group were also treated with oral itraconazole for a total of 2 mo.

It is unknown if any additional animals from this building harbor or will succumb to histoplasmosis. Antemortem disease testing in these species thus far has been unrewarding. Further diagnostic testing is being investigated.

Table 1. Species, necropsy date, and *Histoplasma* lesion location of the animals of the Houston Zoo found to have histoplasmosis.

Species	Common name	Necropsy date	Histoplasma lesion location
<i>Octodon degus</i>	Degu	10 July 1998	systemic with lung involvement
<i>Petaurus breviceps</i>	Sugar glider	18 Jan 1999	skin only
<i>Octodon degus</i>	Degu	30 Sept 1999	systemic without lung involvement
<i>Rousettus aegypticus</i>	Egyptian fruit bat	09 Oct 1999	systemic without lung involvement
<i>Artibeus jamaicensis</i>	Jamacian F bat	19 Oct 1999	systemic with lung involvement
<i>Rousettus aegypticus</i>	Egyptian fruit bat	01 Nov 1999	systemic with lung involvement
<i>Rousettus aegypticus</i>	Egyptian fruit bat	08 Nov 1999	systemic with lung involvement
<i>Rousettus aegypticus</i>	Egyptian fruit bat	17 Dec 1999	systemic with lung involvement
<i>Rousettus aegypticus</i>	Egyptian fruit bat	10 Feb 2000	systemic with lung involvement
<i>Rousettus aegypticus</i>	Egyptian fruit bat	07 Apr 2000	systemic with lung involvement
<i>Vulpes zerda</i>	Fennec fox	27 Apr 2000	systemic with lung involvement
<i>Petaurus breviceps</i>	Sugar glider	10 July 2000	skin only
<i>Ammospermophilus harisii</i>	Ground squirrel	17 Oct 2000	systemic with lung involvement
<i>Octodon degus</i>	Degu	12 Dec 2000	systemic without lung involvement
<i>Ammospermophilus harisii</i>	Ground squirrel	02 Mar 2001	systemic without lung involvement
<i>Rousettus aegypticus</i>	Egyptian fruit bat	16 Mar 2001	systemic without lung involvement
<i>Rousettus aegypticus</i>	Egyptian fruit bat	21 Mar 2001	systemic with lung involvement
<i>Rousettus aegypticus</i>	Egyptian fruit bat	12 May 2001	systemic with lung involvement
<i>Rousettus aegypticus</i>	Egyptian fruit bat	26 June 2001	systemic without lung involvement
<i>Petaurus breviceps</i>	Sugar glider	30 Aug 2001	systemic with lung involvement
<i>Petaurus breviceps</i>	Sugar glider	02 Sept 2001	systemic with lung involvement
<i>Vulpes zerda</i>	Fennec fox	06 Nov 2001	systemic without lung involvement
<i>Rousettus aegypticus</i>	Egyptian fruit bat	14 Dec 2002	systemic with lung involvement
<i>Rousettus aegypticus</i>	Egyptian fruit bat	13 Jan 2003	systemic without lung involvement
<i>Petaurus breviceps</i>	Sugar glider	21 Mar 2003	systemic without lung involvement

WHAT DOES A POLYMERASE CHAIN REACTION (PCR) RESULT REALLY MEAN?: THE DIRTY SECRETS OF MOLECULAR DIAGNOSTICS

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Abstract

Molecular diagnostic tests are now routinely offered by most government, university, and private commercial laboratories. However, because these tests are relatively new, many clinicians and pathologists have limited experience with their selection and interpretation. In addition, these new diagnostic methods bring with them new pitfalls and therefore require new criteria for interpretation.³ The purpose of this presentation is to explain some of the advantages and disadvantages of molecular diagnostics, with the hope that it will be an aid to test selection and interpretation.

There are a number of different molecular diagnostic tools available today for diagnosis of genetic and infectious diseases, but this talk will focus on the use of the polymerase chain reaction (PCR) to detect specific nucleic acid (DNA or RNA) sequences for infectious disease diagnosis. This is by far the most common application of molecular diagnostics in the zoo and wildlife setting. The purpose of PCR is simply to amplify the target DNA to the levels necessary for detection. The amplified DNA can then be identified by its molecular weight (by comparing its migration distance on a gel to controls of known molecular weight) or by its base sequence (as determined by an automated DNA sequencing instrument, or by hybridizing a labeled DNA probe to the amplified DNA – called southern blotting). PCR can also be used to identify RNA by a method called reverse transcriptase (RT)-PCR, which reverse transcribes the RNA into cDNA before amplification. This is followed by identification of the amplified cDNA by the methods described above. Although technically more complicated, this type of PCR is important because many viruses have RNA genomes.

PCR as a diagnostic tool has many advantages, including extremely high sensitivity, potentially high specificity, lack of host-species specificity (very important for zoo animals!), potentially short turnaround time, minimal sample size requirements, and the ability to rapidly develop new tests for unknown or unculturable agents.⁴⁻⁶ PCR can also be applied to many different sample types, including paraffin-embedded tissues, and is useful for molecular strain typing in epidemiologic investigations.⁵ Finally, molecular identification of pathogens is safer for laboratory personnel than in vitro cultivation, which requires more stringent biosafety procedures and special permitting for certain agents.⁶

Less well publicized are the disadvantages of PCR as a diagnostic tool. These include the fact that

although the test itself is not host-species specific, validation of specific tests for diagnostic applications is! What this means is that a laboratory offering PCR for a specific agent in a specific host, such as feline herpesvirus in the domestic cat, has validated that test to reliably identify only that specific agent in that specific host. The validation process involves demonstrating that there are no other common infectious agents, commensal organisms, or host DNA segments that would be accidentally amplified by the PCR primers, thus producing a false positive. This type of validation enables a positive test to be based solely on finding a band of the appropriate molecular weight on a gel, thus foregoing the labor-intensive sequencing or southern blotting required to more definitively identify the PCR product. While this approach is appropriate when it is applied to the agent and host species for which it was validated, using domestic animal tests in zoo animals can result in unpredictable false positives or false negatives. In other words, in most of the testing situations we face, finding a band of the correct molecular weight on a gel is not sufficient to call the test positive. There are many ways in which PCR can accidentally amplify unrelated sequences to create a band of the correct molecular weight, but the wrong sequence. It is important when using commercial molecular diagnostics in zoo animals to have a plan for confirming any positive bands, either by direct sequencing, southern blotting, or by another confirmatory test.

Additional disadvantages of PCR include the fact that it is not usually quantitative, which can make clinical significance of a result more difficult to interpret. For example, the quantitative nature of conventional tests is often useful in establishing the clinical significance of the test result, as when a clinically ill animal has a rising or very high titer to a likely etiologic agent, or a bacterial culture yields heavy growth of a pathogen. In contrast, a PCR result may be reported as positive even though only a few individual organisms or viral particles are present. In many circumstances, infectious agents present in such low numbers could be clinically insignificant. Such “clinical false positives” can also occur because PCR doesn’t distinguish between viable or dead microorganisms. Other disadvantages of molecular diagnostics are the technical difficulty of the procedures, the expensive equipment required, the high risk of cross contamination of amplified DNA in the laboratory leading to false positives, and the contribution of sampling error to the occurrence of false negatives. Finally, sample handling requirements can be complicated. RT-PCR usually requires very fresh samples and special transport media to prevent degradation of the RNA, which is much more labile than DNA. It is always important to scrupulously avoid contamination of samples (e.g., microtome blades must be changed between every block when cutting paraffin sections for PCR). Certain sample types also carry inherent limitations, for example PCR on paraffin embedded tissues is generally limited to identifying base sequences of less than 500 bases, and direct PCR on fecal samples often results in false negatives due to the presence of unidentified inhibitors.

So how can a busy clinician make the most of molecular diagnostics?

1. First, be aware that the dirty secret of molecular diagnostics is that a “positive” test may only mean a band of a particular molecular weight on a gel. It doesn’t necessarily mean that that band corresponds to the specific infectious agent you are looking for.
2. Know what your result means (i.e., try to get a feel for potential sources of false positives or

negatives and have a plan to confirm any positives, either by direct sequencing, Southern blotting, or alternative testing).

3. Know what you are asking for and why. Is the point of the test to determine whether a particular agent is present, to confirm results of another test (e.g., serology), or to characterize and more specifically identify an agent already known to be present? Be aware that test performance will generally be much lower when you are screening healthy animals for a disease of low prevalence as compared to testing clinically ill animals for a likely etiologic agent.

4. Understand how to interpret the clinical significance of a positive or negative result. For example, does the agent cause latent infections or asymptomatic carrier states? If so, a positive PCR result might not be any more informative than positive serology. Does the presence of the agent alone have clinical significance or is quantitation necessary to establish significance? Could a negative result be due to sampling error?

Molecular diagnostic technology is still changing rapidly, with many advances on the horizon. DNA-Chip technology has the potential to provide rapid molecular diagnostics for many different pathogens in one simple on-site test.¹ Real-time quantitative PCR is available now and can provide very rapid results with specific confirmation of positives incorporated into the test.² Other applications include simultaneous bacterial identification and antibiotic susceptibility testing within hours, searching for residual neoplasia following chemotherapy, monitoring response to treatment for infectious diseases, and representational difference analysis for identification of any unknown agents in a sample.^{5,6}

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BIRDS THAT SING AND DIE: BETA AMYLOID PRECURSOR PROTEIN AS A MARKER FOR AVIAN TRAUMATIC BRAIN INJURY

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Abstract

Accurate information regarding the morbidity and mortality of zoo animals is important in their captive management and may affect the success of efforts to reproduce critically endangered species. Traumatic injury is one of the leading causes of death in avian zoological specimens and head trauma is a major subset of these cases. In veterinary patients, the ability to diagnose traumatic brain injury is often hampered by incomplete history and a lack of gross lesions or histologic evidence visible by routine staining and light microscopy. The diagnosis of traumatic brain injury is often tentative and made by exclusion of other causes. Accurate, rapid, and economic methods to document injury to the brain would provide an important tool in avian diagnostics

In the last 10 yr there has been a surge of research regarding the pathophysiology, diagnosis, and treatment of traumatic brain injury in humans.^{1,8} Recent advances have indicated the importance of axonal injury (AI) as a cause of morbidity and mortality. AI in humans and mammalian models can be detected with high specificity and sensitivity by immunohistochemical demonstration of β -amyloid precursor protein (β -APP).^{2,3,5,7,9} A neuronal glycoprotein, β -APP, is carried by rapid anterograde transport within the axon and accumulates in areas where that transport is impaired (i.e., sites of axonal injury). These immunohistochemical methods have been used successfully in forensics to document many kinds of mechanical injury, such as "shaken baby syndrome," with as short a posttraumatic survival period as 30 min.^{2,9} To date, no published efforts have been made to apply these methods to nonmammalian species, though the presence of β -amyloid has been confirmed in an avian model⁴ and a single avian patient.⁶

The hypothesis of this study was that β -APP accumulates within damaged axons in the brains of birds which have died from traumatic brain injury, and that this β -APP can be detected by immunohistochemical methods already developed for mammalian species.

Case material was gathered from archived necropsy cases of the Zoological Society of San Diego. Sixteen adult birds were examined of which three had histologically apparent axonal lesions, and the others had either confirmed gross, histologic, clinical, or historic evidence compatible with head trauma. Two cases also had spinal cord trauma. Species included representatives from six orders (Columbiformes, Psittaciformes, Apodiformes, Passeriformes, Ciconiformes, and Gruiformes).

Three birds euthanatized for unrelated reasons were used as negative controls. Human brain tissue from a patient with Alzheimer's disease was used as a positive control. An additional 14 cases from the archives of the ZSSD in which the birds died suddenly and no diagnosis was found were also examined. Using standard immunohistochemical avidin-biotin complex techniques, two antibodies against β -APP were utilized at varying concentrations: a) β -APP (clone 22, C11; Boehringer AG, Mannheim, Germany),^{2,3,5,7,9} and b) β -APP C-terminus (CT 695; Zymed Laboratories, 561 Eccles Ave. So., San Francisco, CA 94080 USA).⁹

The results of this study confirmed that β -APP is produced in bird neural tissue, that it does accumulate within damaged axons, and that immunohistochemical techniques developed for its detection in mammalian subjects will work in avian species. Subjectively, the use of β -APP is helpful in detecting injured axons that might otherwise be overlooked in standard H&E sections. In addition, the results of at least one case indicate that immunohistochemical staining with β -APP may detect axonal injury earlier than standard histopathologic techniques. AI, however, was not detected in the majority of confirmed head trauma patients in this study and AI was not found in any "sudden death" cases in a population of birds which died suddenly of unknown causes. These negative results most likely reflect some of the limitations of this study, since relatively few serial sections were examined in each case and standardization of the anatomic location, posttrauma to death interval, or character of the original traumatic event was not possible. The subjective increase in neuronal staining with β -APP may also be important, but these results could not be confirmed objectively without carefully matched controls. While further work utilizing cases with a well-defined trauma to death interval is needed to fully develop this diagnostic tool, β -APP immunohistochemical staining can be a potentially important new method to diagnose and study acute brain injury in avian species.

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GLOMERULAR ATHEROSCLEROSIS AND NEPHROSCLEROSIS IN HOWLER MONKEYS

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Abstract

Atherosclerosis and nephrosclerosis have been described in captive and wild howlers.⁵⁻⁷ This report describes the occurrence of glomerular atherosclerosis and nephrosclerosis in five black howlers (*Alouatta caraya*), three red howlers (*Alouatta seniculus sara*) and one golden howler (*Alouatta seniculus straminea*) from two zoological facilities in the United States. Table 1 summarizes the signalment and history for each animal. Two were males and seven were females. Age at necropsy ranged from 5-26 yr, and average age was 16.1 yr. Five animals died and four were humanely euthanatized. The most common reported historic findings included chronic renal failure (three animals), protein-losing nephropathy (two animals), weight loss (three animals), depression or lethargy (three animals) and constipation (two animals). Serum chemistries were available for seven monkeys and abnormal values included azotemia (seven animals), elevated serum creatinine (seven animals), hypoalbuminemia (seven animals), hypoproteinemia (six animals), hypercholesterolemia (five animals), and hyperphosphatemia (five animals). Urinalyses were available for four monkeys and abnormal values included proteinuria (three animals), hematuria (three animals), and glucosuria (three animals).

Table 2 summarizes the gross and histologic findings for each animal. The most common gross findings were small, shrunk or scarred kidneys (seven animals), globoid or hypertrophic heart (four animals), thoracic or pleural effusions (four animals), aortic or valvular plaques (two animals), endocardiosis (two animals) and yellow fat (two animals). Histologically, all animals had varying and generally severe glomerular and renal vascular atherosclerosis, membranoproliferative and mesangioproliferative glomerulopathy, renal tubular protein casts, nephrosclerosis, and chronic interstitial nephritis. Extra-renal arteriosclerosis and atherosclerosis were detected in seven animals, especially in the heart and aorta. Eight animals had varying degrees of myocardial fibrosis. Some additional histologic findings included chronic enteritis or enterocolitis (six animals), pancreatic acinar cell atrophy (four animals), hemosiderosis in lymph nodes gut or liver (three animals), atrophy of fat (three animals), islet cell hyperplasia (two animals), and hyperplastic goiter (two animals).

The renal lesions in these howlers were attributed to atherosclerosis, hypertension, ischemia, and aging. Glomerular protein leakage and tubular protein casts were attributed to glomerular atherosclerosis and glomerulosclerosis. The nephrosclerosis and chronic interstitial nephritis were attributed to tubular atrophy associated with protein casts as well as ischemic change associated with hypertension and atherosclerosis. The presentations in these animals are similar to various nephropathies described in humans that are associated with acquired derangements in lipid metabolism, atherosclerosis, diabetes mellitus, and hypertension.^{2-4,8-10} High circulating levels of low density lipoproteins, glomerular free radical injury, and ischemia are believed to play important roles in the development of chronic renal disease in humans.^{2-4,8-10} Chronic renal disease with protein-losing nephropathy and atherosclerosis appear to be over-represented in captive howler monkeys fed diets that may not be considered atherogenic in other species, indicating that these animals may be exquisitely sensitive to the effects of hypercholesterolemia, atherosclerosis, and associated development of renal disease.^{1,5-7,11} Strict dietary management and periodic monitoring of serum cholesterol levels may be indicated for howler monkeys.

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Table 1. Signalment and history for howler monkeys with glomerular disease.

Case	Species	Age (yr)	Sex ^a	Clinical presentation	Blood chemistry values	Urinalysis	Disposition
1	Black	11	M	Protein-losing nephropathy	Azotemia Hyperphosphatemia Elevated creatinine Hypercholesterolemia Hypoproteinemia Hypoalbuminemia	Proteinuria Hematuria (trace)	Euthanatized
2	Black	19	F	Protein-losing nephropathy Weight loss Vomiting	Azotemia Hyperphosphatemia Elevated creatinine Hypercholesterolemia Hypoproteinemia Hypoalbuminemia Hyperglycemia ^b	Proteinuria Hematuria (trace) Glucosuria ^b	Died
3	Golden	18	F	Chronic renal failure Weight loss Constipation Pica	Azotemia Hyperphosphatemia Elevated creatinine Hypercholesterolemia Hypoproteinemia Hypoalbuminemia	Proteinuria Glucosuria	Died
4	Red	5	F	Found recumbent and hypothermic	NA ^c	NA	Died
5	Red	16	F	Hospitalized for intensive care No further history	Azotemia Hyperphosphatemia Elevated creatinine Hypercholesterolemia Hypoproteinemia Hypoalbuminemia	NA	Died
6	Black	19	F	Lethargy and pulmonary congestion Cardiomegaly	Azotemia Hyperphosphatemia Elevated creatinine Hypoalbuminemia Neutrophilic leukocytosis w/ left shift Mild anemia	NA	Euthanatized
7	Red	9	F	No history	Azotemia Elevated creatinine Hypoproteinemia Hypoalbuminemia Hypercholesterolemia Regenerative anemia	NA	Died
8	Black	22	F	Lethargy Emaciation Kyphosis Anemia Chronic renal failure	Azotemia ^d Elevated creatinine Hypoproteinemia Hypoalbuminemia Mild anemia	Hematuria	Euthanatized
9	Black	26	M	Anorexia Depression Constipation Kyphosis Spondylosis Chronic renal failure	NA	Hematuria Proteinuria	Euthanatized

^aM=male; F=female.

^bAnimal was on fluid therapy at time urinalysis was obtained.

^cNot available.

^dMost recent bloodwork was 5 mo prior to death.

Table 2. Gross and histologic lesions associated with glomerular disease in howler monkeys.

Case	Gross findings	Renal histologic lesions	Other histologic lesions
1	Small kidneys Yellow fat Thick left ventricle	Atherosclerosis MPG ^a Sclerosis CIN ^b Protein casts	Goiter Atrophy of fat Pancreatic fibrosis and atrophy Arteriosclerosis/atherosclerosis (heart valve) Chronic colitis
2	Pale granular kidneys Plaque on pulmonary valve	Atherosclerosis MPG Sclerosis CIN	Goiter Myocardial fibrosis Hemosiderosis Arteriosclerosis/atherosclerosis (ovary, vagina, aorta, kidney) Pheochromocytoma Chronic colitis
3	Small pale kidneys Globoid heart with thick left ventricle Thickened uterus	Atherosclerosis MPG Sclerosis CIN Protein casts	Mycobacteriosis Hemosiderosis Myocardial fibrosis Atherosclerosis/arteriosclerosis (heart) Chronic colitis
4	Small pale pitted kidneys Hypertrophic left ventricle	Atherosclerosis MPG Sclerosis CIN Protein casts	Myocardial fibrosis Myocardial infarction Pancreatic acinar degeneration and fibrosis Thyroid hypoplasia and fatty infiltration Chronic enterocolitis
5	Generalized edema Ascites Hydrothorax Diarrhea Scarred kidneys	Atherosclerosis MPG Sclerosis CIN Protein casts	Atherosclerosis (heart, stomach) Myocardial fibrosis Islet hyperplasia Atrophy of fat Chronic enteritis
6	Obesity Pulmonary consolidation Enlarged left atrium Ascites Hydrothorax	Atherosclerosis MPG Sclerosis CIN Protein casts	Florid pulmonary edema Myocardial fibrosis Pancreatic acinar atrophy
7	Thin pale mucous membranes Yellow fat Hydrothorax Aortic aneurism Left ventricular hypertrophy Endocardiosis	Atherosclerosis MPG Sclerosis CIN Protein casts	Atherosclerosis/arteriosclerosis (aorta, spleen, urinary bladder, uterus, ovary, colon, pancreas) Aortic aneurism Myocardial fibrosis
8	Emaciation Endocardiosis Aortic plaques Gastric ulcers Pale foci in kidneys	Atherosclerosis MPG Sclerosis CIN Protein casts	Atherosclerosis (mesentery, lymph node, spleen, aorta, ovary) Myocardial fibrosis Endocardiosis and arteriosclerosis Gastric mineralization Atrophy of fat Acinar atrophy and islet hyperplasia
9	Ascites Saponified fat Small pale kidneys Constipation	Atherosclerosis MPG Sclerosis CIN Protein casts	Atherosclerosis/arteriosclerosis (heart, spleen, intestine) Chronic enteritis myocardial fibrosis Hemosiderosis

^aMembranoproliferative glomerulonephritis.^bChronic interstitial nephritis.

IRON STORAGE DISEASE IN CAPTIVE EGYPTIAN FRUIT BATS (*Rousettus aegyptiacus*)

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Abstract

Hemochromatosis refers to excess deposition of iron in tissues resulting in injury to those tissues. It is an important disease of several captive zoological species including toucans, tanagers, birds of paradise, and Egyptian fruit bats (*Rousettus aegyptiacus*). Currently, liver biopsy is the only ante-mortem diagnostic technique for this disease. This study was undertaken to evaluate hemosiderosis/hemochromatosis in a captive population of Egyptian fruit bats by determining whether there is a relationship between blood iron values and hepatic iron concentration, and if histologic findings correlate with hepatic iron levels. Twenty-three adult Egyptian fruit bats were used in the study, and six island flying foxes (*Pteropus hypomelanus*) were used as controls. All were housed in indoor-outdoor enclosures at the Lubee Foundation, a private zoological facility in North Central Florida, and fed the same diet of mixed vegetables, fruits, and a commercial vitamin/mineral supplement. Blood samples were collected for complete blood counts, plasma biochemical profiles, serum iron levels, total iron binding capacity, whole blood lead levels, and plasma ferritin assays. Liver samples obtained via laparotomy were divided; one half was placed in formalin, processed for histologic examination, and scored by two blinded observers for iron deposition, necrosis, and fibrosis, and the other half was frozen and submitted for tissue mineral analysis. The Egyptian fruit bats had significantly higher liver iron (mean = 3668.89 ± 1822.82 ppm) and lead (mean = 8.93 ± 5.77 ppm) levels than the island flying foxes (mean [Fe] = 173.89 ± 173.08 ppm, mean [Pb] = 1.87 ± 0.50 ppm). The hepatic iron levels were significantly ($P < 0.001$) correlated with tissue lead levels, histologic grading for iron and necrosis, serum iron, and ferritin. Blood lead levels were not elevated, and blood lead levels were negatively correlated ($P < 0.001$) with tissue lead levels. Evaluation of serum iron and ferritin may be useful and less invasive methods for diagnosis of hemochromatosis in Egyptian fruit bats. Elevation of the tissue iron and lead levels could be related to altered intestinal absorption of these ions in Egyptian fruit bats.

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ACUTE AORTIC RUPTURE IN ANTARCTIC PENGUINS

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Abstract

There was an unusual cluster of unexpected mortality in Emperor (*Aptenodytes forsteri*) and adelic (*Pygoscelis adeliae*) penguins during a 4-mo period from November 2001 to March 2002. Two emperor and three adelic penguins all presented with sudden death. The affected birds ranged from 16-25 yr. Sex distribution was two male emperors and one male and two female adelic.

On gross examination, four of the five birds had a large amount clotted blood around the great vessels and the heart base. One bird had an extensive pericardial hemorrhage with both clotted and unclotted blood distending the pericardium and compressing the heart. Full thickness aortic tears ranging from 0.5–1 cm in length were identified in the proximate aorta of all birds. No birds had evidence of aortic dilation or aneurysm. One bird had firm intimal plaques.

Histologic examination revealed cystic medial necrosis and acute hemorrhage in the aortic wall at the site of the tears and extending into the adjacent wall, primarily within the tunica media. Special stains for elastin demonstrated fragmentation and rupture at the site of hemorrhage but when performed in nonhemorrhagic areas adjacent to the area of rupture these stains demonstrated no significant fiber shortening or fragmentation. The one emperor with intimal plaques noted grossly did have significant multifocal atherosclerotic changes including mineralization in the aortic wall.

Heavy metal analysis was performed on the livers from three of these birds. Copper levels were within published normal values for these seabirds,^{2,3} but also within the ranges associated with aortic rupture in turkeys and ostrich.¹

The etiology of this mortality cluster was not determined. The low hepatic copper in two birds suggests a possible role of copper deficiency. The temporal cluster suggests a secondary factor.

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TAURINE DEFICIENCY ASSOCIATED DILATED CARDIOMYOPATHY IN GIANT ANTEATERS (*Myrmecophaga tridactyla*): PRELIMINARY RESULTS AND DIAGNOSTICS

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Abstract

Recently several cases of adult giant anteaters (*Myrmecophaga tridactyla*) that have died of cardiac insufficiency associated with severe dilated biventricular cardiomyopathy have been reported. The syndrome is similar to that seen with taurine deficiency in domestic felines and canines. Prolonged, progressive exercise intolerance and dyspnea are characteristic clinical signs. Current diagnostic methods include thoracic radiographs and echocardiography. Most affected animals are not diagnosed with dilated cardiomyopathy until clinical signs are present. Treatment at that stage has been unresponsive to oral cardioactive drugs and/or taurine supplementation. The purpose of this study was to evaluate plasma and blood taurine concentrations in giant anteaters with suspected cardiac disease. Initial results suggest that low blood and plasma taurine concentrations are associated with dilated cardiomyopathy and may be used as early diagnostic indicators. We recommend oral taurine supplementation in anteaters with whole blood taurine levels under 300 mg/ml.

Introduction

Dilated cardiomyopathy in giant anteaters resembles taurine deficiency associated cardiomyopathy in other species.^{1,3,4,11,17} Dilated cardiomyopathy is an occasional finding in domestic dogs,^{2,3,6} and cats,^{4,5,15-17} and, less frequently in foxes, raccoons, opossums, poultry, rats, rabbits, hamsters, humans, ferrets, and skunks.^{5,7} Myocardial disease of this type is observed in domestic cats due to insufficient dietary taurine.¹² Taurine, a beta-sulfonic amino acid, is ubiquitous in mammalian tissues, and is the most abundant free amino acid in the heart, retina, skeletal muscle, and brain.^{7,13,15} Dietary taurine is essential for cats due to their limited ability to synthesize taurine from cysteine and methionine.^{3,7-9,13,15} This is associated with a low concentration of cysteinesulfinic acid decarboxylase, an enzyme required for taurine biosynthesis. Cats are unable to conjugate significant amounts of bile acids with glycine and, therefore, must utilize taurine for conjugation of bile acids.⁸ This requirement may result in a taurine demand in excess of supply. Taurine has also been suggested to function in myocyte calcium binding, modulation of contractility (possibly associated with myocardial calcium homeostasis), osmotic regulation, blood pressure control, and platelet resistance to aggregation.^{7,13} Dilation of the heart occurs when the left ventricle fails to generate normal contractile force and ejection fraction is severely reduced. End-systolic and end-diastolic

ventricular volumes increase and myocardial wall tension becomes elevated. Left ventricular relaxation and diastolic compliance alterations occur, along with impaired contractile function. This contributes to elevated filling pressures. The activation of the sympathetic and renin-angiotensin-aldosterone systems further increases cardiac preload and afterload. End-systolic volume increases from impaired left ventricular contractility. The end result of these processes is pulmonary congestion, effusion, and signs of low cardiac output. Biventricular dilation and associated cardiac insufficiency are the hallmarks of a taurine-deficient cardiomyopathy in domestic cats.^{1,8-10,12,13,15} Cats also develop sequelae of retinal degeneration, reproductive and developmental anomalies, and increased platelet aggregation, as well as decreased leukocyte function.⁶ In felines and some breeds of canines, specifically American cocker spaniels, boxers, and doberman pinschers, taurine and L-carnitine supplementation can ameliorate the clinical signs and, in many cases, improve cardiac condition and size to near normal parameters.^{4,6,8,9,11-13,15} Dogs usually don't require taurine in their diet, provided there are sufficient sulfur amino acids present for adequate taurine synthesis.

A globoid heart with thin left and right ventricular walls is apparent at necropsy in giant anteaters with dilated cardiomyopathy. Mineralization of the aorta and stomach has also been found occasionally. Histopathology of cardiomyopathy in anteaters revealed varying degrees of right and left ventricular myofiber hypertrophy, atrophy, and disarray. Passive congestion of the lungs and liver supports the gross finding of bilateral dilated cardiomyopathy. Myocardial fibrosis varied from mild to severe. Occasional mineralization of myofibers and mild chronic inflammation were also seen. Anecdotal evidence in one case suggested a possible role of taurine in the development of dilated cardiomyopathy.¹ The purpose of this study was to evaluate plasma and blood taurine concentrations in giant anteaters with suspected cardiac disease and better characterize this relationship.

Materials and Methods

Whole blood samples were collected opportunistically from eight different giant anteaters from six different institutions that all appeared normal. Table 1 provides signalment information. Further examination, however, revealed that cardiomyopathy was present in all anteaters except anteater A2 in institute 2. Some animals were sampled serially over time. Taurine levels were measured in whole blood preserved in EDTA and in heparin and plasma samples. These samples were frozen and run in as a batch.¹⁴ Complete white blood cell counts were also done when possible. Radiographs of the thorax were performed during routine physical exams on all animals and echocardiography was performed on anteaters A1 and A2 in institute 2.

Results

The results obtained in this study are presented in Table 1. In addition, all animals had subjectively enlarged hearts in radiographs, except for anteater A2 in institute 2.

Discussion

These preliminary results suggest that in the anteaters examined, blood levels of taurine below 300-600 nmol/ml and plasma levels below 60-120 nmol/ml seem to be associated with the presence of cardiomyopathy, and the degree of deficiency may be associated with the degree of disease. Normal canine taurine levels are 60-120 nmol/ml for plasma, 200-350 nmol/ml for EDTA whole blood, and 200-350 nmol/ml for lithium heparinized whole blood.^{5,9} Normal feline taurine levels are 60-120 nmol/ml for plasma, 300-600 nmol/ml for EDTA, and 300-600 nmol/ml for lithium heparinized whole blood.¹⁴

In our study, whole blood was used as an indicator of total body depletion, although it is a slower indicator of changes in systemic taurine levels. Whole blood did not require centrifugation, however, simplifying sample collection. Minimal sample processing after collection decreased the possibilities of technical error in the taurine assay. False elevations in blood taurine levels may occur when an animal presents with leukocytosis, however, since taurine binds to leukocytes. Plasma taurine levels were used as an early indicator for systemic taurine depletion, but thorough centrifugation and careful extraction were required to avoid false elevations due to leukocyte contamination. Both plasma and whole blood samples could be stored, provided there was no bacterial contamination. Whole blood is the recommended sample to submit for systemic taurine determination.¹⁴

Changes in the diets of captive anteaters had been made following reports of hyperostosis associated with hypervitaminosis A and D in tamanduas fed commercial feline diets.² Many institutions had changed commercial cat food-based diets to a paste made of moistened dog food and leafeater (Marion Leafeater, Marion Zoological, 13803 Industrial Park Boulevard, Plymouth, MN 55410 USA). Yogurt, omega fatty acids supplements, and vitamin K (Vetamix Inc. Shenandoah, IA, 51601 USA) were usually added. In at least one instance, the use of commercial dog food may have resulted in taurine deficient dilated cardiomyopathy in giant anteaters.¹ Anecdotal evidence points to disparate taurine levels in commercial dog foods. Nutritional consultants recommend the use of a high quality commercial dog food to ensure adequate taurine levels. Supplementation with pinky mice and meats, as well as access to rotten logs and insects as enrichment, may ameliorate a taurine deficient diet. Oral taurine and carnitine supplementation (taurine, carnitine, 500-mg capsules, Solgar Vitamin and Herb, Leonia, NJ 07605 USA) has been shown to arrest and, occasionally, partially reverse myocardial failure and cardiac chamber dilatation in taurine depleted cats and dogs.⁶ Domestic cats require 1 g/kg of dry matter taurine for expanded diets, 2 g/kg of dry matter taurine for canned diets, and 500 mg/kg of dry matter taurine for purified diets. Relatively new commercial products on the market (Mazuri Insectivore 5MK8, PMI Nutrition International LLC, PO Box 19798 Brentwood, MO 63144 USA) specifically formulated for insectivores can contain taurine concentrations of up to 2.68 g/kg as fed. A few institutions possessing giant anteaters have begun to base their diets on these products. At this time we recommend oral taurine supplementation in anteaters with whole blood taurine levels under 300 nmol/ml. We suggest that, with early detection of taurine deficiency, high-level oral taurine supplementation may provide similar beneficial effects in giant anteaters, and may even reverse myocardial failure and cardiac chamber dilatation. Further studies, however, are required to more precisely define the nutritional

requirement of taurine in anteaters, adequate blood and plasma levels, the most appropriate treatment dosages, and the prognosis for recovery.

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Table 1. Signalment, WBC count, and taurine levels of anteaters with dilated cardiomyopathy.

Animal ID	Sex ^a	Age (yr)	Date of examination	WBC × 10 ³	Plasma (nmol/ml)	EDTA (nmol/ml)	Heparin (nmol/ml)
A1 Inst. 1	F	3	18 Apr 2001	15	33	280	
A2 Inst.1	NA	NA	14 June 2001	10.5		341	329
A2 Inst.1	NA	NA	31 Jan 2002		53		160
A1 Inst. 1	NA	NA	15 Nov 2002		73	168	
A1 Inst. 2	M	5	28 May 2001		27	67	
A2 Inst. 2	F	3	19 July 2001	8.87	48	192	178
A2 Inst.2 ^b	F	NA	15 Jan 2002	7.4	139		279
A2 Inst.2 ^b	NA	NA	24 Apr 2002	7.44	190		350
A2 Inst.2 ^b	NA	NA	20 Dec 2002	13.0	106	325	290
A1 Inst. 3	F	29	27 Dec 2001		18		75
A1 Inst. 4	M	7	3 Feb 2002		22		126
A1 Inst. 5	NA	9	24 Jan 2002		53		143
A1 Inst. 6	F	16	13 Feb 2002		21		240

^aF = female; M = male; NA = not available.

^bFollowing taurine supplementation of 2 g s.i.d. p.o. total dose.

IN SITU CONSERVATION PATHOLOGY: DEVELOPING A FORMAL ZOO-BASED RESIDENCY PROGRAM TO TRAIN VETERINARY PATHOLOGISTS IN LATIN AMERICA

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Abstract

The lack of board-certified veterinary pathologists and wildlife pathologists in Latin America may have a considerable impact on the ex situ and in situ conservation programs in the Latin American countries. Until late 2002 there were no formal residency programs to train veterinary pathologists in these countries so they would be eligible to sit the boards of The American College of Veterinary Pathologists (ACVP). Under these circumstances, the quality of the pathologic diagnoses and their appropriate application to conservation and research efforts, and emerging and re-emerging wildlife disease reporting cannot be assured. Many species involved in conservation programs are indigenous to countries with very limited economic resources or with sociopolitical situations that draw the attention of their citizens from wildlife. For many wildlife field projects in Latin America, these efforts currently depend on foreign support, primarily from North America and Europe, and include the importation of personnel and equipment and the exportation of samples. The cost for such support may be high and is often impractical. As a result, routine gross and histopathologic examinations are not performed or are performed by inadequately trained people, and valuable information is lost.

Currently, two ACVP-board certified veterinary pathologists serve in Latin America, and apparently only one works with zoo and wild animals (at Africam Safari, Puebla (México)). This lack of pathology support has been the subject of much concern among the individuals and institutions involved in this presentation. The initial objective was to establish a formal residency program to train Latin American veterinary pathologists in the United States and, with time allow this program to grow until there is at least one such program in each country in Latin America. This would create a network for true “in situ conservation pathology” and have a great impact on Latin American conservation. In 2001, The Department of Veterinary Microbiology and Pathology at the College of Veterinary Medicine in Pullman, WA, approved a proposal to start a residency program to train Latin American veterinary pathologists in collaboration with Northwest ZooPath, WA. In late 2002, Africam Safari offered a formal, in situ residency program for Latin American veterinary pathologists (two positions) after the ACVP board-certification of its staff pathologist. Now pathologists can be trained in Latin America to become eligible to sit the ACVP boards. A few years ago this seemed far from feasible. Now the expected snowball effect is probable.

The residency is a 4-yr program, and includes a self-teaching study schedule; training in gross pathology, histopathology and writing of formal pathology reports; research; and periodic mock exams similar to the ACVP certifying exam. Teaching materials include the caseload at Africam Safari, histopathology slide collections, gross pathology kodachromes (NOAH's Arkive and Africam's collection), and an on-site library including journals (Veterinary Pathology, Journal of Veterinary Diagnostic Investigation, Journal of Zoo and Wildlife Medicine, Journal of Wildlife Diseases, as well as some 2,000 bibliographic references from scientific journals) and books (including some of the titles covering general and systemic pathology, cytology and zoo animal and wildlife diseases). Resident research activities must include a retrospective study on the causes of disease and death in a single species, a prospective study on a major or new disease that needs special diagnostic testing, and a case report. An annual 1-mo externship at Northwest ZooPath is also an integral part of the program.

A 1-yr pilot internship program started at Africam Safari in June 2002 with a graduate student from the Benemérita Universidad Autónoma de Puebla and was funded by Africam Safari. Apart from the basic training in gross pathology and histopathology, the trainee has completed her contribution to a multi-institutional project on a previously unreported disease in zebras (sarcoïd with demonstrable bovine papillomavirus infection) as well as histoplasmosis in maras, and is currently working on a retrospective study of the morbidity and mortality in the captive mara colony at Africam as of January 2003.

From this beginning, we expect that there soon will be three to four residency positions (two of them in situ at Africam and one or two between Washington State University and Northwest Zoopath) for Latin American veterinarians. These training positions will positively impact the development of veterinary pathology as applied to nondomestic animals in Latin America, in situ conservation programs for Latin American wildlife, and the peoples of Latin America. Our efforts are now directed to stabilize long-term financial support for these valuable training positions. At Africam Safari, we are looking for funding from wildlife agencies, United States and European zoo grant programs, and other resources. Additionally, we will start an external diagnostic service that may eventually cover the expenses of the two residency positions as well as to provide needed exposure to domestic animal pathology.

- INVITED REVIEW -

UNDERSTANDING THE BASIC PRINCIPLES AND PRACTICAL ASPECTS OF VACCINOLOGY

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Abstract

The role of vaccines in preventing disease in domestic animals and humans is reasonably well understood and documented. In zoological species immunity as a result of vaccination is largely assumed, but rarely certain, due to a variety of reasons including unknown species susceptibility and inability to prove vaccine efficacy. Current vaccination protocols in zoological collections are based on limited reports in the literature, anecdotal information, trepidation, animal value and irreplaceability, and the unknown consequences of administering vaccines on a routine basis. Zoo vaccinologists assume that a particular disease in a nondomestic species is caused by the identical etiologic agent and will develop an effective immune response to a vaccine produced for a known human or domestic animal pathogen. Vaccination strategies in zoological institutions should be based on principles of immunology, epidemiology of the disease in a particular species and the safety and efficacy of the specific vaccine. Since little is known about vaccinology in the wide variety of nondomestic species found in zoos, immunologic principles of domestic animal vaccines may serve as a model for vaccination protocols in exotic animals. However, to adequately design safe and effective vaccination programs for our animals there is a need to obtain information on true disease prevalence, species susceptibility and response to vaccination.

Introduction

Vaccinology has changed significantly since many of the vaccines available today for domestic species were originally developed. Our understanding of the immune system has improved and expanded so now many aspects of immunology can be explained at the cellular and molecular level. Additionally, major discoveries have been made in the areas of cell and molecular biology, bacteriology, virology and parasitology which provide greater insight into the nature of the organisms against which the vaccines are designed. Veterinary vaccinologists have begun to take advantage of many of the advances made in the basic sciences. Transferring and applying the new scientific knowledge from modern biology to vaccinology has been slow and difficult because vaccinology, like most of medicine, remains as much an art as a science. Furthermore, vaccinology tends to be driven more by tradition and current wisdom than by scientific principles. It is a discipline that since its beginnings more than 200 yr ago, has prospered through empiric approaches

both for human and veterinary vaccines. The early success in development of vaccines to control many diseases that once caused significant morbidity and mortality clearly demonstrated that an understanding of immunology or any other basic science was not a prerequisite for a vaccine to be successful. Those successes were for diseases that are easily and effectively controlled by the immune system. Unfortunately, the diseases that more recent vaccines have been developed to prevent, present significant challenges to vaccinologists, since the empiric methods of the past will not work as effectively. The diseases that remain are often complex and not as easily controlled by the immune system as diseases for which many of the earlier vaccines were designed to prevent. In fact, some of the new vaccines are for diseases caused by the immune system or the immune system may facilitate survival of the pathogen. To produce the vaccines for the future, modern vaccinology will surely require not only a better understanding of basic immunology, but also a better understanding and utilization of certain advances made in molecular and cell biology, genetics, biochemistry, microbiology and parasitology. Perhaps equally important for modern vaccinologists to understand and consider will be subjects like economics, epidemiology, public health, management practices, animal husbandry and animal (including human) behavior, if new and improved vaccines are to be developed for the diseases which continue to cause significant problems.¹⁻⁸

Thus the challenges for vaccinologists, regardless of the species to be vaccinated, will be to develop vaccines that are truly needed; are safe when administered to avoid immediate or future health problems for the vaccinees and nonvaccinated contacts; are effective in preventing disease in the vaccinees and hopefully effective in reducing the threat of disease for nonvaccinated individuals; are cost effective and; are stable and easily administered so they readily reach the target population that will benefit by receiving them. Vaccines developed for domestic species or humans and used in zoo species will present unique challenges. It will be critical when possible, and if required, that domestic animals vaccines be modified as needed to provide protective immunity in a safe and effective way for zoo species especially those that are rare and endangered. One cannot assume that a bovine vaccine will perform similarly in a Holstein (*Bos taurus*) cow and a gaur (*Bos frontalis*) cow.¹

The Immune System

An understanding of the immune system provides a basis for understanding the nature of vaccine immunity. Two major types of immunity prevent or limit infectious diseases. The two types are nonspecific or innate immunity and; specific or adaptive immunity. Adaptive immunity is characterized by specificity and memory and it is this adaptive immunity which is primarily or exclusively stimulated when an animal receives a vaccine. Ironically, in nature, it is innate or nonspecific immunity that prevents a majority of the pathogens from infecting and/or causing disease in animals. Innate immunity is important because it is the first line of defense and is immediately activated in response to inherent or elaborated chemical substances of the infectious agent. Examples of the innate immune system include skin defense mechanisms such as normal bacterial flora, hair, fatty acids, desquamation; intestinal tract normal flora, anaerobic conditions, pH, fluid flow, presence of lysozyme, mucus, and enzymes; and respiratory tract cilia, mucus,

coughing and alveolar macrophages.

Although current vaccines often do not have a significant beneficial effect on innate immunity, immunomodulators (nonspecific immune stimulants) and some types of vaccines and drugs are targeted toward enhanced innate immunity as a nonspecific method for disease prevention. Certain of the innate factors play a role in specific immune responses. Notable among the soluble factors playing an important role in both innate and adaptive immunity are the interferons (IFNs), certain of the other cytokines (e.g., IL-1, TNF) and complement components. Cells involved in both innate and adaptive/specific immunity are, to a limited extent, neutrophils and natural killer cells, and more critically, monocytes and macrophages.^{1,2,4,8,9}

Ontogeny of the Specific Immune System

The development of the specific immune system has been defined for several mammalian species. In most, if not all species, the genetic information for the specific immune system is present in the fertilized egg and within days the developing embryo has hematopoietic stem cells in its yolk sac. By the end of the first month of gestation in most species, hematopoiesis occurs in the fetal liver. The pluripotent stem cells in the liver are capable of differentiating into mast cells, basophils, megakaryocytes, erythrocytes, eosinophils, neutrophils, and monocytes and, especially critical for the development of the adaptive immune system, they eventually differentiate into the various types of lymphocytes. These include the T helper cell (T_H), T cytotoxic cell (T_c) and B cells, particularly the plasma cells. Early in the second month of gestation lymphoid stem cells migrate from the liver to the developing bone marrow and certain of the cells migrate to the thymus for further maturation into T cells. Shortly after the bone marrow becomes populated with the lymphoid stem cells, the bone marrow becomes the main source of future lymphoid cells that migrate to the thymus for months to years, depending on the species. The B lymphocytes mature in the bone marrow under the influence of the stromal reticular cells and the T cells mature and are educated in the thymus. "Education" refers to the immune system's cell-based discrimination between self and nonself. In the thymus, most T cells with anti-self specificity are eliminated or suppressed so an immune response does not develop to self antigen. An immune response directed toward self antigen can lead to autoimmune disease. Those T cells responsive to foreign antigens that provide cell mediated immunity (T_c and T_{H1} cells), those T cells that regulate (r) or suppress (s) the immune response (T_r or T_s) and those that provide help for B cells (T helper cells) leave the thymus to populate the peripheral lymphoid tissues.

In addition to B cell maturation in the bone marrow, the Peyer's Patches are the site of B cell development for mucosal humoral immunity. The B cells that migrate to mucosal sites from the Peyer's Patches produce primarily IgA that is secreted onto mucosal surfaces. Bone marrow, thymus and special areas in the Peyer's Patches are referred to as central, or primary lymphoid tissues. However, these are not the sites where the lymphocytes encounter antigens. Instead, the primary lymphoid tissues are connected through blood and lymphatic channels to lymph nodes, spleen and lymphoid tissue found in the skin and at mucosal surfaces primarily of the gut, lung, and genital tract. These peripheral, or secondary lymphoid organs, are the sites where lymphocytes meet

antigen. The mature, immunocompetent, but antigen naive cells are continually seeded from the primary lymphoid organs to secondary organs where they encounter antigens and perform their specific effector functions. By at least 1-2 wk prior to birth in most, but not all, species the adaptive systemic immune system is well developed. However, the mucosal system often lags behind, not maturing until after birth.

There is a unique phenomenon that occurs at birth and persists for up to 2 wk in certain species such as the domestic canine and feline. During this period, thermoregulatory control is not fully functional and therefore body temperatures rarely go above 35 °C (95 °F). The specific immune system, particularly monocyte/macrophage and lymphocyte function, is significantly suppressed by low body temperatures. Thus, the newborn puppy and kitten are heavily dependent during this period on their innate immune system, which is also partially affected by low temperatures. They are especially dependent on passively acquired immunity (antibody) which is obtained by absorption of the colostrum from the dam through the intestinal tract for up to 72 hr after birth since only a small amount (5%) of the passively acquired immunoglobulin passes via the placenta in these species. By 1-2 wk of age, thermoregulation is functional and body temperatures become optimal (> 37 °C) for adaptive immunity. Thus, the temperature related immunosuppression disappears.^{2,4,9-15}

However, at approximately 4-5 wk of age, or at the time the puppy or kitten is weaned, another period of transient immunosuppression may occur. This immunosuppression usually lasts for about 1-2 wk and is often due to nutritional deficiencies of vitamin E and selenium that is caused by low levels of these nutrients in the dam's milk prior to weaning. Puppies and kittens fed commercial food supplemented with appropriate vitamins 1-2 wk prior to weaning are more likely to avoid and/or shorten the duration of this immunosuppression. Deficiency in vitamin E and selenium affects cell types important for cell mediated and humoral immunity. It has been demonstrated in animals with severe vitamin E and selenium (Se) deficiency that certain vaccines fail to stimulate protective immunity or they stimulate an immune response which is short lived. In the vitamin E/Se nutritionally deficient dog, vaccines that have a tendency to stimulate a hypersensitivity response are more likely to induce this adverse response and fail to provide protective immunity.^{16,17}

In the bovine and other ruminant species the immune system is very well developed prior to birth and thermoregulation is well developed after birth. However, immunosuppression is still known to occur in these species just prior to and shortly after birth due to high levels of cortisol. Endogenous cortisol, sometimes produced at immunosuppressive levels, is required to initiate parturition. Environmental stress (poor husbandry, inclement weather, etc.) may also cause elevated levels of cortisol.¹⁴ This marked increase in cortisol can be highly immunosuppressive and interfere with effective vaccinal immunity in young animals.

By 6-8 wk of age in most species the adaptive immune system should have recovered from any transient periods of immunosuppression, and if passively acquired maternal antibody (PAMA) is not present to interfere with active vaccine immunity, the young animals should be able to develop optimal immune responses to many of the vaccine antigens. However, most animals will have PAMA to many of the vaccine components, thus vaccines will often not immunize. To ensure

vaccines are given at an age when PAMA no longer interferes, it is necessary to vaccinate until 12 wk of age or older. To help ensure an optimal immune response to those components of a multi-component vaccine that are of greatest concern for the young animal, vaccines with a limited number of components are recommended. For example dogs at 6 wk, should receive a vaccine with canine distemper and canine parvovirus in preference to one with many additional components that are probably not needed at that age. Then at 9 wk and/or 12 wk, vaccines with additional components could be given, if desired. Between 3-6 mo of age, the canine and feline immune system is fully functional, interference by maternal antibody has disappeared, and the immune system should remain at optimal levels until an age associated immune senescence occurs in very old animals (dogs and cats > 9 yr of age). Similarly the immune system of most species is fully functional by 3- 6 mo and there is usually little or no passively acquired antibody after 6 mo of age to interfere with vaccine induced immunity in any of the domestic species. Since the persistence of passively acquired maternal antibody in zoo species has not been well studied it cannot be assumed that vaccines will effectively induce immunity at these same ages. Furthermore, if the maternal antibody titer results from natural infection rather than vaccinal immunity, the higher level of antibody from natural infection will persist for a much longer time than the lower amount of antibody from vaccination. Thus, the age a young zoo species can be effectively immunized may be different than for the domestic species depending on the source of antibody (i.e., infection or vaccination). For example, almost all domestic dog pups can be effectively immunized with canine parvovirus-2 (CPV-2) vaccines by 12-14 wk, whereas the wolf (*Canis lupus*) pup from a dam that has never been vaccinated or infected could be immunized at 6 wk of age. However if the dam's titer was caused by natural sub-clinical or clinical infection, the wolf pup may not respond until 16 to 18 wk of age. Furthermore, the half-life of PAMA may be different among the various species.^{1,2,4,8,18,19}

Immune senescence is a measurable decrease in immune function, primarily of T cells. However B cell function, because of its dependence on T helper cells can also be affected by age. Immune senescence is genetically controlled, thus the extent to which immunity is decreased, and the time that the decrease occurs, varies among individuals, breeds and species. It could be assumed that certain large dog breeds will experience a decline in immunity as early as 7 yr of age, however, in most dog breeds a decline would probably not be seen until 10-15 yr of age or older. Although immune senescence in cats has not been well studied, we would estimate immune suppression in most cats would occur several years later than in the dog, with a decline seen as early as 10 yr for some cats but in most cats it would not occur until 13-18 yr of age or older. In general, immune senescence would occur earlier in those breeds and species with the shortest life span, since it is one important factor contributing to decreased longevity. Because T cells and T cell functions decline more during age related immune senescence than B cell function, vaccinal immunity dependent on B cells and antibody does not seem to be significantly affected in older animals immunized at an early age. This is likely to be the case because memory cells and memory-effector cells present from prior vaccination or infection are less dependent on T cell help than naive B cells or T cells (see discussion later). Unless significantly compromised by various environmental factors that can cause immunosuppression it is expected that during a majority of the life of a healthy individual, the adaptive immune system from vaccination is fully functional and the memory cells stimulated by vaccination should persist for certain, but not all vaccines for months or years. However, differences

exist among species and a wild canid or bovid differ significantly from the domestic counterpart that received the same vaccine.^{7,8,18}

The Cellular Immune Response

The antigen specific defense mechanisms, such as those triggered by vaccines or pathogens are dependent on the T cells and B cells found in the peripheral lymphoid tissues. Initiation of an adaptive immune response requires presentation of antigen by special cells, called antigen presenting cells (APCs), to the T and B cells that have receptors specific for the antigen epitope.

The professional antigen presenting cells include the macrophages, B cells, CD4+ T cells and dendritic cells. Dendritic cells include Langerhans cells and follicular dendritic cells (FDC) with the FDCs being unique in that they present antigen to B cells in the form of antigen-antibody complexes for months after immunization. A critical feature of an APC is the co-expression of class I and II MHC molecules on the cell membrane. Class II MHC molecules are found only on the professional APCs mentioned above, whereas class I MHC molecules are present on all nucleated cells. APCs elaborate a variety of cytokines and have other membrane molecules which are critical for activation of immune responses. Therefore, APCs are critical both for direct antigen presentation and for optimal activation of antigen specific lymphocytes.^{4,7,8}

Antigen processing and presenting cells are critical to the development of an adaptive immune response. Vaccine antigens, like all other antigens, are processed in one of two ways depending on the type of vaccine. Noninfectious (killed) vaccines and modified live vaccines have antigens which are presented primarily in the context of class II Major histocompatibility complex (MHC II) molecules to the CD4+T helper cells. This method of presentation is called the exogenous antigen pathway. In addition to this pathway, a second pathway exists for modified live vaccines with intracellular pathogens in which an infectious agent can infect any of a number of cells, including the APCs. After infection of the cell, new antigens produced in the cytosol moves to the endoplasmic reticulum to combine with the Class I MHC (MHC I) molecules. The MHC-Ag complex then moves to the surface of the cell to be presented to a CD8+ T cytotoxic cell (CTL) which is one of the primary lymphocyte classes important in cell-mediated immunity. This method of presentation is called the endogenous pathway. Although expression of antigen in the context of class I MHC molecules requires a live vaccine, a number of methods are under development to get noninfectious antigens to associate with class I molecules for the induction of CTL responses. Methods that have been partially successful under some conditions have included antigens mixed with liposomes or with certain adjuvants and placing antigens on biodegradable beads. Additional studies are required to find better methods to routinely activate CTLs when a noninfectious vaccine is used for diseases in which protective immunity is partially or completely dependent on CTLs.^{7,8,20,21}

The T and B cells specific for an antigenic epitope will be activated to undergo clonal expansion which results in significant numbers of new antigenic specific lymphocytes that differentiate into effector cells and into memory cells. The effector T cells include T helper cells, T regulatory cells

and T cytotoxic cells. The effector B cells are the antibody producing plasma cells that secrete specific antibody of the IgM, IgG, IgA and IgE classes.^{2,8,22,23}

The antigen recognition receptor on a T cell is referred to as the T cell receptor (TCR) and on the B cell as the B cell receptor (BCR). Many additional receptors and molecules that serve as co-factors or stimulatory factors are also present on the surface of T and B cells to participate in activation, differentiation and expansion of these cells during antigen presentation. The BCRs are monomeric IgM molecules anchored in the B cell membrane. These immunoglobulins have the typical structure with four chains, two heavy chains composed of one variable and three to four constant regions and two light chains composed of one variable region and one constant region. The chains are connected by disulfide bonds. Antigen specificity of the membrane bound immunoglobulin receptor resides in the highly polymorphic amino terminal hypervariable regions of the heavy (V_H) and light (V_L) chains to form the antigen binding site. Memory B cells have receptors similar to the membrane IgM but include other Ig isotypes (e.g., IgG, IgA, IgE). The memory cells have the advantage of not first having to produce an IgM antibody when encountering antigen before a switch to the IgG, IgA or IgE classes take place since the switch has already occurred through previous encounters with antigen and T helper cells. Furthermore, the memory cells seem to be more easily activated during a secondary response than the naive cell stimulated during a primary response. This has the advantage of rapid and often significant increased amounts of the class of antibody providing the most appropriate protective response, notably IgG and IgA. Memory-effector B cells are B cells that survive and secrete antibody for months to years after initial antigenic stimulation.^{7,8,24,25,26}

The antigen receptors on T and B lymphocytes differ structurally but all are members of the immunoglobulin superfamily, as are the MHC class I and class II molecules. An important functional similarity between TCRs and BCRs is they both, in combination with appropriate co-stimulatory molecules, activate the T cell or B cell respectively to undergo differentiation and clonal expansion. These receptors bind distinct epitopes of the antigen. The B cell receptor recognizes the shape of the antigenic epitope which is often composed of five to six amino acids or sugars, and the B cell receptor may bind structures as large as a virus particle. The TCR complex on the other hand binds peptide antigen in association with class I or II MHC molecules. T cell epitopes are processed antigen (peptide fragments) bound in MHC molecules and B cell epitopes are portions of unprocessed antigen in their native conformation. Thus an antigenic epitope recognized by the B cell receptor would always be different from the epitope recognized by the T cells. Furthermore, since it is the T helper cell (CD4+) that recognizes antigen in the context of the MHC class II molecule and the T cytotoxic cell (CD8+) that recognizes the antigen in the context of MHC class I molecule, these two types of T cells would also recognize antigenic epitopes which differ. Additionally, only peptide determinants are presented by APCs to T cells, whereas B cells recognize not only peptides, but glycoprotein, lipoprotein, carbohydrate and lipid antigens as conformational determinants. Antigens as complex as those found in vaccines, and those in nature, have many epitopes. Some are B cell epitopes and others are T cell epitopes, but together they constitute the type and complexity of stimulus required to generate an immune response after vaccination or infection respectively. Virtually all vaccine antigens required for protective immunity are T

dependent, thus T cell and B cells must cooperate to provide a protective immune response, whether that response is cell mediated and/or humoral.^{7,8,21,27}

Vaccine Immunity

The immune response which develops to an infectious viral vaccine (e.g., modified live) will often be far more complete and complex than the response to a noninfectious vaccine with a purified protein (e.g., subunit vaccine) or a limited or large number of antigens (killed vaccine) for the same pathogen. The response to the more complete and complex array of antigenic epitopes expressed during in vivo replication of the attenuated pathogen in the MLV vaccine leads to a greater number of B and T cells being activated by the numerous epitopes presented to the immune system. This is important for induction of a protective immune response to the specific pathogen as well as pathogens that are antigenic variants of the original pathogen or possibly different serotypes of the pathogen. In general, there is more cross-reactivity for epitopes recognized by the TCR than BCR. Therefore, if a T helper cell or a CTL provides some level of protective immunity, a vaccine with appropriate T cell epitopes is likely to provide immunity not only to the specific pathogen, but also to closely related variants or serotypes of the pathogen. However, if antibody is the main protective factor, as is the case for many viral and bacterial pathogens, it is unlikely that immunity will be cross protective, especially when a noninfectious vaccine is used. This would be the case for various serovars (serotypes) of *Leptospira* sp. Vaccinal immunity to leptospira, if present, is serovar specific, thus immunity for leptospira serovars not present in the vaccine would not be expected.^{2,3,5,6,28,29,31} In contrast, there is excellent cross protective immunity between canine adenovirus type 1 (CAV-1) and canine adenovirus type 2 (CAV-2).^{30,31} Similarly, there is immunity to most, but not all, of the serotypes of feline caliciviruses due to cross-reactivity among serotypes. However, cross-reactivity may be improved by using additional or different serotypes of virus than are currently present in commercial vaccines. When disease is caused by a single or limited number of serotypes, development of an effective vaccine is much more easily achieved. When there are many antigenic variants, as with lentiviruses (e.g., HIV, FIV), it is very difficult if not impossible to make an effective vaccine.^{3,5,28} Thus, if a zoo species is infected by an uncommon serotype (or strain) of a pathogen, current vaccines may be poorly protective, if at all. Whereas, if there are no strain or serotypes of the pathogen, vaccinal immunity could be expected.

It has been known for many years that CD4⁺ T cells had two different functions: 1) to help B cells produce antibody (the classic helper T cell activity) and 2) to mediate delayed-type hypersensitivity or cell mediated immune functions ascribed to T effector cells other than CD8⁺ CTL's.^{7,8} Until recently, it was not clear whether these separate functions were accomplished by a single population of helper T cells each at different stage of maturation, or if two different populations of cells existed. The evidence now available would strongly suggest that there are two distinct CD4⁺ T helper cells in most, if not all, mammalian species. The two subsets of T helper cells have been imaginatively given a Th1 and Th2 designation.^{4,7,8} In referring to the cells as Th1 and Th2, it is suggested that the cells can be readily identified and easily separated, which is not the case. The difference between the two subsets of T cells is not based on unique cell determinants but by the cytokines the cells produce and by certain other biologic activities. In general, the Th1 cells facilitate a cell-

mediated response with the production of certain cytokines important for that function, and the Th2 cells provide the classic helper response for antibody production by B cells. Vaccination strategies to selectively stimulate one type of response or T cell subset over another could provide an advantage for development of a protective immune response. Selection can be achieved by administering certain cytokines with antigen (e.g., IL-12), varying the dose of antigen, changing the route of administration of antigen and/or using a specific adjuvant. The potential importance of the role played by these two subsets of T cells is demonstrated by immunity to *Leishmania* infection in two different genetic strains of mice.^{7,8} The resistant strain C57BL/6 elicits a strong Th1 response and subsequent recovery, whereas BALB/C mice have a Th2 type response and develop severe disease. The production of IFN- γ by the Th1 cells was shown to be the important factor in protection since administration of anti-IFN γ monoclonal antibodies (MAb) which prevented IFN γ s from being active, made the resistant mice susceptible. Susceptible mice administered anti-IL-4 MAb which inactivated IL-4, did not develop disease because the IL-4 was not able to block the Th1 cells from forming. It is important to recognize that studies in inbred genetic strains of mice are very artificial and/or exaggerated. After natural infection or vaccination the immune responses are likely to include both Th1 and Th2, but the kinetics and the degree of these responses are likely to be very important for development of protective immunity. Therefore, if a vaccine can produce memory Th1 and Th2 cells at the time of challenge, the response should be protective. However, if a vaccine fails to induce or only poorly induces Th1, there would be little or no memory in this subset. If IFN γ is critical for protection from disease, the vaccine may not be effective. IL-12 and IL-4 are two cytokines that significantly influence the development of Th subset immunity, with IL-4 favoring a humoral response (Th2) and IL-12 a cell-mediated response (Th1). Thus, methods that influence the amount of IL-12 and IL-4 as well as other cytokines (IL-18, IL-6), are being used with some success to enhance protection associated with vaccines to several pathogens. In certain systems, efforts to direct the immune system look very promising. Hopefully, future vaccines designed to favor one T cell subset over another can provide better protective immunity than many current vaccines. This manipulation of T cell subsets might also be used to reduce the possibility of adverse reactions such as a type I hypersensitivity response.

The initial immune response that develops in the young animal after infection with a specific pathogen, or more importantly after vaccination, is of critical importance. This first encounter seems to determine the efficacy of the immune response when and if the pathogen is encountered at a later time. Development of a protective cellular and humoral immune response, and the development of T and B memory cells for multiple antigenic epitopes, is often best achieved by vaccines that most nearly simulate what occurs during natural infection. Vaccinal immunity is most often dependent on immunologic memory and not on the effector cells generated shortly after vaccination since the T and B effector cells stimulated after vaccination are usually short lived (living only for a few days to a few weeks). Memory is one of the two major characteristics of an adaptive immune response, specificity being the other. The success of vaccine induced immunity thus depends almost entirely on the development of memory T and B cells that can be activated to produce a rapid and effective protective response long after the original effector cells have disappeared. When a vaccine is developed it is then tested for its ability to induce cell mediated and/or humoral immunity, but more importantly the vaccine must provide a protective immune response shortly after vaccination. If the

effector cells required for protection are present then it is highly likely that memory cells have also been stimulated by the vaccine. The memory cells, not the effector cells, survive to be activated by the next encounter with the same agent (e.g., virus, bacteria) as was present in the vaccine. Thus, protection shortly after vaccination is critical if the vaccine has any chance of inducing the appropriate memory cells which survive for long periods of time, providing protection for years or a lifetime with certain vaccines and up to 1 yr with other vaccines. A vaccine that fails to protect the animal a few months after it is given will not provide protection a year or more later because the appropriate memory cells were not stimulated.³⁰⁻³² Therefore, some vaccines cannot produce very effective protective immunity. Even worse is a partial, but nonprotective immune response, that can interfere with revaccination with the development of a response at a later time. Thus, no matter how many times the animal is vaccinated, protective immunity fails to develop.

Most of the experimental studies demonstrating that memory cells persist for the lifetime of the animal have been performed in mice, a species that rarely lives more than 3 yr, therefore, studies in longer lived species were required to determine the true life of memory cells. Memory cells and memory effector cells in humans have been demonstrated many years after vaccination in the absence of known antigenic stimulation. Memory cells in most species are not easily identified and only the demonstration of protection after a challenge infection can absolutely show that there are memory cells capable of protecting the animal from disease. There is, however, a general consensus among most immunologists based on experiments of nature, that memory B and T cells for certain antigens persist for very long periods of time in most species that have an adaptive immune system.

Another cell type that is very important is the memory effector B cell that, in the absence of overt antigen stimulation, produces antibody for months or years after infection or vaccination. These cells are more easily demonstrated by following antibody titers. In humans, it has been concluded, based on persistence of antibody and the demonstration of specific CTLs to certain viruses, that B cell and T cell memory can persist for at least 30-50 yr. Studies in dogs have demonstrated that memory B cells, antibody producing memory effector B cells, and presumably the requisite T helper cells for canine distemper virus (CDV) persist for more than least 10 yr.^{3,4,30-34} It was also demonstrated that protective immunity for CDV persisted for at least 7 yr when vaccinated puppies were later challenged with virulent virus. Cats have memory-effector B cells for feline parvovirus (panleukopenia virus) that persist for more than 7 yr. Similarly, animals vaccinated once as pups with CPV-2 were shown to have memory cells that protected them from infection and disease a minimum of 7 yr later.^{2,31} These are a few examples in which modified live or killed viral vaccines were given at an early age then never again. Viral vaccines are generally believed to provide the longest and most effective immunity. Studies with killed vaccines also show long term memory but modified live products show even longer and stronger immunity. Vaccine licensing studies with noninfectious, killed rabies virus vaccines given once at 12-16 wk of age show that dogs and cats have memory cells that persist for a minimum of 3 yr and provide protective immunity from challenge with virulent rabies virus that is equivalent to that seen after 1 yr. It should be noted that rabies vaccine is the only vaccine in which minimum duration of immunity studies are required by the United States Department of Agriculture (USDA).^{5,6} Only a few studies on maximum duration of immunity exist for domesticated species and no studies exist for maximum duration of immunity

with vaccines used in zoo species.

One study where cats were vaccinated as kittens with killed combination vaccine containing feline panleukopenia (FPLV), calicivirus and herpes virus showed that memory and memory effector B cells, and presumably their respective T helper cells, were present at least 7 yr after vaccination.³⁴ An important observation from this feline study is that in spite of there being no revaccination, and the viruses of interest were not present where the cats were housed, the immune system remained stimulated since antibody was continually being produced by the memory-effector B cells.³⁴ These studies show the immune response after vaccination with certain viral antigens can persist for many years. In contrast to studies with many modified live and killed viral vaccines where memory cells can be shown to provide a protective response for many years, it is difficult, if not impossible, to show that the bacterins (killed vaccines) for *Leptospira* sp. provide significant immunity for more than 4-6 mo, if at all. Thus, protective immunity and immunologic memory varies significantly among components of a multi-component vaccine and for different types of vaccines (e.g., bacterial vs. viral) as well as among species.^{3,31} One animal in which there is a relatively short duration of immunity for most vaccine antigens is the horse. It is rare in this species to find vaccines that provide more than 1 yr of immunity. Due to this fact, it is common practice to vaccinate nondomestic equine species on a year-to-year basis as well. Based on the aforementioned published studies, other unpublished studies, field observations, and general knowledge about natural disease and specific vaccines it is apparent that memory cells are capable of providing protective immunity in most animal species for many years or even a lifetime in species as long lived as human beings.^{29,31,35}

Understanding the mechanisms involved in immunologic memory and developing methods to readily measure immunologic memory would facilitate the development and testing of new and improved vaccines. Thus, a number of laboratories are conducting studies to answer some of the important questions on this subject. Experience and observation with vaccines for dogs and cats suggest the following: 1) modified live and killed viral vaccines that prevent diseases caused by systemic infections (e.g., CDV, CPV-2, FPLV) provide memory that persists for many years, probably the lifetime of the animal; 2) modified live vaccines for viruses that cause local mucosal infections (e.g., canine parainfluenza virus, feline herpes) may have shorter lived memory than is seen for systemic infections but memory appears to last for several years although the effectiveness of these vaccines is limited; 3) killed viral vaccines provide poor immunity against mucosal infections, thus there is no or only short term memory; 4) memory cells for some bacterial antigens seem to be reasonably long lived but memory for protection to the infections caused by other bacteria seem to be shorter lived than for most viruses probably due to the complexity of the immune response required for protection against bacteria; 5) bacterins (killed bacterial vaccines like *Leptospira* or *Bordetella*) are not very effective in providing long term immunity/memory; and 6) protection from systemic bacterial infection is often longer lived than protection from local mucosal infections with bacteria. Improvements in bacterial vaccines and the development of parasite vaccines will be an important challenge for the future since the complexity of the organisms and the difficulty of preventing disease will likely lead to problems in developing vaccines that are effective and/or provide a long duration of immunity (e.g., greater than 1 yr).^{30-32,34}

An important factor in long lasting protection from many pathogens of young animals is age related resistance that involves innate and adaptive immunity. This is especially true for those pathogens that primarily cause severe infections in younger animals due to a weakened (poorly developed) immune system. However, most age related resistance against pathogens is totally or partially independent of specific immunity and independent of immunologic memory cells. For example, canine herpes virus infection in susceptible pups less than 3 wk of age causes severe disease and high mortality, whereas infection of susceptible pups older than 6 wk of age causes no significant clinical disease.^{1,2,35} Obviously this difference is not due to specific immunity or to memory cells. Instead, it is due to thermoregulatory control and to a maturation of a functional immune system. The decreased immunity and low body temperatures at less than 3 wk of age makes this very young puppy highly susceptible to disease by this viral pathogen, as well as many other pathogens. When passively acquired maternal antibody is not present the animal is at severe risk of disease. Passively acquired maternal antibody is very important in protecting the young animal because active immunity is less than optimal and the immune system is often unable to adequately protect against viral, bacterial pathogens and certain parasites. These same pathogens are unable to cause disease in an older animal unless the animal is immunosuppressed. In the young kitten, infection with feline leukemia virus (FeLV) will lead to persistent viremia and subsequent disease in a very high percentage of experimentally infected animals. Whereas, in older cats (1 yr of age or older), the infection will generally result in protective immunity with no clinically apparent disease or persistent viremia, regardless of the vaccination status of the cat. Again this example is not one of specific immunity or memory cells, but instead age maturation of innate and adaptive immunity in the cat. Therefore, when vaccinating a young animal to protect it from diseases which most often occur in young animals, specific immunologic memory is of importance - but this memory is not the only mechanism for lasting protection because age associated resistance and memory together will play a critical role in protection. In contrast, for pathogens that cause severe disease irrespective of age, memory cells are of critical and principle importance and age plays little or no role.^{1,3,31,36}

When considering duration of immunity several principles apply: 1) duration of immunity from vaccination will equal but will not be longer than duration of immunity after recovery from natural infection with a given pathogen, 2) duration of immunity is shown to be greater for most viral vaccines than for bacterial vaccines, 3) duration of immunity can be due to specific factors like memory cells as well as innate factors like age, and 4) duration of immunity can be extended for vaccines with short duration of immunity when the pathogen is present in the animal's environment (natural vaccination).

Mucosal Immunity

Mucosal immunity requires special consideration when discussing vaccinal immunity. Although the immune responses that occur systemically can also occur locally, the mucosal immune system is partially independent of systemic immunity. The migration of specific B and T cells from mucosal sites such as the gut associated lymphoid tissue (GALT) and bronchial associated lymphoid tissues (BALT) to various other mucosal sites provides the host with a local immune response which is critically important for protection against certain pathogens. Local T cells, as well as T cells

migrating from the systemic immune system to mucosal sites, provide both cytotoxic and helper functions. Also, large numbers of B cells, produced mainly in mucosal-associated lymphoid tissue (MALT) are responsible for IgA antibodies that are secreted onto mucosal surfaces. This IgA immunity is independent of systemic B cell responses to the same pathogen. The source of antigen naive B cells for local mucosal immunity in most mammals is mainly gut associated lymphoid tissue (e.g., Peyer's Patches). Antigen-specific T cells and B cells, some of which may be activated by antigen in special regions of the Peyer's Patches, and also antigen naive cells migrate through the efferent lymphatics, thoracic duct and systemic circulation to various effector sites to act locally and become part of mucosal immune system. Effector sites include the lamina propria of the gut, the respiratory tract, the reproductive tract and glandular tissues. MALT thus serves as a source of antigen reactive cells that can protect the mucosal surface from infection and invasion of pathogens that cause diseases at that site. MALT may also provide some limited protection from pathogens that enter the body at that site to cause systemic disease. Thus local immunity at mucosal sites would serve as a critical first line of specific immunity. The induction of mucosal IgA responses depends on help provided by Th2 cells and the co-stimulation molecules and cytokines found in the systemic immune system. MALT contains both Th1 and Th2 cells, however, the Th2 cells required to help the large number of local B cells provide IgA antibody probably predominate as the T cell population. Although both CTL's and Th1 cells are present at mucosal sites to provide a cell mediated immune response, it is likely that the IgA antibody plays a more critical role in mucosal protection than do the T effector cells.^{4,7,8,37}

Since a majority of pathogenic viruses and bacteria cause disease after entering the body at mucosal sites or by direct infection of mucosal tissue, methods to enhance mucosal immunity by vaccination could provide improved protective immunity at least to certain pathogens. To date, however, the development of noninfectious vaccines which provide effective mucosal immunity has been a challenge. A significant amount of research is directed toward improved vaccines that induce long term protective immunity in various mucosal tissues. Local immunity is best stimulated by replicating antigens (e.g., modified live vaccine) at mucosal sites, whereas systemic immune responses can be stimulated parenterally by noninfectious vaccines (killed vaccines) as well as replicating antigens. Both routes of administration (parenteral and local) require adjuvants for noninfectious vaccines to avoid multiple injections and to achieve protective immunity. Special adjuvants are required for killed vaccines to be effective when administered locally (oral and/or intranasal) since many of the adjuvants used for parenteral immunization do not work. When one considers immunity to organisms that have mucosal tissue as a primary site of infection (e.g., canine parainfluenza virus, feline viral rhinotracheitis virus) the primary vaccinal immunity would often be local rather than systemic immunity. Therefore, if a modified live vaccine is used parenterally, the animal will develop a systemic immune response but may or may not develop local immunity. However, if the modified live vaccine is given locally the animal will often develop local and systemic immunity. In contrast, if a noninfectious (killed) vaccine is given parenterally the animal will not develop an effective local immune response, but should develop a systemic immune response. The non-infectious vaccines presently available for dogs and cats when given locally (e.g., orally; intranasally) provide neither local nor systemic immunity.^{3,5,6,30,31,37} One major difficulty for immunization strategies with noninfectious oral vaccines, beyond the problems of

eliciting a local antibody and cell-mediated immune response, is orally administered noninfectious vaccines may induce a state of systemic immunologic tolerance or unresponsiveness (anergy). "Oral tolerance," as this process is called, may represent a natural avoidance of immune responses to ingested antigens that could lead to hypersensitivity reactions. Regardless of the reason for this tolerance, it does pose a significant problem and local immunization remains a major challenge to vaccinologists.^{4,7,8,37,38}

For zoo species, oral vaccination may be desired since feeding vaccines would not require capture and restraint. However, special vaccines would need to be developed in order to immunize orally with certain modified live vaccines and with all noninfectious vaccines. Some of those methods are currently available (e.g., baited vaccines with MLV or recombinant vectored vaccines) and methods are being developed for oral administration of noninfectious vaccines (e.g., special immunomodulators like Cholera toxin B).

Types of Vaccines

There are two major types of vaccines: infectious and noninfectious. The infectious vaccines include virulent organisms, attenuated organisms, and live vectored organisms of viral, bacterial or parasite origin. Noninfectious vaccines include killed organisms, sub-units of the organisms, synthetic antigen, and anti-idiotypic antibodies.

In general, the most effective vaccines are the live infectious vaccines because they induce an immune response similar to that seen after recovery from natural infection. However, modified live vaccines are only safe in the host species they have been designed to protect. Attenuation is dependent on an effective immune system in the target species for the vaccine and vaccination of another species may result in severe disease and death. A good example is canine distemper virus vaccine. A very safe and highly effective product in the domestic dog, but capable of causing disease and death in the grey fox (*Urocyon cinereoargenteus*), the black footed ferret (*Mustela nigripes*) and certain other species. When vaccinating zoo species, noninfectious vaccines even though not always effective as infectious, would be the vaccine type of choice. This would be the case for several reasons including the possibility that the infectious vaccine may cause disease in the zoo species; an infectious vaccine in an unnatural host may be under significant mutational pressures, thus the vaccine organism may adapt to the new species to cause chronic disease; and the vaccine may be shed by the zoo species to cause the attenuated organism to become virulent even in the target domestic species. The obvious disadvantage of noninfectious vaccines in zoo species is reduced effectiveness; multiple doses (e.g., two to three) required for protective immunity; shorter duration of immunity and increased chance of an adverse reaction (hypersensitivity).^{3-6,39}

Developing effective and safe vaccination programs for zoo species provide challenges and unique opportunities. First and foremost, it will be critical to show the vaccine will cause no significant adverse reactions in the animal. In general, the greatest concern in zoo species would occur with a modified live vaccine that causes disease and possibly death of the vaccinated animals. The example of canine distemper vaccines causing disease and death in certain wildlife/zoo species was

discussed above. Because this problem is acute, severe and referenced in the literature, zoo vaccinologists recognize its potential danger and thus it is easily avoided. However, for other vaccines the adverse event may be more subtle. For example, a modified live herpes vaccine like bovine herpes type-1 (BHV-1) is used in adult animals without an apparent affect. BHV-1 becomes latent, as all herpes viruses do, and when reactivated by stress (e.g., parturition) the virus is spread to young animals causing significant disease or spread to nonvaccinated pregnant adult animals to cause abortions. In domesticated cattle where most of the animals are immune as a result of natural infection or from vaccination, there are few or no problems when BHV-1 is reactivated. But in a highly susceptible species or nonvaccinated population severe adverse consequences may occur. When certain MLV vaccines are administered to animals, vaccinal virus is known to be shed in secretions and excretions. For example intranasal BHV-1 and PI-3 viruses are readily shed in high amounts for days after vaccination. Whether or not the shed virus would cause disease is unknown, but infection of other susceptible animals could pose a direct risk to a highly sensitive species or it could cause the vaccine viruses to become more virulent through multiple animal passages.^{5,6}

In contrast to attenuated live virus vaccines, discussed above, recombinant live viral vaccines may serve as a safe and effective alternative if available. For example, the canary pox-vectored canine distemper vaccine (Recombitek®, Merial, Inc., Duluth, Georgia USA) should be a safe vaccine for prevention of distemper. However, without studies in the specific species of interest it is difficult to know if the product is effective.

Noninfectious killed vaccines are not without safety problems. In general, killed vaccines are less effective than modified live vaccines in their ability to induce protective and lasting immunity. If immunity persists for only a few months, revaccination becomes an issue as it would not be possible to repeatedly revaccinate animals that need to be captured or darted due to the risk of injury and death. Killed vaccines are especially problematic with regard to causing hypersensitivity reactions. Risk-benefit analysis should always be an important aspect of vaccination, but for zoo species it is likely to be the first issue of concern, not a secondary issue as it is for domesticated species.

Conclusions

In spite of the vast amount of new knowledge in the basic sciences during the past 40 yr, vaccinologists are left with many challenges for developing new and improved vaccines. Recombinant DNA biotechnology provides new and exciting methods that are essential to designing vaccines for the 21st century. This technology has already begun to provide opportunities to meet the challenges of making safe and effective vaccines. We can look forward to a bright future for veterinary vaccines as our understanding of the immune system improves and biotechnology is used to engineer products that stimulate immunity in ways not currently possible.^{3,5,6,31,40}

These comparative immunologic principles and developing biotechnology will prove helpful in establishing rational zoo vaccination protocols only when several basic, unanswered questions are addressed. These include which species are truly susceptible to a particular etiologic agent; what is a protective response to vaccines in these species; which assay(s) should be used for detection of

antibody response and/or presence of pathogen; how often should we vaccinate; how much vaccine should we provide; which is the best route of administration; how do we interpret serologic results; how do we prove efficacy without pathogen challenge studies; who has used what vaccine manufactured by who in what species; where do we find certain vaccines? These are just a few of many questions the zoo veterinarian is confronted with when deciding how to vaccinate a valuable, unique and often endangered species.

Therefore, the goal for veterinary vaccinologists must be to design an effective strategy to answer these vaccine questions for zoo species. A special zoo species vaccine interest group is proposed to organize and implement the effort to answer the aforementioned questions. A centralized information database on vaccine safety, adverse reactions, vaccine efficacy, known disease outbreaks, species serologic response to vaccination and species immunologic idiosyncrasies needs to be established. This basic, yet essential information is necessary if we are to truly provide protection from disease through vaccination for our ecologically, economically and esthetically important zoological specimens.

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SEROLOGIC TESTING: USES AND MISUSES IN DEVELOPMENT OF STRATEGIC VACCINATION PROGRAMS FOR ZOOS

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Abstract

Specific immune responses are divided into cell-mediated immunity and humoral immunity. Humoral immunity is mediated through soluble proteins (antibodies) found in body fluids. Serology is the measurement of antigen-antibody interactions for diagnostic purposes.³ The practical application of serologic methods for evaluation of vaccine responses in zoo animals is the focus of this presentation.

Overview of Serologic Tests

Serologic tests can be used to detect antigen or antibody. The specific methodology determines whether the result reflects the presence of an antigen or the immune response to that antigen.³ Results are usually expressed as “titers” using an endpoint dilution of test material or as “positive” or “negative” at a specified dilution. However, a titer is not a titer – in other words, knowledge of the methodology used in a specific test to measure a titer is important in being able to correctly interpret the results.⁵ Another confounding factor is that humoral immunity is only one aspect of a complete immune response. In some infections, cell-mediated immunity plays a more important role, so measuring the humoral response might not reflect the level of protective immunity.^{5,6} In other cases, antibodies to particular parts of an organism may be present but might not play a role in elimination of the infection. Even when measuring antibodies to a single organism, different test methods can result in variable titers or only detect specific types of immunoglobulins (e.g., IgM vs. IgG).³ A particular problem working with exotic animals is the lack of validation of most serologic tests for these species. In most cases, “protective” titers are unknown.

Despite all these difficulties, serologic tests can be useful in assessing response to vaccination or exposure to an antigen. Current discussions in veterinary medicine have focused on developing strategic vaccination programs based on risk assessment and duration of immunity.^{2,4,6} Use of serologic tests to individually tailor vaccination programs in individual animals has been proposed. Advantages include cost savings in vaccine, avoiding the potential for adverse reactions, and scheduling vaccinations to maintain effective levels of immunity while decreasing the potential stimulation of autoimmune disease. Disadvantages include increased cost associated with serologic testing, multiple veterinary visits, inability to obtain samples at regular intervals (e.g., in many zoo species), lack of validation for species, and difficulty determining “effective” immunity. Since this

presentation will focus on application of serologic testing to vaccination, some commonly used assays that detect antibody will be reviewed.

Immunofluorescence Assays

Indirect fluorescent antibody test⁵ (IFA) is used to detect antibodies. Antigen produced in the laboratory specifically for this test can be in the form of a tissue smear, section, or cell culture on a slide. The patient's serum is incubated with the slide, washed, and immune complexes are visualized using a fluorescent-labelled antiglobulin. The quantity of antibody in the test serum can be estimated by using increasing dilutions of serum to detect the endpoint. The technique used in radioimmunoassay (RIA) is similar but uses radiolabels to detect complexes.

Immunoenzyme Assays

Indirect enzyme-linked immunosorbent assay³ (ELISA) is one of the most common serologic methods used in veterinary medicine. The indirect ELISA technique utilizes an antigen bound to a well or tube, to which the patient's serum is added at varying dilutions (or a single dilution in the case of positive/negative test methodology commonly employed in in-clinic kits). The presence of bound antibody from the patient's serum is detected after adding an enzyme-labeled antiglobulin, followed by enzyme substrate. The amount of color change is proportional to the amount of bound serum antibody. Results can be visualized as positive/negative color change or can be quantified using a spectrophotometer.

Western Blot

This technique is often used when dealing with a complex mixture of protein antigens.⁵ Antigens are separated using electrophoresis and then transferred to a nitrocellulose membrane. The membrane is incubated in the patient's serum with antibodies binding to specific protein bands. Detection of the antigen-antibody complexes are detected using enzyme-labeled antiglobulin (or radioisotope-labeled antiglobulin). A dot blot is a variation of the Western blot technique.

Precipitation

These tests require a solution of soluble antigen mixed with antiserum.³ When the ratio of antibody to antigen is in optimal proportions, a visible precipitate is formed. Results are often read visually as positive or negative at a specific dilution.

Agar Gel Immunodiffusion (AGID)

Instead of using tubes to detect precipitation of antibody-antigen complexes, this technique uses diffusion of soluble antigen and antibody from separate areas of an agar plate.⁵ An opaque line of precipitate appears where the reagents meet in optimal proportions. In the case of complex antigens, multiple lines of precipitation may be visible since the optimal proportions of each component occur

at different positions. The Coggin's test used to detect antibodies to equine infectious anemia virus uses this method.

Agglutination Assays

Because antibodies have multiple binding sites, they are capable of cross-linking particulate antigens, resulting in clumping or agglutination.³ IgM antibodies are more efficient than IgG. Similar to precipitation, there is an optimal zone of antibody and antigen concentrations that result in a visible reaction. Agglutination reactions are more sensitive than precipitation techniques. Passive agglutination tests are performed by chemically linking soluble antigens to red blood cells or latex beads.

Viral Hemagglutination (HA) and Hemagglutination Inhibition (HI)

Certain viruses can bind and agglutinate red blood cells and thus serve as a basis for this assay.^{3,5} This reaction can be blocked or inhibited by the presence of antibody that binds the virus. The higher the dilution of serum that can inhibit hemagglutination, the higher the titer of virus-specific antibody.

Complement Fixation Test

Test serum is incubated with a source of antigen and complement. If antibodies are present to the antigen, binding will occur and activate the complement cascade, depleting the amount of free complement.³ In order to detect free complement, an indicator system consisting of antibody-coated sheep erythrocytes is added in the second step. If complement is present, the cells are lysed and the titer is considered negative. If antibodies are present, the titer is considered the highest dilution of serum in which no more than 50% of the erythrocytes are lysed.

Interpreting Titers

Some considerations to keep in mind when interpreting titers:^{4,6}

- A positive titer does not necessarily mean the animal is actively infected or that the disease is due to this etiologic agent.
- A negative titer does not necessarily indicate that the disease is not present.
- Titers do not always correlate with the presence or absence of a protective immune response in the host.
- Titers to a particular etiologic agent may vary in a single serum sample due to differences in specificity and sensitivity of the test method used.

Often serologic tests are performed early in the course of disease or too soon after exposure and the negative titer is incorrectly interpreted to rule-out the suspected etiologic agent. Ideally, paired serum samples (acute and convalescent) should be compared to determine if there is a rising titer. A positive result is usually defined as a fourfold or greater increase in titer.⁵ Unfortunately,

sometimes only a single sample is available. Extrapolation from other animals or previous serologic results may be used as a basis for comparison with careful consideration of the cautions stated above and in light of the clinical situation. The following examples from zoological practice will be used to illustrate the concepts of serologic test interpretation.

Example 1

First trimester abortion in a southern white rhinoceros (*Ceratotherium simum*). A 30-yr-old female southern white rhinoceros was examined after detection of an aborted fetus (estimated first trimester). Diagnostic tests for equine herpesvirus (EHV-1) and infectious bovine rhinotracheitis (IBR) virus were performed using the serum neutralization (SN) technique. This assay measures the ability of specific antibodies to neutralize the cytopathic effects of virus in tissue culture. SN may not distinguish between infection and vaccination; therefore, diagnosis should be based on rising titers, as well as other diagnostic results. Hemagglutination inhibition (HI) was performed to detect antibodies to equine influenza virus. Brucella titers were measured using an agglutination test method. Results are shown in Table 1.

A review of the medical record revealed that she had been vaccinated annually with a commercial equine vaccine (Fluvac EWT; Ft. Dodge Laboratories Inc. Fort Dodge, IA 50501 USA) containing Eastern and Western equine encephalitis virus, tetanus toxoid and equine influenza virus. The positive titer to equine influenza could be attributable to prior vaccination. However, the positive titer to EHV-1 was not due to previous vaccination, and should be considered in the differential list for abortion in perissodactylid species. Additional steps would include a convalescent titer (approximately 4 wk later) and examination of the fetus for evidence of viral infection. If results indicate a possible link, an equine vaccine that includes EHV-1 should be considered for the breeding herd.

Example 2

Rabies vaccination in hybrid tigers (*Panthera tigris*). Potential exposure to rabies virus warrants vaccination of susceptible species. However, vaccine-associated sarcomas have occurred in domestic cats.² The benefits of monitoring the response of vaccination by serologic testing, instead of repeat vaccination, will decrease the risk of adverse effects and may result in the decreased need for further immunization. Unfortunately, there is not an established protective titer for most species, and challenge studies in exotic species are unlikely to be done. A threshold titer needs to be designated below which booster vaccination is recommended.

Rabies titers in six female tigers are shown in Table 2. Titers are determined by the rapid fluorescent focus inhibition test (RFFIT). In humans, a titer greater than 1:5 is considered a protective titer.¹ During quarantine, these tigers were bled and then vaccinated with a commercially available veterinary product (Imrab 3, Merial Inc., Athens, GA 30601 USA, 2 ml i.m.). Vaccination was repeated in 1999 and 2000. The results demonstrate the significant individual variation in immune response as well as the ability of this product to stimulate an antibody response in this

species. Rabies titers are currently monitored so that booster vaccines can be individually scheduled. An arbitrary threshold titer of 1:50 is the cutoff value when vaccination would be scheduled. Information on larger numbers of animals is needed to develop scientifically based immunization programs for exotic species.

Example 3

Eastern equine encephalitis (EEE) virus vaccination in secretary birds (*Sagittarius serpentarius*) are shown in Table 3. An adult secretary bird was found dead without any premonitory signs. A second bird died acutely approximately 1 wk later. Necropsy findings were consistent with viral encephalitis and EEE infection was confirmed on virus isolation. In an attempt to prevent disease in the remaining two birds, a commercially available killed EEE vaccine (Encephaloid, Ft. Dodge Laboratories Inc., 0.5 ml i.m.) was administered. Titers were measured using hemagglutination inhibition (HI). A greater than fourfold increase in titer to EEE suggests that the vaccine induced an antibody response in these birds (titers to flavivirus, which includes West Nile virus and St. Louis encephalitis virus, were consistently <1:10). Due to waning antibody at 6 mo, the dose of vaccine was increased to 1.0 ml i.m. Quarterly titers are being measured to determine duration of titers using this dose. Although antibodies were detected, without challenge studies (either experimentally or through natural exposure), it should not be assumed that this equates with protective immunity.

Summary

Technologic advances and increased knowledge of the immune system have enhanced our ability to detect and measure responses to natural exposure, infection, and vaccination. Although the development and validation of serologic tests for exotic species is limited, rational application of currently available techniques can be used to aid in diagnosis and disease prevention. Serologic monitoring should be used to develop scientifically based vaccination programs for zoo animals.

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Table 1. Serologic results in a southern white rhinoceros.

Assay	SN	HI	Card agglutination
Etiologic agent			
IBR	Neg 1:4		
EHV-1	Pos 1:24		
Eq Infl ^a A1		Pos 1:32	
Eq Infl A2		Pos 1:16	
<i>Brucella</i>			Neg

^aEquine influenza virus.

Table 2. Rabies titers in six hybrid tigers.

Titer date	1998	1999	2000
Tiger 1	<1:5	1:56	1:280
Tiger 2	<1:5	1:200	1:1100
Tiger 3	1:56	1:1400	1:1500
Tiger 4	1:36	>1:7000	>1:7000
Tiger 5	<1:5	1:210	1:320
Tiger 6	<1:5	1:270	1:280

Table 3. Eastern equine encephalitis (EEE) titers in secretary birds.

Titer date	Vaccination date	Bird 1 titer	Bird 2 titer
Prevaccination	9 March 2002 ^a	1:10	1:10
30 March 2002	30 March 2002 ^a	>1:40	>1:40
17 Oct 2002	17 Oct 2002 ^b	1:10	1:20
11 Jan 2003	-	>1:40	>1:40

^aVaccinated intramuscularly with 0.5 ml EEE vaccine.

^bVaccinated intramuscularly with 1.0 ml EEE vaccine.

VACCINE-INDUCED CANINE DISTEMPER VIRUS INFECTION IN A LESSER PANDA (*Ailurus fulgens fulgens*) THREE YEARS FOLLOWING LAST VACCINATION

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Abstract

A 7-yr-old lesser panda (*Ailurus fulgens fulgens*) had been showing signs of intermittent inappetence, lethargy, mild ataxia, and occasional head tremors for several weeks. The symptoms seemed to be very mild and sporadic, with improvement between episodes, which slowly began to increase in frequency. One month after onset, a head tilt to the left was noticed. The animal was examined under anesthesia and was discovered to be in poor body condition, weighing 4.2 kg, and demonstrated muscle atrophy of the rear limbs, more pronounced on the right. The animal developed rear limb paresis 1 wk after this initial examination, demonstrating minimal motor function and muscle tone.

Complete blood count, serum chemistries, heartworm antigen levels, and skull radiographs at the time of initial exam showed no abnormalities. Serum was tested for evidence of *Toxoplasma gondii* IgG and IgM, *Neospora caninum*, *Sarcocystis neurona*, *Babesia canis*, *Blastomyces*, *Histoplasma*, *Aspergillus*, *Coccidioides*, and *Leptospira* spp. No significant abnormalities were found. Antigen levels of *Cryptococcus* were slightly elevated (1:16), and serum neutralization titers for canine distemper virus (CDV) were significantly increased (1:6144). Magnetic resonance imaging (MRI) was performed and cerebrospinal fluid (CSF) was collected. The MRI of the brain revealed increased water density on T₁/T₂ images as well as no contrast enhancement in the periaqueductal gray matter. Differentials for these findings include inflammation, thiamine deficiency, a vascular accident, or a minor neoplastic lesion. Protein levels in the CSF were increased (43 mg/dl), making inflammation a likely cause. Cerebrospinal fluid was negative for evidence of *S. neurona* and *T. gondii* IgG and IgM. Viral neutralization titers for CDV in the CSF were negative.

Multi-drug therapy was initiated with dexamethasone 1.3 mg i.v. b.i.d., enrofloxacin 45 mg p.o. b.i.d., clindamycin 150 mg i.v. b.i.d., doxycycline 25 mg p.o. b.i.d., and thiamine 300 mg s.c. b.i.d. The symptoms progressed and became more severe despite intensive therapy. Eventually, ventroflexion of the neck, vertical nystagmus and difficulty with mastication and swallowing became evident. The animal became dyspneic with abdominal breathing and increased bilateral lung sounds. Radiographs revealed congestion around the base of the heart and the large vessels. Due to the worsening clinical signs, the animal was euthanatized.

A review of the animal's records revealed that it had been vaccinated once subcutaneously with a

modified-live canine distemper vaccine (FerVac™-D, United Vaccines, Inc., Madison, Wisconsin 53713 USA) 3 yr previously. One month after vaccination, the animal exhibited signs of decreased appetite, mild diarrhea, and head tremors, which improved with treatment. Two years after vaccination, the animal was again showing signs of inappetence and weight loss. Mild neutrophilia and an interstitial pattern on thoracic radiographs were discovered. The animal again improved, although very rare and short episodes of head tremors were observed by keepers throughout the next year until the fatal episode described above began, 3 yr after the vaccine was given.

Postmortem findings included perivascular, lymphocytic encephalitis, lymphoid depletion of the lymph nodes and spleen, interstitial neutrophilic pneumonia, and suppurative hepatitis. Immunohistochemistry of brain tissue revealed scattered neurons that stained strongly positive for morbillivirus. Brain and heart sections were negative for West Nile virus on immunohistochemistry.

In order to determine whether the CDV infection was caused by a wild strain or the vaccine, a morbillivirus phosphoprotein (P) gene fragment was amplified by RT-PCR from frozen brain tissue. This gene contains several highly-preserved areas and varies among the different morbilliviruses, and to a lesser extent among different species, geographic areas, and wild vs. vaccine strains of the virus.^{1,2} A sequence of the P gene was derived from the brain sample, as well as from a sample of the vaccine, and both were found to be not only similar, but identical, confirming that the vaccine strain caused the infection in this animal.

This panda was housed in an outdoor wire mesh enclosure and contact to animals carrying the canine distemper virus, including feral dogs and raccoons, was possible for the entire extent of its 3-yr stay at the zoo. However, after extensive PCR testing of the panda tissue and the vaccine product itself, it was determined that the strain of virus was not wild, but rather vaccine-induced. This is the first report of a lesser panda showing clinical signs and succumbing to canine distemper virus several years after vaccination with a modified live product.

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HEPATITIS B VIRUS VACCINATION OF SILVERY GIBBONS (*Hylobates moloch*) AT THE PERTH ZOO

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Abstract

Hepatitis B virus (HBV) has been identified in a number of nonhuman primate species, including the Silvery gibbon (*Hylobates moloch*). The Perth Zoo has a successful Silvery gibbon breeding program and has developed an HBV vaccination regime to protect offspring of this species. Serologic testing has demonstrated that this vaccination regime has been successful in producing a serologic response consistent with vaccine-induced immunity.

Introduction

Hepatitis B virus (HBV) infections involving strains that are distinct from human HBV genotypes, have been identified in gibbons (GiHBV), orangutans (OuHV), chimpanzees (ChHBV), gorillas (GoHBV) and woolly monkeys (WMHBV).^{4,6,7,13,14} Recent studies have demonstrated wide-spread infection of wild primate populations with these species-specific HBV infections.^{3,5,8,12-14} Recent studies provide evidence that these viruses are indigenous to these nonhuman primate species, suggesting that a transmission event in the Old World between humans and nonhuman primates may have occurred, involving a common ancestor virus from which the nonhuman primate HBV variants and the Old World human HBV genotypes (A to E) have evolved.^{4,5,8,13} However, other researchers favour the theory that these nonhuman primate HBV variants are more likely the result of recent cross-species transmission of the virus between humans and nonhuman primates.^{6,7} Fulminant hepatitis has been reported in a woolly monkey infected with WMHBV,⁶ and elevated alanine aminotransferase (ALT) levels were reported in gibbons infected with GiHBV.¹⁰ However, the pathogenicity of the HBV infections in other nonhuman primate species remains unknown.^{7,14} Experimental transmission of human HBV to chimpanzees and gibbons has been documented,^{1,10,11} and infectivity of gibbon HBV to a chimpanzee has also been demonstrated.⁹ To date there is limited knowledge as to the zoonotic potential of these nonhuman primate viruses. Although it seems likely that these viruses are potentially zoonotic,⁹ studies have not been able to confirm transmission of HBV from nonhuman primates to humans.¹⁰

The Perth Zoo currently houses one of the few successful breeding colonies of Silvery gibbons (*Hylobates moloch*) in the world and the only one in the Australasian region. Serologic testing of the two breeding adults at the Perth Zoo has shown cross-reactions with human HBV antigens and

both are considered chronic carriers of an HBV-like virus (i.e., positive for HBV surface antigen [HBsAg+ve] and negative for HBV surface antibody [anti-HBs -ve]) (Tables 1 and 2). The breeding female is further classified as a high-infectivity chronic carrier, due to the cross-reaction of her sera with HBV e antigen (HBeAg). The likelihood that human babies born to high-infectivity chronic carrier mothers will develop HBV infection and become chronic carriers is greater than 90%, as compared to the 10-15% risk of infection associated with babies born to low-infectivity chronic carrier mothers.^{2,3} Although transmission studies of the disease in nonhuman primates have been limited, previous studies have demonstrated a similar perinatal transmission pattern to that of HBV in humans.^{4,7} DNA sequencing currently being undertaken by the author has demonstrated that the HBV-like virus isolated from the breeding female at the Perth Zoo is closely related to the previously identified GiHBV rather than human HBV.^{7,9,10}

An unrelated adult male the Perth Zoo Silvery gibbon, who was also classified as a high-infectivity chronic carrier, died recently after a period of liver-related illness. This individual was found on postmortem to have evidence of liver cirrhosis consistent with pathology seen in humans with chronic hepatitis B infection, and had demonstrated elevated levels of ALT on multiple occasions (up to 613 U/L).

Based on this information, the Perth Zoo was concerned that there would be a high probability of offspring born to this Silvery gibbon breeding pair becoming infected with the virus, developing chronic carrier status, and possibly developing liver pathology associated with the infection in later life. Given the critically endangered status of the species however, and the obvious compatibility of the pair, it was decided to continue breeding these animals. A vaccination program to immunize newborn Silvery gibbons against HBV was developed with the help of human virologists, to reduce the risk of transmission of the disease to future offspring.

Development of an HBV Vaccination Regime

The Silvery gibbon pair has given birth to four offspring since 1995, the most recent birth was in March 2003. All four offspring have been vaccinated against HBV. The initial recommended vaccination regime involved vaccinating newborn gibbons within the first 24-72 hr of birth, with 0.5 ml of recombinant Hepatitis B vaccine (Energix B®, SmithKline Beecham Biologicals, B-1330 Rixensart, Belgium) administered intramuscularly (i.m.) and 0.3 ml HBV Immunoglobulin (100 IU/ml) administered i.m. at a separate site. Booster vaccinations of 0.5 ml Energix B® vaccine i.m. were to be given at 4 mo and 12 mo of age. The immunoglobulin administered with the initial vaccination served to provide passive transfer of immunity against potential viral exposure during the birthing process. Recommended doses to be used were extrapolated from human doses and reduced according to body weight differences between the species.

The vaccination regime was used on the first offspring (OF1), however the second booster was delayed to 15 mo for management reasons. Blood testing at the time of the second booster demonstrated that this animal had developed a serologic response consistent with immunity to HBV. However, the presence of HBV core antibody (anti-HBc) as well as anti-HBs indicated that this

immunity was derived from past exposure rather than vaccination (Table 2). As the vaccine contains surface antigen only, the presence of core antibodies suggests that the animal has been exposed to actual virus rather than just vaccine. It is not possible from these results to determine if the anti-HBs has resulted from exposure to vaccine, virus or a combination of both. Repeat blood testing 5 yr after the initial vaccination showed that immunity had been maintained. Transfer of this animal to another institution shortly after this time meant that further testing could not be performed.

Due to the lack of serologic response to vaccination attained by the initial vaccination regime in OF1, the regime was amended for the second offspring (OF2) to involve a vaccination at 24-72 hr with 0.5 ml Energix B® vaccine i.m. and 0.1 ml HBV Immunoglobulin (100 IU/ml) i.m., followed by boosters of 0.5 ml Energix B® vaccine i.m. at 1 mo and 6 mo. Due to management and animal factors, the initial Energix B® vaccine and HBV Immunoglobulin injections were given at 72 hr, the first booster at 6 wk, and the second booster at 5 mo. Blood testing 1 yr after the second booster demonstrated that this animal had no evidence of exposure to the disease and had developed a serologic response consistent with vaccine-induced immunity (anti-HBc -ve, anti-HBs +ve). Serum from this individual was collected recently and has shown that this immunity has been maintained (4.5 yr after the initial vaccination).

The third offspring (OF3) was vaccinated with 0.5 ml Energix B® vaccine i.m. and 0.1 ml HBV Immunoglobulin (100 IU/ml) i.m. at 48 hr, with a single booster of 0.5 ml Energix B® vaccine i.m. given at 8 mo. This vaccination regime had been modified following discussions with human virologists, and the initial Energix B® vaccine booster (given previously at 1 mo) was eliminated due to problems encountered while darting the dam in order to vaccinate the previous offspring (OF2) at 1 mo of age. Blood collected from OF3 2 yr after initial vaccination demonstrated that this animal had also developed a serologic response consistent with vaccine-induced immunity and had no evidence of exposure to the disease (anti-HBc -ve, anti-HBs +ve).

Due to the success of the modified vaccination regime used for OF3, this regime was repeated for the fourth offspring (OF4), born in March 2003. This baby was vaccinated with 0.5 ml Energix B® vaccine i.m. and 0.1 ml HBV Immunoglobulin (100 IU/ml) i.m. at 48 hr. A single booster of 0.5 ml Energix B® vaccine i.m. will be given at 8 mo.

Discussion

Based on serologic testing, the vaccination regimes used for OF2 and OF3 appear to have been successful in producing vaccine-induced immunity that has been maintained for at least 2 yr since the initial vaccination. The regime used for OF3 was preferred since the booster vaccination at 8 mo was able to be performed without the need for anesthesia of the dam. All previous vaccinations given prior to this age required general anesthesia of the dam to allow access to the baby and to ensure good acceptance of the baby when returned to the dam.

Although the initial regime used for OF1 did not produce a serologic response consistent with vaccine-induced immunity, serology from this animal is suggestive of immunity secondary to

exposure to infection, and therefore OF1 can now be safely involved in a breeding program without the risk of transmitting the disease to future offspring or other in-contact animals.

Although the number of animals in this study is low, it appears that the modified regime, involving vaccination at 24-72 hr with 0.5 ml Energix B® vaccine i.m. and 0.1 ml HBV Immunoglobulin (100IU/ml) i.m., followed by a booster vaccination of 0.5 ml Energix B® vaccine i.m. at 8 mo, is successful in providing a serologic response consistent with vaccine-induced immunity. It is recommended that this vaccination regime be used for any future offspring born to this pair of Silvery gibbons at the Perth Zoo. Virologists have also recommended that repeat boosters of Energix B® vaccine are given every 5 yr, on an opportunistic basis, in order to maintain immunity.

Despite the apparent success of the program, there are inherent risks to the dam, offspring and staff associated with the vaccination procedures, especially when darting and general anesthesia are involved. However, the benefits of the vaccination procedure in successfully establishing vaccine induced-immunity in Silvery gibbon offspring at the Perth Zoo was deemed to outweigh the risks involved in the zoo's situation. The suitability of such a vaccination regime for other institutions would depend on the number and status of the animals involved.

The effectiveness of vaccinations used in exotic species has often been questioned, as it is difficult to ascertain the extent of true protection afforded by vaccines that have been designed for other species (in this case, humans). This is certainly applicable in this situation, and whether these animals are truly "immune" to infection is likely to be debatable. In a critically endangered species such as the Silvery gibbon, we do not have the luxury of being able to run challenge tests to determine whether or not the immunity is truly protective. It would appear though, that in the cases at the Perth Zoo true protection has been achieved as both OF2 and OF3 developed immunity that has been maintained for several years while living in very close contact with two chronic HBV carriers. The fact that OF1 showed evidence of exposure-induced immunity demonstrates that the virus is transmissible in a captive situation, and as such there is a real risk of infection to all future offspring. Neither OF2 nor OF3 have shown evidence of exposure however, suggesting that their vaccinations provided true protective immunity. An alternative explanation could be that the dam had seroconverted to become a low-infectivity carrier during the time between the birth of OF1 and OF2. In this scenario, the risk of transmission of the virus to the later offspring would be reduced from > 90% to 10-15%. If this were the case, the serologic immunity demonstrated may not be indicative of true protective immunity. However, serum samples collected from the dam at the time of vaccinating OF4 have confirmed she is still a high-infectivity carrier, supporting the theory that the immunity attained in OF2 and OF3 is truly protective.

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Table 1. Serologic markers of hepatitis B virus infection.

Marker	Significance
HBsAg	Indicates presence of HBV surface antigen in serum and current HBV infection. If present for > 6 mo without the development of anti-HBs, considered to be chronic carrier.
anti-HBs	Indicates presence of antibody to HBsAg in serum. Considered to be the <i>protective</i> antibody. Usually appears after the disappearance of HBsAg from the serum and can persist indefinitely. May be the result of vaccination or actual infection.
HBcAg	HBV core antigen. Remains within the hepatocyte and does not circulate in the serum.
anti-HBc	Indicates presence of antibody to HBcAg in serum. Appears in the serum shortly after HBsAg and before the appearance of anti-HBs. Can persist indefinitely in the serum. Indicative of actual infection rather than vaccination.
HBeAg	Indicates the presence of HBV “e” antigen in serum, a qualitative marker of HBV replication and relative infectivity. High levels of HBeAg in the absence of anti-HBe indicate high infectivity and the presence of circulating HBV DNA. Appears concurrently with HBsAg and becomes undetectable before the disappearance of HBsAg in acute infections. Can persist indefinitely in chronic infections. Not routinely tested for if HBsAg negative.
anti-HBe	Indicates the presence of antibody to HBeAg and conversion to lower infectivity. In acute infections is indicative of resolution of infection (combined with anti-HBs). Can persist indefinitely in chronic infections. Not routinely tested for if HBsAg negative.

Table 2. Typical serologic patterns.

Classification	HBsAg	anti-HBs	anti-HBc	HBeAg	anti-HBe
Never exposed	-ve	-ve	-ve	-ve	-ve
High infectivity chronic carrier	+ve	-ve	+ve	+ve	-ve
Low infectivity chronic carrier	+ve	-ve	+ve	-ve	+ve
Current acute infection	+ve	-ve	+ve	±	±
Vaccine immunity ^a	-ve	+ve	-ve	-ve	-ve
Past exposure (exposure immunity)	-ve	+ve	+ve	-ve	±

^aHBsAb > 10 IU/L considered protective in humans.

VACCINATION OF ANDEAN CONDORS (*Vultur gryphus*) AND CALIFORNIA CONDORS (*Gymnogyps californianus*) WITH A WEST NILE VIRUS DNA VACCINE

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Abstract

During the late summer and early fall of 1999, an outbreak of viral encephalitis occurred in New York, resulting in fatal neurologic disease in humans and a variety of native and exotic birds, and horses. West Nile virus (WNV), a mosquito-borne flavivirus never before identified in the Western Hemisphere was shown to be the cause of disease.¹ The virus has spread across the United States in the past 3 yr, killing thousands of birds of many species, and is expected to cross the Rocky Mountains imminently reaching the home of the last 200 plus members of the endangered species, the California condor (*Gymnogyps californianus*).

The only commercially available vaccine is one manufactured and approved for horses. It is a killed vaccine in a lipid adjuvant (MetastimTM) (West Nile-Innovator, Fort Dodge, Fort Dodge, IA USA) with variable results in some species of birds^{2,3} and of unknown efficacy in most species. The Centers for Disease Control and Prevention (CDC) has developed an experimental recombinant DNA plasmid vaccine in an aluminum phosphate adjuvant, that in experimental studies has shown promise in protecting two very sensitive crow species, Fish crows (*Corvus ossifragus*) and American crows (*Corvus brachyrhynchos*) (Bunning et al., personal communication).

The CDC included the California condor in its evaluation of their experimental DNA plasmid vaccine. Eight Andean condors (*Vultur gryphus*) and 27 California condors residing at the Los Angeles Zoo were vaccinated twice, 21 days apart. Serum was collected at day 0, day 21, and at 3 mo (or longer) post vaccine. There were no adverse reactions, and preliminary evaluations show good immune response to this vaccine, and further titer evaluation is pending. Vaccination of the rest of the population is in progress.

The candidate vaccine looks to be a promising one for future avian vaccination for WNV; however, it is currently for research only and not commercially available at this time.

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PROTOZOAL PATHOGENS IN FECES OF FREE-RANGING MARINE-FORAGING RIVER OTTERS (*Lontra canadensis*)

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Abstract

Cryptosporidium spp. and *Giardia* spp. are ubiquitous, highly infectious protozoal parasites that have been identified in humans and in numerous species of domestic animals and wildlife. Infected humans and animals shed millions of environmentally-stable infectious oocysts, making environmental contamination a concern. Within the inland waters of Washington State (Puget Sound and Northwest Straits), river otters (*Lontra canadensis*) forage in marine waters where they eat numerous species of fish and invertebrates and play an important role in the nearshore marine ecosystem. We evaluated marine-foraging river otters as regional sentinels for environmental contamination with *Cryptosporidium* spp. and *Giardia* spp. Fresh river otter scat samples were collected from shoreline latrine sites throughout the inland waters and tested for *Cryptosporidium* spp. and *Giardia* spp. oocysts via immuno-magnetic separation and fluorescent antibody testing. *Cryptosporidium*-like and *Giardia*-like oocysts were identified in a small percentage of samples and identification of these parasites to species is pending. Preliminary evidence suggests that river otters may be good sentinels of environmental contamination with these protozoal parasites. A larger sample size and a better understanding of the home range of these animals in this marine ecosystem are warranted.

PHARMACOKINETICS OF PHENOBARBITAL IN CALIFORNIA SEA LIONS (*Zalophus californianus*)

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Abstract

Domoic acid, a potent excitatory neurotoxin produced by the diatom *Pseudonitzschia australis*, has recently been reported as a cause of stranding in California sea lions (*Zalophus californianus*). Clinical signs in affected sea lions include ataxia, disorientation, scratching behavior, seizures, coma, and death. Lesions caused by domoic acid in these animals have included neuronal necrosis, particularly in the area of the hippocampus. Since first being reported in 1998, approximately 400 live-stranded sea lions suspected of having domoic acid toxicity have been admitted to the marine mammal center, a private nonprofit marine mammal rehabilitation center in central California. Treatment of affected animals has been directed at attempting to stop seizures with the use of benzodiazepines such as diazepam and lorazepam, and preventing further seizure activity with the use of phenobarbital. Rapid initiation of therapy in affected sea lions may have been responsible for increased survival rates reported in a previous study. Few studies have evaluated the use of anticonvulsants in marine mammals, and dosages used are frequently empirically derived by extrapolation from other species. However, absorption, distribution, and elimination in different species may be highly variable. Although phenobarbital dosing is often titrated to clinical effect, clinical response is difficult to assess in sea lions affected by domoic acid toxicity because clinical signs of domoic acid toxicity including lethargy, disorientation, and ataxia may closely resemble signs associated with phenobarbital overdose. The pharmacokinetics of oral phenobarbital were investigated in order to determine a safe and efficacious dosage for use in treating seizures induced by domoic acid in California sea lions.

Nine California sea lions (six male, three female) weighing between 29 and 43 kg were included in the oral phenobarbital study. At the time of the study, the sea lions were judged to be in good body condition, showed normal behavior, and had normal hematology and serum chemistry parameters. None of the animals had been suspected as having been affected by domoic acid toxicity at the time of stranding. The animals did not receive any other medication during the course of the study. Sea lions were housed in three groups of three animals each and had access to a freshwater pool at all times. The animals were fed a diet consisting of whole, frozen Atlantic herring (*Clupea harengus*) thawed and given at a rate of approximately 5% of the body weight of each animal per day. The sea lions were fed approximately every 12 hr at 08:00 and 20:00. Each group of three sea lions was given a dosage of 2, 4, and 6 mg/kg respectively of phenobarbital (Qualitest Pharmaceuticals, Inc., Huntsville, AL 35811) orally b.i.d. for 7 days. Blood samples were drawn from the caudal gluteal

vein using 1.5-inch, 21-ga needles directly into blood collection tubes containing serum separation gel and clot activator (Vacutainer Systems, Becton Dickinson, Franklin Lakes, NJ 07417). Between 3 and 5 ml were drawn per sample. Blood was drawn on day 0, 4, 7, 10, and 14 of the study. Animals in the third group, which was given the highest dosage of phenobarbital, were also sampled on day 16. Mentation was scored using a standardized protocol. Oral dosages of phenobarbital (2 mg/kg) resulted in peak plasma concentrations of 10-30 mg/L which are considered therapeutic levels for most species receiving phenobarbital for treatment of seizures. Dosages above 2 mg/kg (4 and 6 mg/kg) resulted in plasma concentrations above 30 mg/L and dosages of 6 mg/kg were associated with significant central nervous system side effects. The time to maximum plasma concentrations (11.9-78.2 mg/L) was 7 days suggesting that a loading dose is required to bring plasma concentrations into therapeutic range rapidly. The average terminal half-life was 5.8 days, indicating that a minimum of 24 days (four half-lives) is necessary before steady state can be achieved. In conclusion, a loading dose of phenobarbital followed by an oral maintenance dosage of 2 mg/kg appears to achieve plasma concentrations in the range of 10-30 mg/L which may be therapeutic without further exacerbation of domoic acid induced central nervous system side effects.

IMAGING IN WHALE FETUSES: ULTRASONOGRAPHY AND COMPUTED TOMOGRAPHY IN DIFFERENT CETACEAN SPECIES OF THE FAMOUS HISTORIC COLLECTION OF WILLY KUEKENTHAL

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Abstract

Willy Kueckenthal (1861-1922) was a German zoologist, a disciple of Ernst Haeckel, director of the museum of natural history in Berlin. Kueckenthal's research concentrated on whale species. He was the founder of the *Handbook for Zoology*. With his studies, he confirmed the biogenetic law of Haeckel (1834-1919; From 1862-1909 Haeckel was professor and director of the zoological institute of the University of Jena and probably the most well-known German zoologist of his time, for his renowned works on the systematics of radiolarians, medusae and sponges, which he himself beautifully illustrated [Kunstformen der Natur, 1899-1904] and for his Biogenetic Law and the Gastraea theory; he coined the zoological terms "gastrula" and "coelom").

The collection of Willy Kueckenthal is a very famous historic compilation of cetacean fetuses. We had the opportunity to examine fetal specimens of several species of *Odontoceti* (toothed whales) and *Mysticeti* (baleen whales) including the largest recent animal, the blue whale, two species of the highly intelligent and communicative dolphins, the tusked narwhal, the sperm whale, and the humpback whale; a representative selection out of the nearly 80 living species. We were delighted getting access to this historic collection for applying modern imaging techniques such as high end ultrasonography and computed tomography, in these valuable specimens of the museum of natural history of Berlin. The *Cetacea* are one of the most distinctive and highly specialized orders of mammals. We were able to visualize in situ some specialized adaptations for their aquatic life, like the highly compressed neck vertebrae, the inner ear with its heavily ossified structures, the skeleton of the flipper shaped forelimbs, the greatly elongated anterior skull bones, and the nostrils, located on top of the head, forming the blowhole. With a special CT window we were able to visualize for the first time the upper respiratory tract within the head and performed a "virtual endoscopy" along this course. In some specimens of the baleen whales, the vestiges of the pelvis and the fetal teeth, lacking completely in the adult, could be imaged.

CT has proved to be an excellent technique for imaging various structures, organs and whole organ systems of the fetuses in situ. The implementation of ultrasonography was limited to special questions. The resolution of parts of the soft tissue was higher, so the ultrasonographic imaging technique was able to cover examinations of defined regions of interest of soft tissue. Both imaging

techniques provide the advantage of nondestructive examination of rare and valuable museum material. The findings are compared to the findings and notations of Kuekenthal¹ in his 1893 monograph.

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ANGIOGENIC GROWTH FACTOR THERAPY USING RECOMBINANT PLATELET-DERIVED GROWTH FACTOR (REGRANEX) FOR LATERAL LINE DISEASE IN MARINE FISH

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Abstract

Wound healing in vertebrates is a multi-step process involving a host of molecular and cellular mediators. Angiogenesis, the growth of new capillary blood vessels, is well established to be critical for complete healing in acute wounds, and is defective in many chronic wounds.¹ Among the critical mediators of wound angiogenesis are cytokine growth factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF). PDGF is a potent angiogenic stimulator that promotes: 1) endothelial proliferation and migration, 2) vascular tube formation, and 3) vascular stabilization.² Deficiencies in PDGF occur in diabetic chronic wounds, and PDGF knock-out mice exhibit aberrant, dysfunctional wound angiogenesis and delayed healing.^{3,4} One iso-form of this growth factor, PDGF-BB, has been developed by recombinant technology and was approved by the Food and Drug Administration in 1997 for accelerating wound angiogenesis and healing in human diabetic patients.^{5,6}

Because the receptor for PDGF-BB is highly conserved among vertebrates, the drug (rhPDGF-BB, becaplermin, tradename: REGRANEX) has been effectively prescribed in extra-label fashion to heal chronic wounds in the exotic veterinary setting, including those occurring in raptors (avian pododermatitis), reptiles (traumatic wound), armadillo (pressure ulcer), and rhinoceros (pressure ulcer). At the New England Aquarium, we hypothesized that rhPDGF-BB would accelerate healing in fish with chronic lesions secondary to head and lateral line disease (HLE).

The first two cases were sailfin tangs with fairly extensive HLE present for more than 14 mo with no improvement despite sharp debridement for 3 mo of the treatment time. Other background treatments included supplemental oral vitamin C and a broad-spectrum ultraviolet light. Over 10 mo of therapy, there was no change in disease progression. Topical angiogenic therapy with REGRANEX began in June 2002.

Materials and Methods

The fish were anesthetized using 90 ppm of MS 222, placed on a padded surface, and maintained with anesthetic water flowing across the gills. One fish had bilateral fairly symmetric lesions. We initially chose to treat the right-side lesion, and to use the left-side lesion as an untreated, internal

control. Both lesions were rinsed with sterile fresh water and surgically debrided by using either a #15 blade or a curette. The rationale for debridement is to remove necrotic tissue and senescent cells, to stimulate a proangiogenic wound microenvironment, and to expose endogenous receptors for PDGF-BB. A thin layer of REGRANEX gel (~0.1 cc) was then applied using sterile technique directly on the wound with a cotton applicator. A 3-min observation period was strictly enforced following each application, to allow the exogenous growth factor to bind to endogenous receptors in the wound bed, prior to placing the fish back into water. REGRANEX was applied weekly and the lesion focally debrided on a biweekly basis. The left-side lesion was subsequently treated with REGRANEX after the other lesion had healed completely.

Results

Treatment with REGRANEX led to visible granulation (angiogenesis) in the wound bed within days of application. After 3 wk of therapy, there was an approximately 50% reduction in size of treated lesions. In contrast, untreated control lesions did not improve. Complete resolution of treated HLLE lesions occurred in 8 wk, with return of all normal melanophore patterning and no clinically evident scarring. Fish tolerated treatments well. There was no compromise in behavior following treatment.

Conclusion

Angiogenic growth factor therapy using REGRANEX can accelerate healing and wound closure in severe HLLE. We speculate that the PDGF-BB receptor is conserved in marine fish, and that stimulation of these receptors by an exogenously applied recombinant growth factor activates signal transduction pathways, leading to granulation and healing. Our results suggest that REGRANEX may be a useful treatment for HLLE in the aquarium setting. Further studies are underway.

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CLINICAL ILLNESS IN TWO HARBOR SEALS (*Phoca vitulina*) AND ONE GREY SEAL (*Halichoerus grypus*) CAUSED BY THE WEST NILE VIRUS

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Abstract

The West Nile virus (WNV) caused clinical illness in three seals housed at different institutions during the summer of 2002. The course of illness varied among the three, but all eventually succumbed to the virus or other illness thought to be secondary or concurrent with the virus. The cases described are the first known incidences of clinical illness due to WNV in marine mammals.

Clinical signs in each included twitching of the face, head/neck and body, reluctance to move, increased respiratory rate and effort, and anorexia. The tremors were evident when the seals were at rest, but were more pronounced when they moved or reached for food items. The seals also sometimes demonstrated violent twitching in response to noises or touch. The first harbor seal (*Phoca vitulina*) began improving after a few days of illness and was nearly normal after day 5 with only occasional, mild tremors. He developed sneezing and epistaxis on day 12 of illness, which appeared to be resolving. He then developed dyspnea on day 23, and died on day 25. Gross and histologic exam determined the cause of death to be bronchopneumonia, possibly secondary to WNV. The second harbor seal had similar clinical signs and also had difficulty manipulating feed items. When force-fed, he gagged and appeared to be unable to swallow. The anorexia and twitching were progressive and his motor abilities deteriorated. He died on day 10. The primary cause of death was judged to be WNV encephalitis. The grey seal (*Halichoerus grypus*) presented with facial twitching, lethargy, anorexia and an increased respiratory rate. Ultrasound revealed an enlarged, globoid heart. She succumbed after 3 days of increasing respiratory distress and neurologic signs. The cause of death was determined to be dilative cardiomyopathy. In all three cases the histopathologic lesions in the brain and spinal cord were mild and alone not useful to identify affected seals.

All three seals tested positive for WNV with the plaque reduction neutralization test (PRNT) at a titer of 1:640 and were negative for St. Louis encephalitis virus (SLE) at a screening titer of 1:20. Both serum samples and pooled tissue samples from all three seals tested positive on RT-PCR. Two of the three were also positive on immunohistochemistry for WNV; one was focally positive only in the cerebrum and one was strongly positive in the spinal cord. Virus isolation was performed on the grey seal and WNV was isolated.

A second harbor seal at the first facility had very mild tremors of the head and neck on the third day of seal #1's illness but appeared completely normal the next day. Serum was collected on day 2 and was positive for WNV on PRNT at a titer of 1:8192 and negative on RT-PCR.

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MEDICAL MANAGEMENT OF NECROTIC DERMATITIS SYNDROME (“BELL ROT”) IN A COLLECTION OF JELLIES

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Abstract

Within a 9-mo period, a number of jellies from a mixed species gallery at the Aquarium of the Pacific in Long Beach, California (USA) presented to the veterinary department with ulcerative lesions of the umbrellar epidermis^{2,6,7} of the bell, often called “bell rot.” Affected jellies were housed in species-specific exhibits, and included West coast sea nettles (*Chrysaora fuscescens*), East coast sea nettles (*Chrysaora quinquecirrha*), lagoon jellies (*Mastigias papua*), blubber jellies (*Catostylus mosaicus*), sandaria jellies (*Sandaria malayensis*), moon jellies (*Aurelia aurita*, *Aurelia labiata*), and white-spotted jellies (*Phyllorhiza punctata*).

All jellies were kept in re-circulating “kreisel” tanks that had oval shapes, smooth plexiglass walls, and a continuous circular flow. Tanks contained approximately 132 – 1,325 L of natural sea water conditioned by sand and ozone filtration prior to use within the exhibit gallery. Additional types of filtration varied between individual kreisels and included protein skimmers and bio-towers. The following water quality parameters were checked weekly and were within normal limits: pH > 8.10, salinity 31.0-34.5 ppt, alkalinity > 2.0 mEq/L, NH₃ < 0.05 mg/L, NO₂ < 0.08 mg/L. Water temperature ranged from 12.8-23.9 °C according to species requirements. Jellies were fed a variety of diet items, including nauplii (adult and juvenile brine shrimp), moon jelly slurry, bloodworms, small krill, and cyclopeeze (zooplankton derived from copepods). Although water flow was adjusted as needed for optimal circulation, jellies in several exhibit kreisels were routinely observed hitting tank walls or getting trapped in water outflow valves.

Each affected jelly was isolated into a small, round, glass container of tank water for visual examination. Lesions ranged in depth and severity from erosions and ulcers of the peripheral edges and exumbrellar⁷ surfaces of the bell to full-thickness tears of the subumbrella⁷ with subsequent internal organ herniation. Jellies with severe “bell rot” and/or herniation were culled and euthanatized by hypothermia at approximately 5 °C. Jellies with lesions less than 3 cm in diameter were isolated within hospital tanks for treatment and observations.

Jellies have an extraordinary ability to regenerate epidermal and mesogleal tissue, with a high degree of muscle-cell plasticity.^{1,3,5-8} Treatment of the bell lesions consisted of sharp excision of necrotic, damaged tissue plus 3-5 mm margins of healthy tissue using Brown-Adson forceps for stabilization

and iris scissors for trimming. No jellies exhibited adverse responses to excisional procedures, and thus, no anesthesia was deemed necessary in these cases. Care was taken to keep dissection and trimming techniques as superficial as possible. Invertebrate pain reception should be considered during any procedure that might inflict pain,⁴ and thus, the use of anesthesia may be advisable for the management of future cases.

After excision of abnormal tissue, jellies were treated with medicated baths (nitrofurazone) (10 mg/L for 1-2 hr, followed by 2 mg/L continuous immersion) until healed or improved, generally 5-7 days. Jellies receiving both tissue removal and antibiotic immersion regained normal appearance of their bell surfaces within 8-12 wk and could be placed back on exhibit; however, several individuals with permanent superficial scarring of the bell following procedures were culled due to a lack of exhibit quality. Nerve function following excisional therapy and regeneration^{1,5,8} remained intact based on observed ability of the bell to continue normal propulsion movements.

Bell tissues from several specimens were preserved in 10% neutral buffered formalin for histopathologic evaluation. Biopsies were embedded in paraffin, sectioned at 5.0 μ m, and stained with hematoxylin and eosin (H&E) stain. There was multifocal sloughing and loss of epithelium (ulcers) and moderate to marked multifocal coalescing necrosis of the epidermis (ectoderm) covering the exumbrella. The epidermis lining the subumbrella was less severely affected. Necrotic epidermis was covered by cell debris and few degenerate, necrotic hemocytes. In several cases, the necrosis extended deep into the underlying mesoglea with multifocal infiltration by granulated and un-granulated hemocytes. On the exumbrellar surfaces of two of the jellies there were multiple, round, 10 – 20 μ m algal-like organisms with thick, refractile cell walls. These organisms infiltrated into areas of epidermal and mesogleal necrosis. Two different jellies had rod-like to undulating filamentous bacteria infiltrating the areas of epidermal necrosis; however, a sandaria jelly with bell necrosis had neither bacteria nor algal-like organisms evident within its lesions.

We hypothesize that traumatic bell lesions were sustained by jellies as a result of colliding with tank walls, either accidentally or due to undetected, irregular currents within the kreisels. Damaged epithelium supported invasion by opportunistic bacteria and algal-like organisms, further penetration into the mesoglea, and inflammation (hemocytic infiltration) leading to necrosis. In some tanks, the extended stingers (nematocytes) of hydroid colonies⁷ present on exhibit walls, likely originating from food sources, may have directly contacted and damaged the delicate bell surfaces of affected jellies.

The algal-like organisms associated with moderate to marked epidermal and mesogleal necrosis were more abundant and microscopically different from commensal algae, or zooxanthellae,^{2,7} naturally found in certain jellies. Possible sources of these algal-like organisms include: addition of newly acquired wild-caught jellies without adequate quarantine, contaminated water supplies, equipment, or food sources, and undetected suboptimal water quality leading to overgrowth of opportunistic noncommensal algae. Further evaluation of the algal-like organisms by electron microscopy is underway in order to better determine treatments or management techniques indicated in the prevention of this syndrome.

Efforts should be made to maintain proper husbandry for captive jellies. Ultraviolet filtration, timed lighting cycles, and dilution by water changes may help reduce presence or numbers of potentially pathogenic microorganisms, including algae. The live food sources should be examined for possible hydroid contamination before adding them to the exhibit. In addition, water flow within jelly tanks must be quite frequently monitored and adjusted to regulate currents and prevent erratic swimming patterns in resident jellies. General guidelines for captive jelly husbandry are available at the website, <http://www.jellieszone.com>.

To our knowledge, this is the first report documenting multiple cases of necrotic dermatitis associated with algal-like organisms and bacteria in captive jellies. Surgical excision of the affected tissue, followed by topical anti-bacterial therapy, may potentiate healing and improve cosmetic appearance of the bell surfaces. Frequency of necrotic dermatitis syndrome in jellies from this collection prompted re-evaluation of water flow design in each system in order to prevent contact with tank walls and subsequent damage to delicate epidermal layers.

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SPRING VIREMIA OF CARP VIRUS: ANIMAL HEALTH POLICY CONSIDERATIONS

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Abstract

Ornamental fish comprise the third largest sector of the multi-billion dollar aquaculture industry in the United States. In 2002, the value of ornamental fish imports into the United States was estimated at \$41 million. As the aquaculture industry grows, there is increasing awareness of the vulnerability of the industry to introduced diseases from foreign imports. While exotic disease introductions have already had major impacts on food production aquaculture, until recently, the ornamental fish trade has remained relatively unaffected. However, the 2002 outbreak of spring viremia of carp (SVC), previously undocumented in the United States, at a koi hatchery in North Carolina and in Cedar Lake in Wisconsin, has numerous current and potential consequences for native fish, natural resources, the ornamental fish industry, and food production aquaculture. SVC is listed as a notifiable disease, by the Office International des Epizooties (OIE), in the International Aquatic Animal Health Code. The potential economic and environmental impacts associated with the discovery of SVC have caused the current policies governing ornamental fish aquaculture to come under scrutiny. Presently, there are no inspection or quarantine requirements for ornamental fish imported into the United States. There are multiple agencies that have jurisdiction over the aquaculture industry, including the U.S. Department of Agriculture (USDA), Department of the Interior, Department of Commerce, Department of Health and Human Services, Environmental Protection Agency, and state agencies. All of these agencies, industry stakeholders and the public may be involved in the development of regulations for the importation of ornamental fish. Under the Farm Bill 2002, the USDA's Animal Plant Health Inspection Service (APHIS) has statutory ability to regulate ornamental fish—the extent that APHIS will be involved in ornamental aquaculture has not been determined. In March 2003, APHIS announced that approximately \$11.7 million had been authorized to implement an SVC control and indemnity program for farm-raised fish in the United States, to assist the states of North Carolina and Virginia with epidemiology, surveillance and indemnification. Zoological parks and aquariums may be impacted by regulatory changes that are implemented in the aquaculture industry.

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Whitaker, National Aquarium, Baltimore; and Roy Yanong, Tropical Aquaculture Laboratory, University of Florida.

DIAGNOSIS OF ERYSIPELOTHRIX SEPTICEMIA IN A LITTLE BLUE PENGUIN (*Eudyptula minor*)

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Abstract

In summer 2002, a 5-yr-old little blue penguin (*Eudyptula minor*) was presented to veterinary clinicians by husbandry staff agonal and died within 10 min despite emergency treatment. Microscopic examination of formalin-fixed paraffin-embedded tissue sections revealed intravascular Gram-positive, non-acid-fast bacilli in multiple organs, with necrosis of tips of intestinal villi and histiocytic infiltrates in pulmonary interstitium and hepatic sinusoids. Frozen samples of lung, liver and intestine were cultured on blood agar under microaerophilic and anaerobic conditions at 37°C and 42°C. Cultures from lung, liver and intestine yielded isolates identified by standard methods as *Erysipelothrix rhusiopathiae*. Genomic DNA was extracted from paraffin tissue blocks and cultures from lung, liver, and intestine, and a previously published protocol was modified slightly to allow amplification of *E. rhusiopathiae* DNA from formalin-fixed tissues.¹ Single-band amplicons of comparable molecular weight to positive control products were obtained from bacterial cultures of lung, liver and intestine and from formalin-fixed paraffin-embedded sections of lung and intestine. Standard nucleotide-nucleotide BLAST sequence comparisons of PCR products against NCBI nucleotide databases demonstrated 99% nucleotide sequence identity with 16S SSU ribosomal DNA of *E. rhusiopathiae* and *E. tonsillarum*. To our knowledge, this is the first confirmed case of erysipelas in a penguin and expands our knowledge of the range of aquatic animals considered susceptible to acute *Erysipelothrix* sp. septicemia. Application of a previously published PCR protocol allowed retrospective analysis and correlation of culture results with formalin-fixed paraffin-embedded tissues, which may be pertinent to cases from other aquatic species wherein this diagnosis is suspected.

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INK EMBOLISM IN FRESHWATER ORANGE SPOT STINGRAYS (*Potamotrygon motoro*) FOLLOWING TATTOOING PROCEDURE

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Abstract

Tattooing is a common practice in animals for identification purposes. Intravenous injection of India ink has been reported to cause thromboembolism in rabbits, mice, and rats.¹ The toxicity of ink is dependent on the type of ink used with ink containing shellac being more pathogenic. In June 2000, three, approximately 3-mo-old, captive, freshwater orange spot stingrays (*Potamotrygon motoro*) died following routine tattooing as part of an animal identification program. One stingray died postrecovery following anesthesia with MS222, and the other two stingrays were found dead in their tank 2 days following the tattooing procedure. At necropsy, all three stingrays had gills that were blackened. Histologically, all stingrays had many, ink emboli within one or more of the following tissues: gills, kidney, heart, and liver. Complications associated with the leakage of ink into the circulation following tattooing are believed to have caused the death of these three stingrays.

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LESSONS FROM A CASE OF CAPTURE MYOPATHY IN A PELAGIC SILKY SHARK (*Carcharhinus falciformis*)

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Abstract

Public aquaria and zoos have historically had difficulty maintaining elasmobranchs, particularly pelagic sharks. This difficulty is often attributed to "poor acclimatization." Numerous factors play a part in this poor adaptation to captivity: transportation, exhibit design, lack of established normal biologic parameters in husbandry and medicine, and poor medical databases of diseases and treatments. Additionally, these animals may begin disadvantaged due to capture stress and possibly capture myopathy (CM). A case of exertional myopathy in a silky shark (*Carcharhinus plumbeus*) and the critical care and treatment options pursued is described.

Case Report

In summer 2002, a 5.56-kg, juvenile female pelagic silky shark was caught by hook and line with gear designed to ensure a brief capture with minimal stress. The animal was immediately put into a circular live well (within 5 min of initial contact time with capture gear) and transported to a local holding facility. The animal had adjusted well to captivity and remained at the facility for 5 mo. There was no additional handling of the animal because the basic philosophy of the capture group is to minimize handling and intervention. The animal was apparently healthy prior to shipment. After a 4-day fast, the animal was transported in a semi-truck fashioned with a specialized fiberglass tank and an oxygenated filtration system.¹³ Ammonia, pH, dissolved oxygen and temperature were repeatedly evaluated during the transport and stayed within acceptable ranges. Total transport time was 36 hr; anticipated transport time was 24 hr but due to vehicle mechanical problems, was delayed.

On arrival at the Shedd Aquarium, the animal was assessed in the transport container and deemed normal. Transport water quality was also normal. Due to the fractious nature of pelagic sharks and the lengthy transport event, the animal was anesthetized (to reduce further capture stress) in the transport container with 100 ppm tricaine methane sulfonate (MS222, Argent Chemical Laboratories, Inc., Redmond, WA 98052 USA) then moved to a maintenance dose of 50 ppm. The anesthetic was buffered with an equal weight of sodium bicarbonate (Dwight and Church, Co., Princeton, NJ 08543 USA). Induction was approximately 6 min in duration and uneventful. A physical exam (PE) was performed, morphometric measurements taken, and blood was collected.

Nothing unusual was found on PE except for myosis of the left eye secondary to trauma sustained during shipment. A water pump attached to an oxygen source was placed in the oral cavity. Standard blood processing for elasmobranchs at the aquarium includes I-STAT (cartridges EC8+ and EC7+, Heska Corporation, Fort Collins, CO 80525 USA), blood culture, CBC, and serum chemistries (IDEXX laboratories, Elmhurst, IL 60126). Total anesthesia time was 13 min and recovery was normal in the animal's quarantine tank by 20 min after initial contact with anesthetic. At this time blood gas parameters were considered normal in comparison to in-house data from black tip reef sharks (*Carcharhinus melanopterus*), another pelagic species.

At 9 hr post arrival, an abnormal, wobbly swimming gait was noted, and the shark would not maintain a normal swim-glide pattern despite being housed in a tank appropriately designed for the animal.

At 16 hr the shark's swimming slowed and it began bumping into the tank walls. A mild water current was noticed in the tank and flow rate was decreased but resulted in no improvement. The patient was removed from the exhibit for supportive care and blood gas assessment under 50 ppm buffered MS-222. Capture stress and myopathy were strongly suspected. Blood gas analysis indicated a slight metabolic acidosis (compared to entry values) and decreased oxygen saturation. Hematocrit indicated anemia. Supportive treatment was initiated. A 150 ml bolus of 0.9% NaCl (Baxter Health Care Corporation, Deerfield, IL 60015 USA) with 40 mg sodium acetate (anhydrous, Fisher Biotech, Fairland, NJ 07410 USA) and 5% dextrose (50%, Burns Veterinary Supply, Westbury, NY 11950 USA) added, and 3 mg/kg sodium prednisolone succinate (500 mg, Pharmacia and Upjohn, Kalamazoo, MI 49001 USA) were given intravenously; 30 mg/kg ceftazidime (Fortraz, 100 mg/ml, Glaxo, RTP, NC 27709 USA) and 2 IU vitamin E (Compounded as vitamin A/D/E at Martin Ave Pharmacy, Naperville, IL USA) were given intramuscularly. Normal cardiac contractility and heart rate (70 beats/min) were noted with cardiac ultrasound. Recovery was rapid and the shark was re-released to the enclosure.

At 18 hr the shark was unresponsive to her environment, had postural abnormalities (tail down) and appeared ataxic. Additional critical care was initiated. There was no resistance to restraint, and anesthesia was unnecessary. Cardiac ultrasonography revealed poor contractility and bradycardia. Repeat blood gas analysis and ionized calcium were monitored. Acidosis progressed, ionized calcium fell, and oxygen saturation was reduced despite oxygenated water flow over the gills. Treatments over a 3-hr period included: repetitive doses of 0.1 mg/kg i.v. calcium gluconate (10%, American Regent Labs, Inc., Shirley, NY 11967 USA) administered slowly, 1 mEq/ml i.v. sodium bicarbonate (8.4%, American Regent Labs, Inc., Shirley, NY 11967 USA), 0.1 mg/kg i.v. atropine sulfate L.A. (15 mg/ml Neogen Corporation, Lexington, KY 40505 USA), and 20 ml/kg i.v. hetastarch (6% in 0.9% NaCl, Abbott Laboratories) mixed with additional i.v. fluids.

Response to treatment in the first hour was initially positive. Blood gas values improved, calcium increased, heart rate increased and contractility improved. The shark began exhibiting swimming motion and an increased awareness of her environment. Despite initial stabilization, the patient ceased all independent swimming behavior by the second hour of supportive care and continued to

decline over the next 2 hr. Cardiac arrhythmias ensued, pH continued to drop and oxygen saturation could no longer be maintained. Euthanasia was elected. Serial bloodwork (Table 1) was obtained.

A complete necropsy examination was performed. There were no gross lesions. Histologically there was moderate, multifocal ventricular myocardial contraction band necrosis, and mild, multifocal rhabdomyolysis of skeletal muscle. Additional incidental lesions were mild, multifocal, lymphocytic meningitis and minimal lymphocytic perivascular cuffing in the olfactory bulb. The final diagnosis was exertional rhabdomyolysis.

Discussion

Rhabdomyolysis was probably a function of stress and overexertion during confinement in-transit and was exacerbated by conditions in the holding tank. Despite novel transport techniques^{13,14} and efforts to provide the correct environment, the mild current in the tank likely resulted in additional exertional effort by an already compromised individual as the shark persistently swam against the current, perhaps in an effort to increase exposure to oxygenated water. The tank design lent itself to a swim/glide pattern, but the shark never established this pattern, critical for a normal 'rest' phase in the swimming cycle.

Marked increases in CPK and LDH were present at the time of entry, indicating myocellular necrosis had occurred prior to arrival at the aquarium. Only mild skeletal muscle necrosis was noted histologically, possibly a function of sampling as lesions with rhabdomyolysis can be sporadic in distribution. Muscle enzyme levels were indicative of moderate skeletal muscle damage, and thought to be higher than would have been expected from myocardial necrosis alone. Notably, cardiac contractility and heart rate declined during clinical course, and myocardial necrosis secondary to physiologic stress, acidosis, and possibly anoxia could have been a secondary sequela of rhabdomyolysis, though cardiac necrosis as a primary lesion of capture myopathy has been described.

Transporting pelagic sharks is a difficult process and until recently¹⁶ rarely resulted in long-term survival post transport. Silky sharks have been successfully shipped on long-term (> 24 hr) transports.¹⁴ Nevertheless, as in this case, unforeseen incidents such as equipment failure and obstacles encountered in travel can prolong an already stressful event. Further, many pelagic sharks are fractious in nature which may predispose them to capture myopathy.

Myopathy in elasmobranchs likely occurs more often than previously documented or recognized. This is due largely to the lack of biochemical or necropsy data on captive animals that succumb to 'poor adaptability.' At Shedd, clinically inapparent and mild myopathies during short transports between exhibits have been observed, evidenced by moderate elevations of LDH and CPK as well as prolonged recovery from anesthesia when compared to less agitated con-specifics.

Diagnostics for Antemortem Evaluation of Capture Myopathy in Elasmobranchs

Shark muscle is comprised of a predominant white portion and a smaller amount of red muscle for bursts of activity. While most muscle uses aerobic glycolysis, shark white muscle predominantly uses anaerobic glycolysis, which means local lactic acid production is higher than in other species.¹ In elasmobranchs, lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK) are the enzymes known to seep into the bloodstream in muscle necrosis¹ and thus are the key enzymes required for antemortem diagnosis of rhabdomyolysis. The published normal values for LDH are no higher than 263 IU/L, comparable to other species.^{5,6,10} However, limited data precludes determination of levels of prognostic value for recovery or demise. At Shedd, routine use of the ISTAT lactate cartridge in normal animals has recently been implemented with the hope that this will assist with tank-side assessment of elasmobranchs pre, during, and post shipment. For all elasmobranchs, preshipment bloodwork, in-transit tank-side monitoring, and follow-up bloodwork upon arrival are strongly encouraged. Blood work should at a minimum include blood-gas analysis, and serum LDH and CPK.

Treatment

Treatment should be targeted symptomatically. Acidosis occurs frequently when elasmobranchs are handled. Correction of acidosis should be attempted when values drop rapidly; however, it should be noted that most fish that develop mild acidosis resolve spontaneously.¹² In CM, hypoxia and shock (hypovolemia) are the most critical conditions to correct. Fluid therapy is essential but caution must be used with fluids in saltwater elasmobranchs due to the high blood osmolality (secondary to greatly elevated blood urea nitrogen); in this report we used a modified sodium chloride solution and there is an 'elasmobranch ringer's' recipe available.⁴ Intravenous calcium supplementation produced favorable clinical responses in critical animals at the Aquarium. Muscle pain is inevitable and use of anti-inflammatories and analgesics could be contemplated. There are no published doses for analgesia in elasmobranchs so again, caution must be exercised. Steroids are of questionable efficacy but are often used in critical situations. At doses described in this text, there have been no adverse effects. Antioxidants may be useful and are usually not harmful; 0.1 ml/kg i.m. vitamin A/D/E has been used at the Aquarium without adverse effects, although efficacy in exertional myopathies has been questioned.^{9,11}

Preventive Recommendations

- During initial capture, ensure all gear is suitable for rapid placement into a stable holding facility; minimize any handling prior to final shipment.
- Decrease stress before, during and after shipment.
 - Chemically: 1) anesthesia decreases the stress of handling during processing, but carries it's own risks in compromised individuals. 2) psychotropic drugs are of potential interest in transporting elasmobranchs (azaperone, zuclopenthixol).^{2,7,8} Diazepam (1 mg/kg p.o.) has been used as a premedication at the Aquarium.
 - Behaviorally: condition sharks to transport by desensitizing them over time to nets, capture,

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- and actual transport if possible.
- Use well-trained staff that can rapidly and safely capture animals.
 - Maintain an oxygenated water pump at all times during transport and handling.
 - Keep out-of-water time minimal; air-exposed, exercised fishes show greater mortality rates.³
 - Use appropriate containers.¹³
 - Fast animals prior to transport to avoid poor water quality.
 - Once an animal undergoes a stress event avoid moves/transport for 3 wk.
 - Consider antioxidant therapy (vitamin E/selenium, vitamin A).

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Table 1. Select blood chemistry values for (*Carcharhinus falciformis*) with capture myopathy.

	Entry	16 hr post	Pre Euthanasia
AST (IU/L)	264	676	358
LDH (IU/L)	21840	3939	27478
CPK (IU/L)	25884	11604	18018
Ca (mg/dl)	14.3	12.5	8.7
K (mEq/L)	3.3	3.7	10.5

THE NEXT GENERATION IN ELECTRONIC MEDICAL RECORD KEEPING: THE ZOOLOGICAL INFORMATION MANAGEMENT SYSTEM

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Abstract

The Medical Animal Record Keeping System (MedARKS) was created in 1986 by Dr. Andrew Teare and later supported by the International Species Information System (ISIS). It has served the zoo veterinary medical community well for many years, but is not adaptable to advances in software technology. The AAZV created an Ad Hoc Electronic Medical Records Committee to begin to address these concerns. At the same time, similar constraints were identified in the ARKS software used by registrars and animal managers of zoological collections. The American Zoo and Aquarium Association (AZA) first created a Strategic Software Taskforce in 2000 to assess the status of current data management practices and determine the feasibility of creating a new system. In 2001, the AZA created the Animal Data Information System Committee (ADISC) and hired a technology consultant, the Inteq Group, to begin the initial planning for a new global animal information system for both zoos and aquariums. Representatives of AAZV and the zoo and aquarium veterinary community were invited to participate with a broadly representative group in the initial scope and design phases of a new Zoological Information Management System (ZIMS).

During Phase I (July 2001 to March 2002) of the ZIMS Project, ADISC, with the assistance of the Inteq Group and from a series of stakeholder workshops, produced a number of products including a Project Mission and Charter, a Technology Assessment, a high-level plan and estimate, and a conceptual data model (CDM). The ZIMS Project Mission is to develop, deploy and maintain a comprehensive information system to support a wide range of people and activities associated with the management and care of animals in zoological (aquariums and zoos) institutions and the zoological community. The charter defines stakeholders, risks, constraints, the technologic environment and potential deployment scenarios. ZIMS Project deliverables and updates are posted at <http://www.zims.org>.

The CDM of Phase I, produced after four design workshops attended by a representative group of zoo and aquarium professionals, helps to define the scope of the proposed system by documenting the many diverse data requirements. It models over 350 entities identified as desirable for information collection and storage (animals, people, places, events and the relationships between them), all of them necessary to support the high-level processes of inventory, veterinary, husbandry, and management.

Phase II of the project (July 2002 to Dec 2002) included a second round of workshops that involved

both national and international participants from six global regions. The scope was refined to focus on the core and veterinary needs, and a Request for Proposals to develop a technical design and architecture. A technology survey was distributed to the potential user base: 230 surveys were distributed with 121 respondents. The preliminary results revealed that 95% (115/121) of the responding institutions have internet access, and 63% (76/121) stated that they have or will have broadband connections within 18-24 mo.

Phase III of the project began in early 2003 and entails documenting and analyzing at a much greater level of detail, the business processes, data and data standards that the system must support. Prototyping, design and development will continue into 2004 and 2005 with implementation planned for late 2005-early 2006.

ZIMS is being designed to support all of the information that is in the current ISIS software (ARKS, MedARKS) plus a great deal more. A plan is underway to review the quality and content of existing data so that the historic value can be retained in the conversion to a new system. The goal is to develop an integrated, scalable and extensible database system that is accessible via the worldwide web. The database and application will support the development of new information technologies such as handheld data collection devices and image capture. The application will be seamless across business needs so that veterinarians can easily navigate different modules such as inventory, keeper reports, or environmental monitoring in addition to veterinary specific data. End-users will be able to set up their preferences for which modules they access most frequently. The new system will have better security features built in to control access. The core, centralized database architecture will provide the foundation for institutions to create add-on specialized applications to enhance productivity at their particular facilities. The web technology will also facilitate remote, 24/7 technical support. The special data requirements of aquatic organisms and groups have been addressed to support environmental variables and taxonomic challenges.

In recognition of the need for an infrastructure to support both the ongoing ZIMS Project and ensure the long-term sustainability of the system as it goes online, several organizations have been working towards building the capacity, resources, and governance that will be necessary. A Memorandum of Understanding was signed between AZA and ISIS to facilitate a partnership for fundraising and technical support. The International Animal Data Information Systems Committee (IADISC) was formed to ensure global participation and representation from the user community. ISIS has undertaken a significant organizational restructuring in order to take on the management of the project and be staffed for future support. The initial core and veterinary modules of ZIMS are forecast to cost approximately \$10 million dollars to develop. A capital campaign was initiated to raise funds from zoological institutions in several regions and from the corporate sector. Over \$1.75 million dollars has been committed by AZA member zoos to date, and major grant-writing and fundraising efforts are underway to solicit funding from government and donor groups. It is anticipated that the international community represented in IADISC will also put forth a financial commitment to the product.

FURTHER OPTIMIZATION AND VALIDATION OF THE ANTIGEN 85 IMMUNOASSAY FOR DIAGNOSING MYCOBACTERIOSIS IN WILDLIFE

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Abstract

Mycobacteriosis caused by *Mycobacterium bovis*, *M. tuberculosis* and *M. avium* has been a well-documented health problem for zoological collections since the late 19th century. Prevalence estimation in these captive wildlife populations has been hampered by diagnostic test methods that are often difficult or impossible to conduct and/or interpret due to the requirement for multiple immobilizations for measurement of response, the occurrence of nonspecific results with methods such as the intradermal skin test, and/or the near-total lack of validation, optimization and standardization of any of the available test methods in the species of interest. Additionally, because intradermal skin testing is the primary screening method for many of these species, the ability to compare exposure in captive wildlife to exposure in free-ranging populations has been limited due to the difficulty with follow-up in free-ranging populations. Lastly, unlike testing methods that use serologic techniques, skin testing precludes retrospective studies of banked samples to determine onset of reactivity.

Recently, human tuberculosis researchers working with tuberculosis in humans have developed an immunoassay that detects a serum protein complex (the antigen 85, or Ag85, complex) produced by mycobacteria in the early stages of mycobacterial infections¹. Previous work has shown that this method is a promising diagnostic tool in the evaluation of tuberculosis exposure in some primates, including orangutans (*Pongo pygmaeus*), a species known for nonspecific tuberculin responses², and captive hoofstock species³. In order to determine the feasibility and applicability of this method for captive and free-ranging wildlife species, we have undertaken a number of pilot studies on different populations of interest, with the goal of optimizing and validating the immunoassay through analysis of serum from known infected and noninfected individuals, and through comparisons with other diagnostic methods. Thus far, we have begun evaluating the applicability of the Ag 85 immunoassay in various avian, primate, rhinoceros and hoofstock species for detecting mycobacteriosis, including paratuberculosis. Preliminary results, a summary of which will be presented, indicate that this method may be a valuable adjunct to other testing methods (including gamma interferon and multiple-antigen ELISA) to allow a better evaluation of true mycobacterial status in these species.

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HIGH RESOLUTION ULTRASONOGRAPHY: A NEW DIAGNOSTIC TOOL FOR SMALL RODENT MEDICINE

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Abstract

Despite recent rapid advances in ultrasound imaging technology, the application of ultrasound imaging as a diagnostic tool in small rodent medicine remains limited, especially in nondomestic rodents. The aim of this study was to establish a reliable noninvasive examination protocol for monitoring prenatal development and ovarian morphology during pregnancy in small rodents. Specifically, we wished to evaluate the utility of high-resolution ultrasonography for imaging reproductive tract structures and pregnancy progress in a small hystricognath rodent species, wild cavy (*Cavia aperea aperea*).

We examined 19 adult female wild cavies from the breeding colony of the University of Bielefeld which had been introduced to a male at different periods. The animals varied in age (127-896 days old) and weight (309-654 g). The entire reproductive tract of each animal in each group was imaged using a Diasis™ ultrasound machine (Dynamic Imaging, Livingston, Scotland, U.K.) two or three times during a 4-wk period. All animals were examined in dorsal recumbency under isoflurane anesthesia (Forene™, Abbott GmbH, 65205 Wiesbaden, Germany).

Three different ultrawide-band electronic linear array probe types with frequencies from 8-16, 10-22 and 28 MHz were used. The miniaturized size of the transducer (active length: 26 mm) allowed small contact areas. Frequencies from 8-16 MHz were used to visualize ovaries, uterus, number of concepti as well as embryonic and fetal development. Placental integrity and amniotic fluid quality were also evaluated. The application of higher frequencies (18-28 MHz) permitted imaging of structures down to 0.4 mm. This allowed reliable pregnancy diagnosis shortly after implantation, on day 8 after copulation. Ovarian functional structures (e.g., corpora lutea and follicles) could be distinguished and measured. Changes during pregnancy were followed. The 8-16 and 10-22 MHz transducers were used to identify fetal organs and to record precise measurements of crown-rump length, biparietal distance, cerebellum, ventricle, lens, orbita, long bones and umbilical cord. Evidence of embryonic or fetal resorption was identified in four female guinea pigs, but no disturbance of general or reproductive health could be recognized.

With high resolution frequencies up to 28 MHz, the applied ultrasound system was able to image even the slightest morphologic changes in reproductive tissue in small rodents. The use of these

techniques could well replace the standard methods for pregnancy monitoring in small rodents (i.e., carcass dissection), and thereby provides new option in small animal diagnostics.

DEVELOPMENT OF RIGID LAPAROSCOPY TECHNIQUES IN ELEPHANTS AND RHINOCEROS

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Abstract

Diagnostic and surgical laparoscopy has become a routine procedure in human and veterinary medicine and has similar potential uses in zoological medicine. Surgical telescopes and fiberoptic cables allow the veterinarian to look inside body cavities of the patient and specialized instruments provide the ability to perform a wide variety of diagnostic and surgical procedures. Rigid laparoscopy is considered minimally invasive surgery and is associated with a more rapid postoperative recovery rate and an improved prognosis as compared to conventional surgical techniques.³ Laparoscopic surgery is commonly being utilized in horses and other zoo animals for a variety of abdominal surgical procedures including tubal ligation and ovariectomy.^{7,8,12} Recent advances in technology now provide the ability to perform laparoscopy in the largest mammalian species maintained in zoological collections.¹¹

A variety of disease problems and reproductive disorders have been documented in the rhinoceros and elephant. Due to their size and anatomy, many of the standard diagnostic tests available in human and veterinary medicine are not routine in these animals (radiography, advanced imaging techniques, liver biopsy, abdominal tap, etc.) at this time. Although conventional abdominal surgery has been performed in rhinoceros and elephants,^{1,5,10} survival rates have been extremely low. Laparoscopy can provide a variety of additional diagnostic options, and may also provide an avenue for performing surgical procedures that would otherwise be impossible in these animals.

A multi-institutional collaboration has been organized to address common goals with regard to enhancing diagnostic capabilities and improving surgical techniques in elephants and rhinoceros. Specifically, we are aiming to:

- Develop laparoscopic techniques and equipment for use in rhinoceros and elephants, which can be used to significantly expand our diagnostic and treatment capabilities in these large mammals.
- Improve international conservation efforts in both rhinoceros and elephants by helping improve animal health and welfare of these species in captive and free-ranging situations.

This collaborative effort has been organized to critically review current equipment, techniques and uses with the ultimate goal of overcoming some of the inherent difficulties with laparoscopy in these

large vertebrates. This includes further development and modification of equipment, investigation of surgical techniques, and expansion of clinical applications.

Cooperation in the development of innovative surgical equipment for the anatomic variety of our zoological species is necessary for the advancement of zoological medicine. Karl Storz Veterinary Endoscopy of America (KSVEA, Goleta, California 93117 USA) was instrumental in the initial development of this specialized equipment at the San Diego Wild Animal Park and has been generously providing research and development for our continuing investigations. The availability of appropriately sized equipment has limited the application of laparoscopic techniques to animals of a size consistent with the intended species of its production. The largest laparoscopy equipment commercially available is marketed for use in equine medicine (57-cm telescope and accessories). This system has been used with limited success in rhinoceros¹¹ and elephants. In many cases, to adequately visualize and manipulate visceral organs, a longer telescope and associated instrumentation is required. We continue to work closely with KSVEA on the production of specialized equipment (Table 1). Standard light source, fiberoptic light cable and electronic insufflator units manufactured for use in domestic large animals have been used successfully in megavertebrates. Due to its compact size and versatility, the authors suggest the use of a portable, battery operated laparoscopy kit (Techno Pack, KSVEA) (monitor, light source, camera and digital recording device) especially under field conditions.

To date we have evaluated the utility of specially designed laparoscopic equipment in approximately seven elephants and four rhinoceros. Experiences from these limited cases have identified several technical and procedural challenges, which need to be overcome if megavertebrate laparoscopy is to be successfully performed.¹¹ Some of these challenges are summarized below:

Technical Challenges

- Equine laparoscopic equipment too fragile and/or short for certain surgical applications in rhinoceros and elephant
- Size and disposition of megavertebrates (> 1000 kg)
- Thick, nonpliable, pachydermatous skin puts unusual pressure (and risk of damage) on equipment
- Insufflation and illumination of large abdominal cavity
- Great depth to visualize and physically reach/manipulate large organs
- Thick, redundant, fibro-elastic peritoneum which is difficult to puncture during surgical attempts to enter the abdominal cavity
- Influence of patient positioning: unable to use conventional laparoscopic positioning techniques in these species
- Both rhinoceros and elephants are hindgut fermentors and have very large and extensive lower intestinal tracts. Gas dilation of these bowel loops combined with limited positioning options, can make laparoscopic visualization of certain organs a problem

Procedural Challenges

- Cost of developing new and specialized laparoscopic equipment is high; duplicate equipment is not available at this time
- Charismatic nature of megavertebrates makes it difficult to perform surgery on such important and high exposure species
- Limited clinical cases for testing equipment due to the relatively low number of megavertebrates in zoological facilities
- Use of laparoscopy in zoo mammals may be out of the comfort zone for many veterinarians and curators due to lack of experience with this instrumentation
- Lack of published surgical procedures in these species
- Inability to perform laparoscopic procedure in surgical suite as with most other species
- Risks associated with megavertebrate anesthesia and sedation including difficulty in providing safe anesthetic procedures, proper restraint, and safety of personnel

A multidisciplinary, systematic approach has been initiated to critically review current instrumentation and procedures with the objective of overcoming these technical difficulties.

The initial phase of our project has been to develop laparoscopic techniques and equipment (Table 1) for use in rhinoceros and elephants by utilizing individuals that have died of natural causes, or live animals with medical conditions that warrant abdominal surgery. We have had the opportunity to perform laparoscopic surgery on both live and deceased white rhinoceros, black rhinoceros, African and Asian elephants. From our initial studies, it is apparent that laparoscopy in these megavertebrates is possible and may greatly enhance our ability to care for and manage these animals in captive and free-ranging situations. The authors have received some funding and institutional support that allows us to travel to institutions which may benefit from the use of this equipment and/or our experience. We are also interested in those zoological institutions which may find themselves dealing with a terminal case in an elephant or rhinoceros, to please contact us directly and to consider allowing laparoscopy to be conducted on the animal prior to a postmortem examination.

Ultimately, we envision the results of our studies on captive animals to be applicable to the management and conservation of elephants and rhinoceros in the wild. A variety of medical disorders are commonly reported in black and white rhinoceros. Many of these medical conditions are difficult to diagnose, monitor and treat. Furthermore, there is a paucity of information on the incidence of these disease conditions in free-ranging populations. The use of minimally invasive laparoscopic techniques will greatly enhance our diagnostic abilities in this species and would be extremely valuable to the understanding of medical conditions of captive and free-ranging rhinoceros and to conservation efforts overall.

Throughout many parts of Africa, wildlife professionals are seriously concerned about the negative effects large elephant herds are having on the native flora and fauna within parks and reserves.^{2,6,9} The historic rangelands of the elephant have become interrupted by national borders and artificial

barriers. Habitats surrounding wildlife parks are increasingly being converted to agricultural lands. The encroachment of human populations has caused a dramatic increase in the number and severity of human-elephant conflicts.^{2,9} These conflicts are commonplace in many parts of East and Southern Africa. Although there have been a variety of plans to reduce human-elephant and elephant environment impacts, little overall success has been achieved in most countries.⁹

Historically elephant population control has primarily been limited to culling and translocation of small groups.^{2,6} Immunocontraception has been attempted with a small population of elephants but is not currently realistic in many situations.^{4,6} In wildlife parks where large herds of elephants exist there is currently no effective, humane method of population control. One of our long-range goals is to develop laparoscopic techniques, such as ovariectomy and tubal ligation, in free-ranging African elephants that can be used to sterilize reproductive females. Once these techniques have been developed, it is our intention to train local wildlife veterinarians and health professionals to perform laparoscopic sterilization of elephants in the field and thus provide local wildlife officials with a tool to help manage elephant populations. It is our hope this will improve conservation efforts across Africa by reducing human-elephant conflicts and helping to save critical ecosystems.

ACKNOWLEDGMENTS

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Table 1. Current laparoscopy equipment specifically manufactured for use in megavertebrates.^a

Instrument	Description
Rigid telescope	1.24 m × 1.0 cm, forward, oblique, rigid, 30 degree
Trocar Cannula	67 cm × 0.5 cm, pyramidal tip
	64.5 cm × 1.0 cm, double-walled
Trocar Cannula	60 cm × 1.0 cm, pyramidal tip
	56.6 cm × 1.5 cm, double-walled
Cup biopsy forceps	1.0 m × 1.0 cm
Babcock grasping forceps	1.0 m × 1.0 cm
Metzenbaum scissors	1.0 m × 1.0cm
Palpation probe	1.0 m × 1.0 cm, with cm markings
Palpation probe	1.0 m × 0.5 cm, with cm markings

^aAll equipment was specially manufactured by Karl Storz Veterinary Endoscopy of America (KSVEA, Goleta, California USA) and is not commercially available at this time.

A VIDEO-ENABLED, RADIO-CONTROLLED REMOTE TELINJECTION SYSTEM FOR FIELD APPLICATIONS

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Abstract

Though the market now supplies several safe and efficient remote drug delivery systems for immobilizing nondomestic animals in captive and free-ranging situations, the range and accuracy of available products is still limited. These limitations prevent the successful immobilization of some free-ranging wildlife by remote injection, as some animals can be difficult to approach. For example, within the framework of the Przewalski's horse (*Equus przewalskii*) reintroduction to Mongolia (www.takhi.org) we are faced with immobilizing animals in a flat steppe habitat without adequate cover for an approach, and animals typically flee at distances in excess of 300 m. In order to overcome this problem, we developed a remote gun that can be controlled from a distance (Fig. 1), and which therefore has greatly superior functional "range" than the actual range of the gun.

We mount a modified CO₂-powered gun (Daninject JM™, Wildlife Pharmaceuticals, Fort Collins, CO 80524 USA) on a heavy-duty tripod (Gitzo Compact 5 VA™, Gitzo S.A., F-94044, Créteil, France) with a motorized video-head (PH 886™, B&H Photo, NY 10001 USA). We use a bi-directional radio link for remote system control from a blind (average distance 300 m). We fix a video camera (Conrad™, A-4600, Wels, Austria) at the ocular of the gun spotting scope which transmits the picture through a 2.4-GHz radio link to the control station. A receiver decodes the information and a 5.5-inch TFT display enables us to utilize the view field of the spotting scope. Our spotting scope has an integrated laser rangefinder (Swarovski LRS™, Swarovski Optic K.G, A-6067, Absam/T, Austria), which relays information on the distance of the chosen target from the gun. This is important in order to ensure that the selected pressure of the gun corresponds to the target distance. An eight-channel radio remote control is used to: 1) control direction and inclination of the video-head (six channels: right and left with two speeds, up and down with one speed); 2) to trigger the laser distance measurement; and 3) to activate the pressure release mechanism (shot). The entire hardware (monitors, radio receivers and transmitters, switchbox) is mounted in two boxes: one close by the gun (distance up to 50 m) and one at the control station. Both boxes are housed in waterproof cases (Pelicase,® Water Safety Europe, D-13353 Berlin, Germany) and weighing approximately 5 kg, including batteries). The distance between gun and control station depends on the power output of the radio links and the antennas used. Presently we are restricted to a distance of several hundred meters as we are using licensed radio bands available to the public. In the future employing other frequencies can easily extend the control range. A second video camera providing a panoramic overview of the entire situation is installed behind the scope.

These modifications do not impair upon the accuracy of the original gun. Time delay due to image

transmission is not appreciable to the naked eye. Training is initially of utmost importance to enhance eye-hand coordination.

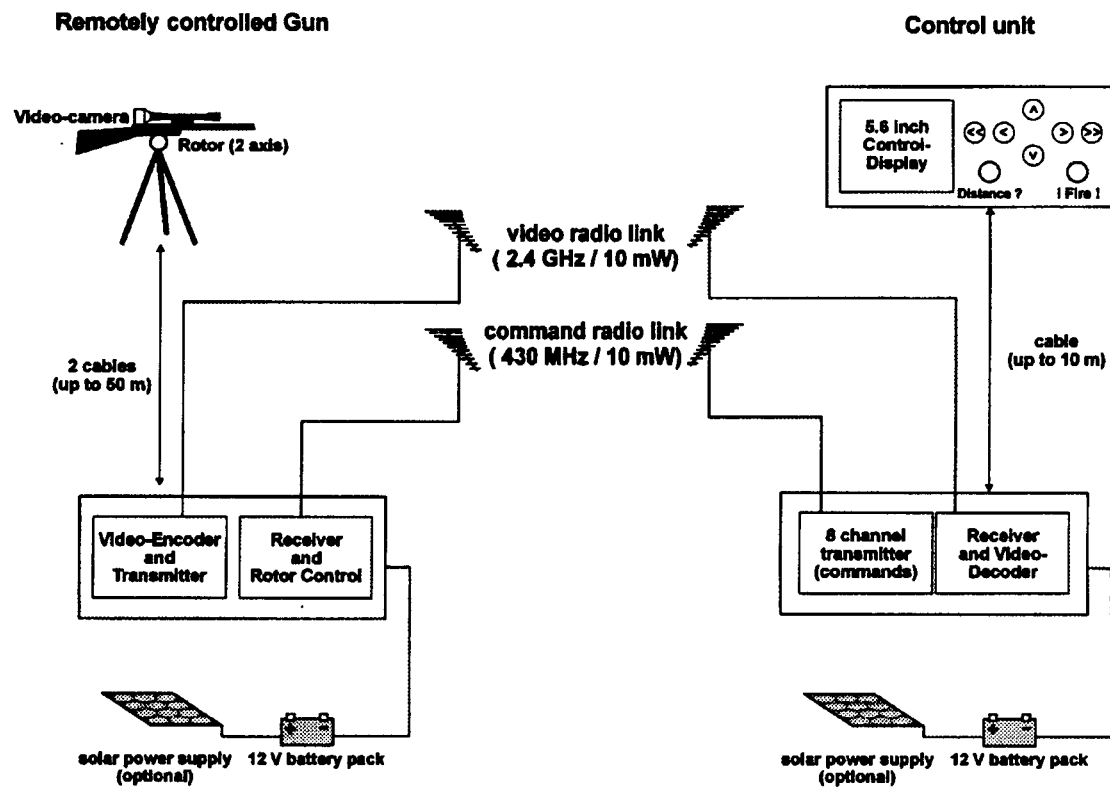


Figure 1. Radio-control setup for the remote gun. The second video link is not shown, for simplicity.

WILDLIFE TELEMETRY FOR A NEW CENTURY: INNOVATION AND FRUSTRATION

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Abstract

Wildlife telemetry came into common use in the latter half of the last century. The factors that allowed for its increased use were the development of solid-state electronics, allowing for miniaturization of components, and the development of smaller, more powerful batteries, particularly lithium batteries. Initially, the focus was on VHF transmitters, but satellite transmitters were first used on wild animals in the early 1970s.

Transmitters have been attached to a wide variety of wild animals, ranging from insects to whales. Currently, the smallest, commercially-available VHF radio transmitter weighs 0.35 g (LB-2N, Holohil Systems Ltd., Ontario, Canada) and has a transmission lifetime of 12 days. The primary use of VHF transmitters remains the determination of the location of the host animal. However, there is an increasing application of telemetry for measurement of physiologic variables, including heart rate, ECG, body temperature, EKG, electromyography, and blood pressure. Telemetric measurement of heart rates has particular application in determining metabolic rates and in the study of human disturbance of free-ranging animals. Besides biologic information, environmental parameters such as temperature and pressure can be measured, and recently it has been suggested that marine mammals could function as mobile oceanographic monitoring platforms.

Satellite telemetry was a technologic forward leap which permitted following animals far beyond the range of human tracking. Oceans, mountain ranges, deserts, ice sheets and rain forest were no longer impediments for animal tracking. Satellite transmitters designed for external mounting now weigh as little as 18-20 g, and can be equipped with solar panel rechargers (PTT-100—Pico PTT™, Microwave Telemetry, Inc., Columbia, MD), giving them a lifetime measured in years. Satellite transmitters designed for implantation into an animal weigh 40 g (PTT-100—Implantable™, Microwave Telemetry, Inc., Columbia, MD), and have a lifetime of slightly more than 1 yr (depending on the duty cycle selected). Several manufacturers offer programming that limits transmissions to periods when the satellites are overhead, preserving battery power. Implantable satellite transmitters typically send a record of the body temperature of the host animal, which can be used to determine survival of the host. Recently, a pressure reading has become available from implanted satellite transmitters. The transmission of additional physiologic parameters must await expansion of the message length allowed for satellite transmitters.

Implantable VHF and satellite transmitters have overcome some of the problems encountered using external attachments. This has been especially true for diving birds, where more than 20 species have been implanted with transmitters having a percutaneous antenna. In addition to allowing for

placing instruments in some birds that will not tolerate an external transmitter, implantable transmitters permit a higher loading factor, with some transmitter-to-body weight ratios reaching 7%.

Transmitters, especially satellite transmitters, have been made smaller in part by using smaller batteries or eliminating one or more of a bank of batteries. Unfortunately, there is almost no ongoing research on battery technology that will permit either a decrease in the size of a battery without reducing its output, or an increase in the duration of output of an existing battery of small size. Likewise, it is doubtful that the spontaneous depletion of lithium batteries that occurs at elevated temperatures (such as those experienced inside birds) can be further slowed or eliminated. In effect, this means that it is very unlikely that satellite transmitters will be decreased in mass much below 20 g, and that decreases in transmitter mass will come at the expense of the duration of battery life.

Recent and planned launches of additional satellites will extend the coverage of the planets. The new satellites have multiple gates which allow for simultaneous detection of more transmitters. Global Positioning System satellites have permitted the development of new generations of positioning instruments accurate to within 30 m of the actual location.

Note: Mention of trade names does not imply government endorsement. Mention of specific model numbers and manufacturers is only done to give examples, and is not meant to be an all-inclusive list of products available.

THE TAIL OF TECHNOLOGY: ZOOLOGICAL MEDICINE

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Abstract

What new technologies hold significant promise for zoo and wildlife medicine? Physical diagnosis, clinical pathology and new interventional strategies will see fairly dramatic changes as new technologies develop over the next several years. In the area of diagnostics, new sensor technology will provide access to bioelectric potentials without the need for skin contact. Such technologies will provide continuous monitoring of electrocardiograms, new methods for assessing fetal health and the ability to monitor post surgical cases with greatly reduced risk to personnel. Thermo physiology and thermal imaging will become a critical element in understanding neonatal physiology, the stress of lactation, and the complex physiology associated with hibernation, torpor and the partition of nutrients. Thermal imaging provides a rapid method for looking at heat production and metabolism following birth and will provide early indications of maternal infant failures. In addition new systems for controlling core body temperature will minimize postsurgical hypothermia and provide smoother recovery from anesthesia. Will there be a time when we can induce a state of stasis with a controlled reduction in metabolic activity that provides an anesthesia like state for capture, immobilization, and surgery or to temporarily reduce stress or conflict? New systems are becoming available for rapid DNA typing which will greatly facilitate paternity testing and aid in genetic management. These technologies will also see applications in the diagnosis of infectious agents as specific gene expression patterns are identified for both innate and acquired immunity. A greater understanding of toll-like receptors will provide insight into methods for enhancing early immune responses and for negating the paradoxical cytokine release seen by a growing number of pathogens. Vaccines will change and become an *ex vivo* process where engineered dendritic cells carry antigen messages to the host without exposure to the pathogen or the antigen. For field work, greater use of GIS and GPS will expand the ability to track animals in their native habitat. Batteries will become smaller and last longer, providing instrumentation that can be adapted for species less than 300 g with tracking capabilities for periods in excess of 1 yr. Communications technology will allow GPS tracking via cell phones and satellite communication systems affording near global coverage of animal activity with information coming to the investigators desk on demand. The last area of progress will be in communication and sensory systems used by animal species adapted to multiple environments and our ability to enhance the range of human sensory capability as anatomy, structural chemistry and neural anatomy are integrated.

BUILDING AN EFFECTIVE HOSPITAL TEAM IN A ZOOLOGICAL INSTITUTION

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Abstract

Building a cohesive and productive group practice in a zoological institution is a worthy goal. Veterinary professionals have considerable education, training, and practical experience with animal management and therefore have significant contributions to make towards developing the best animal care practices for their institutions. With the right character, mutual respect, and cohesiveness, the hospital team, including technical and administrative staff, can become one of the most valued assets to a zoological facility. The purpose of this presentation is to offer examples of specific practices and general principles to consider in developing an effective hospital team in a multi-veterinarian zoo practice. Many of the ideas described in this abstract were born from individuals on our veterinary staff looking to improve the way we do things.

Planning and Team Purpose

- Align the purpose and goals of the department team with those of the organization. Make periodic planning sessions an ongoing priority for the department.
- Identify the critical needs of the organization that the team is uniquely suited to meet. Organize all activities in an effort to fulfill those needs.
- "Service," both clinical and strategic, should be a key element in the purpose of animal health care in a zoo.
- Position the veterinary team such that it can monitor animal care practices and influence the animal welfare standards of the institution.
- Maximize the effectiveness of the existing team, in terms of quality, credibility, and service, in order to strengthen requests for additional labor.

Communications

- A team's effectiveness is directly related to the time and effort given to communication and daily planning.
- Invest time at the beginning and end of each day for planning procedures, follow-up, and other activities. Include veterinary technicians, hospital keepers, and other animal care staff as partners in planning the day.
- Meet each morning with the hospital and animal care staffs to make final plans for the day.
- Together with the hospital staff, observe each hospitalized animal in the morning to briefly update each other on the animals' condition.
- Develop a group decision-making process that strives for consensus but also supports reasonable

independent thinking.

- Develop a common understanding among clinicians for when it is appropriate to make changes to one another's diagnostic and therapeutic plans.
- Resist the temptation to establish case ownership (beyond 1 or 2 days) for the purpose of saving time and reducing the need to communicate. This approach is often counterproductive and divisive.
- Make every effort to communicate with dignity and respect. Insist on professional decorum even in the heat of the battle. Never accept verbally abusive behavior regardless of the justification.
- Hold monthly staff meetings where all team members are encouraged to contribute to the agenda, regardless of how trivial or contentious the issues may seem. Make sure someone is responsible for seeing each issue through to resolution.

Case Management, Tracking, and Follow-up

- Designate a rotating daily "duty veterinarian" who is responsible for general communications, organizing the day, and assigning tasks to the veterinary staff.
- Use a shared calendar to organize all planned hospital and field procedures. Review with animal care staff regularly.
- Plan, prioritize, and then "calendarize" as many of the daily activities as possible. Schedule realistically to avoid frequent cancellations. Develop a reliable system for rescheduling cancelled procedures. Adhering to rigorous calendar planning makes it clear to all parties what can be accomplished in a given day. Further, it discourages spontaneous and trivial requests.
- Produce a master "To do" list each day listing each procedure and identifying who is primarily responsible for each case that day. This can be the responsibility of the duty veterinarian.
- Track all active cases on a master list; keep it current; and share it with animal care staff for their reference and validation. Keeping this list current can be the responsibility of individual clinicians.
- Provide rapid attention to new, urgent, and existing cases. Develop a system of triage and follow-up that provides reasonable attention and response time to the animal care staffs' needs.
- Consider using a transcription service or dictation software to expedite and digitize medical records entries.
- Include medical assessments and plans in the record entries so that other clinicians will understand the reasoning and be better able to implement the strategy.
- Have monthly morbidity and mortality review meetings with the veterinary, pathology, nutrition, and animal care staffs. Maintain a list of action items for follow-up.

Protocols and Procedures

- Use written protocols and procedures to take some of the guess-work out of the job and provide consistency among the veterinary staff. Written protocols are particularly important to have in place for compliance with governmental regulations, accreditation, and high-risk situations or any situation where procedural deviation cannot be accepted. Examples of situations where

written procedures are important include primate handling, quarantine, animal escape, shipping requirements, narcotic handling, etc.

- If protocols are not used regularly, eliminate them. If they are used, even if controversial, continually revise them making use of input from the users.
- Write protocols to help others do their job better, rather than to transfer responsibility.
- Even though protocols are written well and distributed properly, do not assume they will be implemented. Often, those responsible for implementation will need training, encouragement, reminders, and the opportunity to participate in revisions. Ultimately, supervisors must hold their employees accountable for implementation.
- Consider developing a generic procedure checklist form for organizing complex medical procedures and communicating needs to technical staff.

Responsibilities and Delegation

- Avoid a “caste” system of responsibilities where some job positions are regarded as less important than others.
- Enable veterinarians and technicians to use their skills and training to the fullest extent possible. Whenever appropriate, have technicians perform higher-level procedures (as allowed by state law) that free veterinarians to focus on their own higher-level activities. Properly trained veterinary technicians, with a clinician present in the vicinity, can perform unsupervised blood sampling, neonatal examinations, bandage changes, anesthesia induction, procedure scheduling, and many others.
- Strive to schedule an equal or greater number of technicians to veterinarians on a given day for the most effective use of skills and resources. This may take a change in mindset for administrators and veterinarians.
- Rotate routine responsibilities such as daily planning, task assignment, treatments, after-hours call, etc.
- Delegate the responsibility for independent projects to veterinary team members. Assure that each project helps to fulfill the purpose of the team. Highlight to the administration each project and its contributor when significant progress is made.

As the size of veterinary staffs grow in zoological institutions, difficult interpersonal issues can become enormous detractors in accomplishing our goals. Such conflicts undermine the credibility and power of the department within the institution. No group can expect to perform flawlessly and without conflict, but paying attention to and placing a high value on the individual team members can avert many problems. Forward thinking and planning must be balanced with good day-to-day communications and the need to accomplish daily tasks. Individuals must feel valued, challenged, and accountable. In the zoo environment, the ultimate reward for successfully building a cohesive, credible, and productive team will be the enthusiastic desire of the institution to seek veterinary advice and influence in strategic decision-making related to animal care and management.

MOVING OUTSIDE THE CLINICAL ROLE: LEADERSHIP WITHIN THE ZOOLOGICAL COMMUNITY

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Abstract

Zoo veterinarians naturally, and fairly often, step into leadership roles outside of the veterinary clinic. Our colleagues hold a wide variety of job titles that come with job descriptions that are distinctly different from “veterinarian.” These titles include department heads and professors (animal care, veterinary medicine, research and conservation, exotic/nondomestic/special species medicine) as well as assistant directors and directors (of veterinary schools, state or federal organizations, and nonprofit organizations, including zoological parks and aquaria). Clearly, each of these jobs requires a skill set that extends beyond clinical medicine.

Why do zoo veterinarians make the step (or leap) from clinician to administrator or teacher or Chief Executive Officer? What new skills are needed, and how are they acquired?

The first question can be answered quite simply; zoo veterinarians are highly trained problem solvers. They are also leaders who seek challenges. Moving from clinical medicine into an administrative role happens gradually for some, and suddenly for others. But the motivation is similar: to meet new challenges, and solve new problems on a larger scale (the “bigger picture”).

The second question is more complicated. Zoo veterinarians already possess many of the skills needed to be successful leaders outside of the clinic. These include the ability to assess problems (diagnosis), prioritize them (triage), find solutions (treatment) and execute a plan (restore health). Zoo veterinarians are creative and flexible when necessary, but they also demand that certain rules be followed. They are good communicators and good collaborators. They have the ability to draw staff together as a team working toward a common goal, and they rely on a network of colleagues for consultation and advice.

In many ways, zoo directors perform similar functions and require similar skills. They must be able to identify problems and craft solutions. They must ensure the health of the entire organization including plants, buildings, staff, and visitors as well as animals.

But there are also a number of skills that zoo veterinarians lack, that are critical to successful leadership outside the clinic. These include fundraising, marketing, financial planning, and public relations. Gaining such skills requires a combination of on-the-job training, mentoring, and professional training. Short courses and workshops are excellent ways to gain many of these skills. Some of the most useful include media training, building donor relationships, management training,

team building, and executive coaching. Travel to other zoos, participation in professional organizations, and exchange of information are other important ways to build experience and capacity in these areas. In the end, there is always more to learn.

Opportunity at the National Zoo

There are a handful of great zoos in the world and the National Zoo used to be one of them. The terrible state of its physical facilities has made it impossible to classify the zoo as being world class today. Nevertheless, the zoo is known as a leader in zoo-based research, veterinary medicine, and professional training. Its attendance ranks among the top ten for all zoos in the United States, with 2-3 million visitors each year. Its animal collection includes 2,800 specimens, representing 350 species from around the world, many of them threatened or endangered.

In January 2000, the (then new) Smithsonian Secretary established priorities for the future of the Institution as a whole, including the renovation of its oldest facilities. The 100-yr-old National Zoo was placed at the top of the list for physical renewal. As a science unit of the Smithsonian, the Zoo was also charged with defining its areas of scientific expertise, both in Washington and at the Conservation and Research Center in Front Royal. At the same time, the search for a new zoo director was launched. In June 2000, the zoo's head of animal health (the author) was selected for this tremendous, but challenging, position.

To use the veterinary analogy, this position required a full year to assess and prioritize the problems (failing facilities, outdated financial systems, limited financial resources) and to begin to map out potential solutions. In June 2001, we established a new mission statement: to study, celebrate, and protect the diversity of animals and their habitats. We described our vision for the future: to transform the zoo's various animal collections, exhibits, education and research activities into a select group of "animal programs" by integrating each element into a more focused and exciting, zoo experience. And, we mapped out a plan to renew our role as a leading center for zoo animal care and medicine, reproductive science, conservation biology research, and public outreach.

The following year, travel to other zoos and formal training (media and donor relations) was extremely helpful. Solutions to problems were refined, and we began to execute plans (including raising funds both public and private). During the current year, strategic planning (organization-wide) and executive coaching will be important additions as we continue our efforts to renew the Zoo with a focus on science. The future promises a steady flow of problems to solve, new challenges to meet, and new skills to master.

LEADERSHIP THROUGH LIAISONS

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Abstract

Often we look at leadership as the role played by the person who is out in front of the troops, the one leading the “charge” towards a goal or achievement. At times, that is certainly a necessary method of management. However, I also think that a sometimes- overlooked leadership method is building liaisons and connections to others in ways that achieve the goals set for ourselves and for our institutions or organizations. Obviously the key to this strategy is determining mutually beneficial (“win, win”) situations where all gain from the effort.

I will focus on four personal examples in which I believe building liaisons was beneficial in advancing goals of the projects or organizations with which I was working. First is the animal health research network that was developed by the Black Rhinoceros Species Survival Plan and the Rhinoceros Taxon Advisory Group. Second is expanding the sponsorship of the Journal of Zoo and Wildlife Medicine (JZWM) to include the European Association of Zoo and Wildlife Veterinarians (EAZWV), and the support of the American Zoo and Aquarium Association (AZA). Third is a current effort to more closely link the AAZV and the AZA in areas of common interest. The last is the liaison-building exercises that are part of my current position as Director of Conservation at the Saint Louis Zoo.

In summary, although building liaisons can sometimes be perceived as passive activity, one that is less likely to produce results when compared to that of leadership by directive. However, I believe that the accomplishments of successful, combined efforts reflect well on all participants, and most often produce greater results than solitary efforts. In the end, if “credit” is an issue, the improved results also reflect on the leader that coordinated them. Most importantly, successful liaisons allow each of us to leverage our limited resources into larger efforts to achieve our goals.

PHILANTHROPY IN ZOOS AND AQUARIUMS: CONNECTING THE DONOR COMMUNITY WITH WILDLIFE HEALTH PROGRAMS

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Abstract

The important work performed by zoo and aquarium health professionals is fundamental to the responsible care of wild animals. Frequently core funds to support these efforts require supplementation in order to acquire new technology, initiate or maintain innovative programs or build facilities. The donor community serves as essential supporters and valued advisors. They are partners on a team committed to a joint mission. The process of achieving fund raising goals can be long and difficult. A clearly defined vision and mission are essential to gaining donor interest in health activities. Understanding the potential donor base and applying development techniques can assist in positive outcomes. Thereafter a commitment to on-going education and attending to the needs of this special donor community are primary to long-term success.

Introduction

Donors to wildlife programs are mission partners that provide both funding and a connection to the broader community. In an environment where competition for donor attention is often intense, it is important to develop sound methods to secure operating, capital and endowment support. These methods include developing a strategic plan, creating a compelling vision and mission that includes connecting wildlife health with the greater organizational goals, integrating wildlife health with the communities interests and matching donor wishes with programmatic efforts. To institute development plans one must define the type of funding to be sought and define the type of donors one wishes to attract to the mission. Lastly, it is important to apply those tools and development strategies that will enhance the potential for sustainable funding.

Creating a Compelling Mission and a Fundraising Strategy

Prior to approaching the broader external community for support it is essential that there be a strong internal commitment to the importance of wildlife health to the institutional mission. A strategic planning process is the first step in gaining this pledge. The process should be representative of key wildlife health staff and stakeholders while creating a design that maintains small, productive working groups. The stakeholders may include other departments within the institution, trustees, government officials, donors or potential donors that have proven their interest in and understanding of the wildlife health program. The goal of the process should include the delineation of a compelling vision and mission statement as well as clearly articulated objectives and strategies. The

vision and mission should truly reflect the drive and commitment of the program and serve as a sincere expression of the dedication of the health staff. As trite as such processes may sometimes appear, a dynamic and passionate mission can be a very powerful tool to engage both staff and donor partners.

It is also essential that the wildlife health mission be closely aligned with the overall mission of the organization. A divergent direction will serve to create drift within the organization, may undermine institutional support and ultimately confuse the donor community. It is therefore of critical importance that the chief executive officer or director of the institution be engaged in the strategic planning process. Further it is important to provide leadership with the information they will need to understand the value of your work and ultimately express ardent support.

The mission should coincide with the perceived needs of the community that the institution serves. Inviting community leaders from the institutions board, allied professions or collaborating partners into the strategic planning process will help ensure broad support for the goals of wildlife health. The outcomes could include a strong emphasis on education, a commitment to the health of wildlife in the region or a more global vision that reflects well on your constituencies and builds pride among your supporters.

Having defined a strategic plan, one must then create a detailed list of needs that reflect the mission, goals and objectives. This may take the form of a relatively narrow fundraising initiative or in its most ambitious form, a large capital campaign. In either case the undertaking will require extensive efforts in disseminating the plan and educating the donor community. The process of cultivation of potential donors and maintenance of existing donors is one built on a solid foundation of education, making the mission come to life in the form of the good works of wildlife health. Proper visualization of the wildlife health efforts ultimately serve as an extension of the donor's desire to have a positive impact- whether it be on the life of an individual animal or the preservation of wildlife in a broader conservation context. Since they may not have the training to do the work themselves, they want to see that their contributions have that same effect. Keeping the donor informed and continually enhancing their level of understanding and involvement will result in an intimate connection that conveys that they too have a stake in the program's success.

Defining the Types of Funds

A basic knowledge of accounting and development principles are important to directing and managing the donor's philanthropic interests. The head of the wildlife health program is not expected to be either an accountant or a development officer. However, in order to interface with those specialists within the organization, a fundamental understanding is essential. Ultimately the donors want to be close to the action and feel that they are interfacing with the people that get the job done. The health workers are the only ones who can fill this niche. Therefore, the program head must anticipate some development responsibilities within their job description. There are some basic decisions as to types of funds that will be sought.

Restricted or Unrestricted Funds

Unrestricted funds do not stipulate how the money is to be spent. In any nonprofit these monies are most highly sought after since it provides the organization with the discretion to direct the funds as the institution deems best. Unrestricted gifts are typically provided by those with a high level of institutional knowledge and are those that believe strongly in its good works. Restricted funds are gifts that the donor stipulates must be used for specific purposes.

New or Existing Program Support

New initiatives can be thought of almost as a type of venture capital investment. They are often attractive to the more entrepreneurial donors since they are typically very exciting and innovative. However, much like any other venture capital investment they contain a higher level of risk for failure. In general, measurements of success or failure have are becoming increasingly more important in conservation. The outcomes may be measured in lives saved, welfare insured, diagnostics performed, diseases discovered, scientific publications written, etc. In contrast, funding existing programs are in a sense lower risk since the goals and achievements have been historically defined. Securing on-going support for such programs can be a challenge as donors are frequently attracted to the new initiatives. Often it requires greater education of the donor to connect them with the long-term and essential needs of ensuring the health and welfare of wildlife.

Equipment or Facilities

As opposed to program costs that are on going, pieces of diagnostic equipment or the building of facilities are discrete one-time needs. It is helpful to maintain a prioritized list of relatively low cost diagnostic equipment needs that can be quickly referenced should interest be expressed. A gift of \$1,000 to \$5,000 that connects the donor with a tangible purchase can be a great start to a long-term relationship. Capital needs include more expensive equipment as well as construction projects. Such items, especially a building, can provide naming opportunities that will clearly connect the significant donor to your work over time. It may also be an opportunity for the donor to build their legacy if that is of importance to them.

Endowment

These are perhaps the toughest funds to secure. Building endowment is an important long-term financial strategy and is the best source of protection against fluctuations in institutional revenues or broad economic downturns. Typically endowment funds are invested by the organization and provide a 5% return on the principal. A \$1 million dollar gift ensures an annual cash flow of \$50,000 dollars. In the most effectively managed endowments, interest earned in excess of 5% is added to the principal and will over time result in larger annual payouts as a hedge against inflation. It can be difficult to find endowment donors because a large gift does not equate with an immediate commensurate product. It does however ensure a sense of perpetuity that is lacking in the large gift that is spent over a relatively short period of time.

Defining the Types of Donors

It is equally important to understand the type of donor to which funding requests will be directed. Each area has particular characteristics that make the fundraising strategy specific and unique.

Individuals

Whether it is \$10 or \$10 million, philanthropic individuals have choices about where they give. Typically they will want to know that their investment is sound and that there will be a defined return of some measure. Trust is fundamental to this relationship and must be established early on. It is important to determine what enlivens this person and in what way they can effectively partner. To ascertain where they find fulfillment in the mission and then seek to fashion a relationship that meets their needs without drifting from the department's goals. It is most important to establish the connection such that the donors see themselves as enabling the wildlife health staff's best efforts. Typically individuals demand the same level of reporting as that required by foundations or government. Therefore, one is well advised to provide such reports in a manner that is complete, detailed, truthful and customized to the donors needs.

There are numerous ways to identify potential individual donors. These may include local media stories on individual giving, donor databases such as Lexis-Nexis and internet web-search sites such as Prospect Research Online (www1.rpbooks.com). Trustees or established donors may help identify and cultivate potential givers. One may also research donor giving lists in annual reports of other nonprofits, many of which are now available on the internet. Periodicals such as *Forbes Magazine* (Forbes 400 annual listing) and the *Chronicle of Philanthropy*, a monthly publication that provides an annual list of the biggest gifts to nonprofits, are also valuable aids.

Foundations

The traditional foundation is one where the original donor is deceased and there is a paid staff that adheres to a defined mission. A family foundation is one where family members are integral to the decision making process. In both cases the foundation usually seeks to maintain or grow the principal and uses the interest earned to provide grants to worthy recipients in a manner that is stipulated by their mission. The grant range varies considerably but in general the larger the grants, the more information one must provide initially and over time. The program officers are charged with ensuring that the resulting product of the grant reflects the foundations mission and in the case of small family foundations, the wishes of the family. Often with larger gifts, naming opportunities are an attractive incentive for funding. In recent years many foundations have incorporated collaboration requirements into their missions. As such, where possible, finding multiple partners in a larger effort will improve the chances of funding. Research tools for finding foundations that fund wildlife health are Prospect Research Online (www1.rpbooks.com) and the Foundation Center (www.fdncenter.org).

Corporate

Direct corporate donations typically require a more public recognition to satisfy their need to be visible in the community or with their stockholders. Naming opportunities are frequently a higher priority. Other forms of recognition such as media articles, in-house publications or use of images and descriptions for annual reports may also be appealing. Reporting is most similar to that of individual donors and often a connection with a corporate officer may establish a long-term relationship with the company as well as the individual. Corporate foundations typically derive their funds from the company's profits. Their needs are less like those of traditional foundations, being more focused on connecting the gift to corporate marketing priorities.

Gifts-in-kind are another form of corporate giving which can be used to great advantage. Corporate representatives may find the novel application of their technology a compelling reason to donate equipment. Alternatively, there may be an interest in adapting their product to a new market. Often, due to the limited market potential of wildlife health products, this is of lower corporate value. It is important when such offerings are made to seriously consider whether the equipment fulfills the needs of the program. The goal should be to fulfill the programs needs while building a long-term relationship.

Government

Local, State or Federal funding can be an important cornerstone for both programmatic and capital initiatives. These funds may come in the form of budget appropriations or grants. Proper review of this subject is beyond the scope of this paper.

Choosing the Right Strategy

Once one has a well-defined strategic plan, charted a fundraising initiative or capital campaign, identified the types of funds and donors, there are a number of strategies for engaging the potential donor community.

Personal Meeting

Creating an intimate connection between your efforts and the donors interests is the most powerful way to cultivate a philanthropic relationship. This may take the form of a presentation in a location of the donor's choosing or an invitation to visit the hospital and facility grounds. The decision on approach should be driven by the donor's desires, however, whenever possible, it is preferable to host the donor at the facility to give a first hand view of the action.

Prepared Information

Brochures, architects renditions and case statements are valuable tools that serve to connect the potential donor with your efforts and typically work best as an adjunct to a personal visit.

Newsletters, timely reports and/or electronic updates can be important ancillary products that serve to keep the donor community connected and educated about your work. They are not a replacement for personal contact but can enhance the relationship.

Special Events

Lectures, tours, luncheons and dinners can serve to cultivate the potential donor as well as maintain the engaged donor over time. Once a core group of donors has been established it may be helpful to create a forum for interaction such as a wildlife health committee. This may serve as a forum for information dissemination as well as to gain feedback on program direction.

Direct Mail Campaign

An impersonal undertaking where program or initiative information is distributed to a cross-section of the community along with a request for donations. Such a solicitation can serve to connect with many more individuals than can be done with personal meetings but typically will only result in a small percentage of respondents. This can be used as a low effort first step to garner entry-level support. Once such donors have made themselves known, a more personalized follow-up can be initiated.

Defining Success

Building a donor community for wildlife health programs can be a very rewarding process, but not without challenges. It is essential to nurture these positive relationships based on trust in order to fulfill the important mission of wildlife health and the conservation of biodiversity. Establishing a strong foundation of support is a long-term process. Cultivation of donors can take years and it is important to remain on mission and positive about the value of the effort. Maintaining these relationships over time is equally important. Demonstrate that your donors are your mission partners. Keep them informed, engaged and most of all, be certain to express sincere appreciation for their contributions to your shared vision.

ZOO VETERINARIANS AND CURATORS: BUILDING A BRIDGE BETWEEN THE CAMPS

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Abstract

How do we define a successful zoo hospital team? Is it the quality of animal care that is provided? What factors determine the quality of animal care? What makes a productive and enjoyable work environment?

Technical competency is one of the critical factors, which most medical, academic and scientific facilities place highest on their priority list. We often judge ourselves and evaluate our departments strictly on our medical and scientific expertise. While this is critical to the success of any team, it is not the only item that is important for success. Most people are becoming increasingly aware that many other factors besides medical expertise, are critical to providing excellent animal care. Some of these might include:

- Effective communication within the hospital team and with other members of the zoo's animal care staff.
- Developing effective working relationships with other partners in the organization.
- Understanding the different roles each person plays and outlining who are the decision makers under various circumstances.

In 1997, when Disney's Animal Programs was created, more than 200 individuals from over 80 different zoological institutions were hired and all placed in the same institution. Each person brought with him or her different experiences and different ways of doing things. This unique opportunity forced the Animal Programs staff to focus on creating a unified team, which was less encumbered by some of the issues that had faced us at other zoological institutions. Since 1997, we have faced a variety of obstacles and our teams have worked through many growing pains. Our ultimate goal is to build a united Animal Programs team where excellence in animal care is achieved not only through technical expertise but also through great communication and effective working relationships.

In order to help build relationships between the veterinary and curatorial team, we have used several tools and processes. Some of these include:

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- Discussing agendas and biases of each team.
 - Group development of a list of “Norms & Agreements” that we have all committed to follow.
 - Working through role clarification sessions and having open discussions about who are the decision makers in different situations. This not only includes situations when animals may be ill, but also such topics as collection planning and exhibit design.
 - Scheduling routine “face time” with each team so that issues can be discussed in a timely manner.
 - Developing “contact veterinarians” for the various teams within animal care so that curators and managers have a single source “go to” vet for various issues and concerns. The contact veterinarian does not get involved with daily clinical cases but acts as a point of contact for larger issues.
 - Physical proximity of our office working areas – regular, unplanned contact can help build good relationships.
 - Utilize objective, experienced, leadership professionals to help us work through difficult discussions, develop common goals, and identify individual and team roles.

By using the above tools and processes, we have greatly improved our communication and the relationships between the veterinary and curatorial teams. This has not always been easy and requires a true commitment to build and maintain good working relationships. We are fortunate to have an organization that understands that even though problems are inevitable, they must be dealt with effectively and not allowed to fester or be swept under the rug. Resources and expertise need to be available to assist in these challenging processes. Without leadership’s support and objective input, it is doubtful this much progress would have been possible.

An improved working relationship not only allows for a more enjoyable work environment but also translates into improved animal care. We have worked to become better advocates for each other during difficult discussions and we are much better at assuming good intent when questions and concerns arise. Our experience in the problem-solving process helps us deal effectively and in a timely manner with the problems that arise. Catching these problems early and having a format to discuss them prevents the big blow-ups (mole hills are fine, it’s the mountains we hate).

Disney's Animal Programs has more than 400 cast members and working with a large group of people will always bring challenges. It has been our intent to minimize these challenges and to not only focus on the technical portion of our jobs but also address and improve the many other aspects of our roles that will allow us to be a highly effective animal care and conservation facility.

PRELIMINARY REVIEW OF AN EPIDEMIOLOGIC SURVEY OF BLACK RHINOCEROSES IN CAPTIVITY IN THE UNITED STATES

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Abstract

In 2000, a doctoral training program in veterinary epidemiology was established focusing on the health of rhinoceroses in captivity. This program was created through a partnership between the International Rhino Foundation, the American Zoo and Aquarium Association's (AZA) Rhinoceros Advisory Group, and the College of Veterinary Medicine at The Ohio State University. As part of this program, a general survey was designed to gather information on the medical and husbandry status of black rhinoceroses in captivity in the United States.

Review of the medical records of the 242 animals included in the survey found the repeated occurrence of several clinical signs as well as some necropsy findings. These findings included epistaxis (20%), dental calculus (10%), necrosis or sloughing of the tail (5%), loose horn (5%), and unexplained swelling of the legs or shoulders (8%). necropsy findings from 147 necropsy reports included mineralization of major blood vessels, organs or soft tissue (16%), thyroid cysts (12%), hemosiderosis of liver, spleen, bone marrow and intestines (30%), and hepatopathy (18%).

Evaluation of data obtained from the survey and the international studbook for the African black rhinoceros revealed a significant difference between the age of wild-born black rhinoceroses and captive-born black rhinoceroses that have died in captivity in the United States. Controlling for time spent in captivity and neonate mortality still showed that wild-born black rhinoceroses lived significantly longer in captivity than did the captive-born rhinoceroses. This suggests that some characteristic associated with captivity may be a risk factor influencing morbidity and mortality in the black rhinoceros.

AN OVERVIEW OF THE NATIONAL ZOOLOGICAL TUBERCULOSIS MONITORING SYSTEM FOR HOOFSTOCK

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Abstract

Tuberculosis, particularly *Mycobacterium bovis* and *M. tuberculosis*, has always been considered an important health issue in zoological collections. In 1996, upon the diagnosis of active *Mycobacterium tuberculosis* infections in several zoo elephants,^{1,2} the Tuberculosis Committee of the United States Animal Health Association recommended “the USDA ...pursue the formation of an inter-industry working group to address the issues of tuberculosis in exotic animal collections.” Thus, the National Tuberculosis Working Group for Zoo and Wildlife Species was formed, consisting of members representing the zoological, wildlife, regulatory, and diagnostic fields. The mission of this group has been “to control and ultimately eradicate tuberculosis (*M. tuberculosis* complex) and control other mycobacterial diseases in zoo and wildlife species.”

The Working Group has proceeded along two parallel paths to date, one focuses largely on elephants, the other on ungulates. In 1998, the group developed the *Guidelines for the Control of Tuberculosis in Elephants*; these were updated in 2001 to deal with treatment of isoniazid resistant strains of *M. tuberculosis* recently isolated from elephants. In 2000, Ziccardi et al. conducted a survey of 139 AZA affiliated institutions (92.7% of those holding ungulates) to determine the level of tuberculosis diagnosed in several categories (bovids, camelids, cervids, elephants, giraffes, hippopotamus, rhinoceros, ruminants, swine and tapir).³ These data were used to finalize the guidelines for ungulate species in zoo environments.

Currently, the Working Group is finalizing the “Tuberculosis Surveillance Plan for Nondomestic Hoofstock.” The purpose of this surveillance plan is to:

- Establish a standard for intradermal tuberculin testing of hoofstock species (other than cattle, bison and deer) housed in zoological collections;
- Collect prospective data on intradermal tuberculin testing and other diagnostic tests in order to determine sensitivity and specificity; establish guidelines for the interpretation of results and for the course of action in the interim period before the skin test is validated or other methods of diagnosis are identified;
- Accurately estimate the true prevalence and incidence of tuberculosis in zoological collections and assess the risk of transmission to program species;
- Provide guidance for state and zoo veterinarians, curators, keepers and other staff exposed

-
- Prevent the transmission of TB within and between zoological collections.

Currently, Lincoln Park Zoo has developed and piloted a mechanism aimed at collecting the information required to accomplish the goals stated above. The requirements of the Working Group for this system were that it:

- Should be web-based, although it should also be available in paper format where needed;
- Should be extremely user friendly, lending itself to rapid, no hassle data entry;
- Provide assurance of confidentiality for both users and their data – each user will need to register and be given permission to have a user name and password;
- Should integrate with other data/record keeping systems such as the proposed ZIMS record keeping system;
- Provide real-time summaries of data entered – access to this information will be limited to those users entering data and all information will be coded to protect institutional confidentiality;
- Should provide an easy mechanism for sharing data with collaborators approved by the Working Group, while maintaining confidentiality.

Lincoln Park Zoo's Davee Center for Veterinary Epidemiology has created a website to house this surveillance and monitoring system and others like it. In the future, we plan to assess the viability of adding primates and expanding the elephant component of the TB database as well as adding an additional West Nile virus database. We hope that this site will provide a useful tool for the collection of long-term monitoring of many diseases of importance to the captive and free-ranging wildlife communities.

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NAIL ELONGATION AS A MEDICAL TREATMENT OF CHRONIC PODODERMATITIS IN GREATER ONE-HORNED RHINOCEROS (*Rhinoceros unicornis*)

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Abstract

Chronic pododermatitis is a poorly understood but common medical problem in adult captive *Rhinoceros unicornis*. A survey of European zoos found 100% of breeding males and approximately 50% of adult females affected.¹ Chronic pododermatitis most commonly affects the hind feet, and is described as nonhealing fissures and ulcers between the pad and nail, pad overgrowth, pad bruising, and in severe cases, inappropriate overgrowth of the middle nail with separation of that nail from the pad.² All of these conditions can lead to chronic infections and continued maceration of the affected tissue. Possible factors contributing to this condition most importantly include inappropriate substrate and limited access to water or mud wallow.^{1,3} Diet, massive body weight and genetic predisposition are also suspected as contributing factors.^{1,3}

Management of this condition is based mostly on improvements in husbandry, cultures, antimicrobial therapy, and trimming of the affected tissue or nail.¹ According to observations of wild *R. unicornis*, normal foot conformation consists of nails that are long and slope around the pad, allowing most weight bearing on the nails, while the pad becomes hardened and concave in appearance.¹ This natural conformation must be taken into careful consideration when trimming is utilized as a form of medical therapy.

A developing technique in the medical management of chronic foot disease in *R. unicornis* is nail elongation prosthetics. The goal of nail prosthetics is to mimic the naturally elongated nail of wild rhinoceros. When the nails are short, excessive weight bearing, ulcerations, and bruising of the footpad occurs. Nail prosthetics were first explored in Europe. Subsequently, the Los Angeles Zoo has medically managed a 33-yr-old female *R. unicornis* utilizing Equi-thane™ super fast hoof adhesive (Vettec, Oxnard, CA 93033 USA) to build up all nails on both front and back feet. The National Zoo has utilized a similar product and technique on a male *R. unicornis*, adding metal horseshoes to each nail. Both of these cases were successful at decreasing the trauma and continued infections of the pad, allowing the conformation to more closely resemble their wild counterparts. The technique is best utilized in cases where there is only damage to the pad, and separation between the pad and central nail has not yet occurred. This developing technique of nail elongation may be a promising option for managing some forms of chronic pododermatitis in *R. unicornis*. However, long term preventative care, appropriate husbandry, combined with medical care is the cornerstone

to improvements in foot care of *R. unicornis*.

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NOVEL USE OF TWO TRANQUILIZERS, ZUCLOPENTHIXOL AND HALOPERIDOL, TO FACILITATE THE TRANSPORTATION OF TWO OKAPI (*Okapi johnstoni*)

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Abstract

This is the first report of the use of two tranquilizers, zuclopenthixol and haloperidol, in okapi (*Okapi johnstoni*) to facilitate transportation. Two okapi, a male and a female, were transported out of the zoo and back again 3 mo later using this protocol. Normally, these animals require training to enter and remain calm in the transportation crate, such training taking 6-8 wk and loading taking approximately 4 hr despite such training. Due to the foot-and-mouth disease outbreak in the United Kingdom in 2001, the okapi were required to move from the zoo site at short notice, without such training. Each animal was given 100 mg of zuclopenthixol 15 hr before the move then 10-20 mg haloperidol to facilitate loading; both administered intramuscularly by dart. Both animals loaded into the crates calmly and within 20 min. Unloading was equally without incident. The animals remained in a "calm and tame" state for approximately 3 wk following dosing.

GLUCOSURIA IN CAPTIVE OKAPI (*Okapia johnstoni*)

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Abstract

Recent findings suggest that glucosuria is present in a number of clinically normal okapi in captive collections in the United States, Europe,⁷ and Japan. Preliminary urine glucose levels in captive U.S. okapi ($n = 10$) at the White Oak Conservation Center (WOCC) were determined using glucose Chemstrips (Roche Diagnostics Corp., Indianapolis, IN 46256 USA; range: undetectable - 1000 mg/dl) and compared to glucose levels of okapi ($n = 10$), held captive in situ in Epulu, Democratic Republic of the Congo. Captive U.S. okapi had urine glucose levels ranging from undetectable to 1000 mg/dl of glucose, which was in contrast to the in situ okapi, none of which had detectable urine glucose. Additional urine samples on captive U.S. okapi ($n = 10$) were then obtained and glucose concentrations were determined using Chemstrips and quantitatively determined using an automated chemistry analyser and comparing results against urine creatinine levels to minimize the effects of concentrated urine samples. Of the 10 samples, three were negative using both Chemstrip and automated analysis, and the remaining seven okapi were positive using automated analysis (27-868 mg/dl) compared with the appropriate ranges of the Chemstrip. Urine glucose/creatinine ratios on positive samples appear to be elevated, ranging between 1 and 20, (< 1 assumed to be normal). This is comparable to work completed in Belgium where urine glucose/creatinine ratios on a single okapi ranged between 6.32 and 13.56.⁷

Serum was collected from two U.S. captive okapi during annual exams for additional testing of glucose, insulin and fructosamine. Insulin levels of $< 2 \mu\text{IU/ml}$ appear to be normal when compared to insulin levels of cattle (0-5 $\mu\text{IU/ml}$),⁵ however corresponding glucose was normal to high-normal (99-180 mg/dl; normal = 115 ± 21 mg/dl). Serum fructosamine concentrations were comparable (362 and 221 $\mu\text{mol/L}$) to concentrations reported in horses ($257 \pm 60 \mu\text{mol/L}$) and for half-bred zebu calves: (215-232 $\mu\text{mol/L}$).^{2,6}

Diet may play a role in the incidence of glucosuria, as the Epulu okapi are fed only a natural browse diet of over 60 species of plant, whereas both the European and U.S. okapi are fed produce, though less produce is fed in the United States.⁷ Certain specialized feeders, such as the 'concentrate selectors' (e.g., royal antelope, *Neotragus pygmaeus*; moose, *Alces canadensis*) may be able to bypass the rumen with higher quality food, such as fruits, allowing the direct utilization of glucose. This may result in a transient serum glucose increase,^{3,4} which may explain the higher serum glucose concentration. If this is the case, it is possible that the glucose renal threshold (100-140 mg/dl for ruminants) could be reached and excess glucose may be excreted in the urine.¹ Currently, the glucose renal threshold for okapi is unknown. Other possible etiologies, such as renal insufficiency

and renal tubular problems, might be considered. Additional testing including verification of insulin and fructosamine levels, renal glucose threshold and fractional excretion also would prove useful information. It is recommended a urine dipstick test along with quantitative laboratory analysis of urine for glucose and creatinine and electrolyte levels should be carried out to build a database of normals for captive okapi.

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NONINVASIVE MONITORING OF STRESS IN NONPREGNANT AND PREGNANT OKAPI (*Okapia johnstoni*)

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Abstract

Stress has long been recognized as having a significant impact on animal health. An acute stress response is designed to help an organism deal with unexpected abnormalities that upset its homeostasis. Chronic stress, on the other hand, can impair immune function and reproductive cycles, among other things. There is also some question as to whether normal variants in an animal's life cycle, like pregnancy, may also cause a stress response. Scientists have used fecal cortisol levels as a noninvasive measure of stress with success in many species. We used enzyme immunoassay (EIA) technology for fecal cortisol monitoring in six female okapi to determine whether pregnancy could be considered a physiologic stressor that affects the hypothalamic-pituitary-adrenal (HPA) axis and to begin establishing baseline fecal cortisol levels. We found in most cases (four of five pregnant females) no significant difference between pregnant and nonpregnant states. We did find significant differences in baseline cortisol levels between individuals. We also noticed a cortisol spike just prior to parturition that may have fetal origins.

Introduction

Okapi (*Okapia johnstoni*) are secretive, solitary individuals endemic to the rainforests of Zaire (Democratic Republic of the Congo). It is impossible to create a captive habitat for okapi that mimics well the size or diversity of their home ranges. Okapi, therefore, may be particularly susceptible to the stresses of captivity and manipulation. They reproduce with difficulty in captivity at some institutions, particularly urban zoos, while they reproduce quite well at other institutions that have more restricted access to the public. A noninvasive method of monitoring stress in this species is particularly relevant and useful (L. Penfold, unpublished data).

The aim of this study was to establish baseline fecal cortisol concentrations in nonpregnant and pregnant female okapi, and investigate whether pregnancy is considered a stressful event in the okapi.

Scientists and veterinarians have been using fecal corticosteroids as a noninvasive measure of stress in wild and captive exotic¹³ and domestic species for several years. Measuring stress in captive, exotic species can be a valuable aspect to a preventive medicine regime since chronic stress has been shown to contribute to impaired immune system function and, hence, greater incidence or severity of disease.⁹ Monitoring stress may also help us to understand and remedy difficulties found in

captive breeding of some species since stress may disrupt or impair reproductive cycles.⁸

Cortisol is secreted into the blood stream by the adrenal glands in response to ACTH.¹ Cortisol is then, in part, filtered by the liver and secreted with the bile into the ingesta within the intestines. Older methods of evaluating HPA axis function by measuring cortisol levels in the serum or plasma are being supplanted or supplemented by methods of measuring fecal cortisol or its metabolites.¹¹ Both enzyme immunoassay (EIA) and radioimmunoassay (RIA) technologies have been used successfully for fecal hormone monitoring in many domestic and nondomestic species.¹³ These measurements are potentially a much more accurate reflection of the true level of stress an individual experiences since drawing blood for serum or plasma measurements causes stress in and of itself. These assays (including this study) have been validated using ACTH stimulation trials, known stressor trials, and/or demonstrating parallelism between serial dilutions of fecal extracts and the standard curve.

Materials and Methods

Feces (~10 g) were collected from six adult female okapi, three times weekly for 1.5-2 yr and stored frozen (-20°C) until analysis. Fecal samples (0.5 g) were boiled in 10 ml of ethanol for 20 min to extract fecal metabolites^{4,13} and the supernatant recovered and dried completely. Dehydrated residue was re-dissolved in methanol, diluted (1:40) in PBS and assayed in duplicate using a heterologous EIA with a polyclonal anti-cortisol antiserum and a cortisol conjugate (cortisol:horseradish peroxidase). Fecal extracts were diluted (1:40) for assay. Intra-assay coefficients of variation were <10%, and assay sensitivity was 3.9 pg/ml. Hormone concentrations are expressed as mass units of hormone per gram feces. Assays were validated by demonstrating parallelism between serial dilutions of fecal extract and the standard curve and by recovery of known hormone added to the standard (accuracy check). Previous studies validated the use of measuring cortisol in okapi feces by an ACTH challenge (Acthar gel, Rhone-Poulenc Roler Pharmaceuticals Inc, Collegeville, PA).¹²

Mean (SEM) fecal cortisol concentrations were determined for individual females, before and during pregnancy, except for one female (Animal 1) who was not bred during the course of this study. Differences in cortisol concentrations before and during pregnancy, and between females were examined using One-way ANOVA on ranks, to account for data that were not normally distributed, and Dunn's method for all pairwise comparisons. Significance was determined at $P < 0.05$.

Results and Discussion

We found no significant difference in stress levels as measured by fecal cortisol levels between pregnant and nonpregnant stages in 4/5 of the females. However, one female (Animal 3) had significantly higher cortisol concentrations during pregnancy than when nonpregnant (Table 1). Pregnancy-specific rises in plasma cortisol levels have been documented in late pregnancy in the bitch.⁹ In species of artiodactyls the case is not clear as to whether pregnancy is a physiologic stressor affecting the HPA axis. Some studies have shown that there is no significant difference between pregnant and nonpregnant states,^{5,14} whereas other studies have found significant

differences in ACTH or cortisol levels between pregnant and nonpregnant states,² with the higher levels recorded during pregnancy.

Regardless of whether or not pregnancy is considered a physiologic stressor in and of itself in okapi, it may still be useful to monitor cortisol levels during pregnancy. Stressful events that occur during pregnancy may detrimentally affect the fetus. Prenatal exposure to maternal glucocorticoids may negatively affect the fetal liver, causing reduction in birth weight and postnatal alterations in glucose hemostasis and function of the HPA axis.³⁷ Fetal growth, cardiovascular function (particularly hypertension), endocrine status, ability to adapt to stress, and metabolism, in general, can suffer long term effects from chronic, or elevated acute, exposure to maternal corticosteroids.

One female okapi (Animal 4), who has a history of early pregnancy losses, did not have significantly higher fecal corticosteroid concentrations before or during pregnancy compared to the other females (Table 1). Females with significantly higher fecal cortisol than the others were subjectively considered temperamentally more 'stressed' than the other females by the keeper staff.

A distinct (fivefold) rise in cortisol immediately prior to parturition was observed in one of our females (Animal 6, see Fig. 1). It has been shown in other mammalian species that an increase in fetal cortisol is essential for the initiation of parturition.⁶ Thus it is likely that we are detecting the excreted cortisol produced by the fetus, observable as a transient spike immediately before birth. If this is the case, then this is the first time that it has been shown that okapi fetuses provide the signal to stimulate the initiation of parturition. As the fetal release of cortisol is transient in nature, and feces were only collected three times weekly, it is not surprising that we missed detecting this prepartum rise in the other females.

Wildlife species are subject to many sources of stress. The physiologic response to acute stress is a healthy, adaptive response and does not suppress the immune system.¹⁰ When stressors become chronic, however, the body's normal stress adaptations have detrimental effects and can suppress the immune system, cause a decline in growth and atrophy of tissues, and interrupt the normal reproductive cycle.⁸⁻¹⁰ Sources of acute stress in the wild may include such things as predatory attacks or brief conflicts to establish social dominance. Sources of chronic stressors in the wild may include habitat encroachment, disease, pollution, climate extremes, and hierarchal social position. Captive animals have added sources of acute and chronic stress including immobilizations and restraint, transport, introduction of new individuals or separation from familiar individuals, unfamiliar environments or enclosures that fail to mimic crucial aspects of their natural habitat, confined spaces, new diseases, inadequate nutrition, boredom, and the constant presence of the viewing public and staff. Some of these may not have a direct effect on the HPA axis, but may indirectly contribute to the animal's overall stress levels.

Because chronic stress has a detrimental effect on immunity, monitoring fecal cortisol or cortisol metabolites is a potentially valuable component of preventive medicine protocols in a captive environment. Considering the wide range of possible stressors, and the great variability in baseline levels between individuals,⁵ high fecal cortisol levels may be relatively nonspecific, but can still be

useful by alerting the veterinarian that something is causing undue stress to the animals. Many of our zoo animals only pass through our hands during their annual exams due to time constraints and the stress of immobilizations. Fecal monitoring causes no stress to our animals and can help to focus our attention on potential problems before more serious illness ensues.

Measuring fecal cortisol levels does have its disadvantages. Hormone metabolism and excretion differs between species so that different assays and protocols will need to be developed for each species of interest. Variability in data may also occur depending on time and circumstances surrounding collection, methods of storage, and processing protocols. Standards to govern these practices will need to be established and followed.

As workers in this particular aspect of the conservation biology field (zoo medicine), we work with nature's creatures in an unnatural setting: captivity. Workers in our field have done much to try to make the captive environments of our charges more comfortable by making enclosures more like natural habitats, taking strides to reduce stereotypic behaviors, and improving nutrition and medical care. Quantifying stress via fecal cortisol measurement, identifying the stressors, and eliminating them, can bring us even closer to providing a comfortable, healthy environment for our captive animals. This concern becomes more pertinent with animals, like the okapi, that are adapted to secluded and/or large home ranges.

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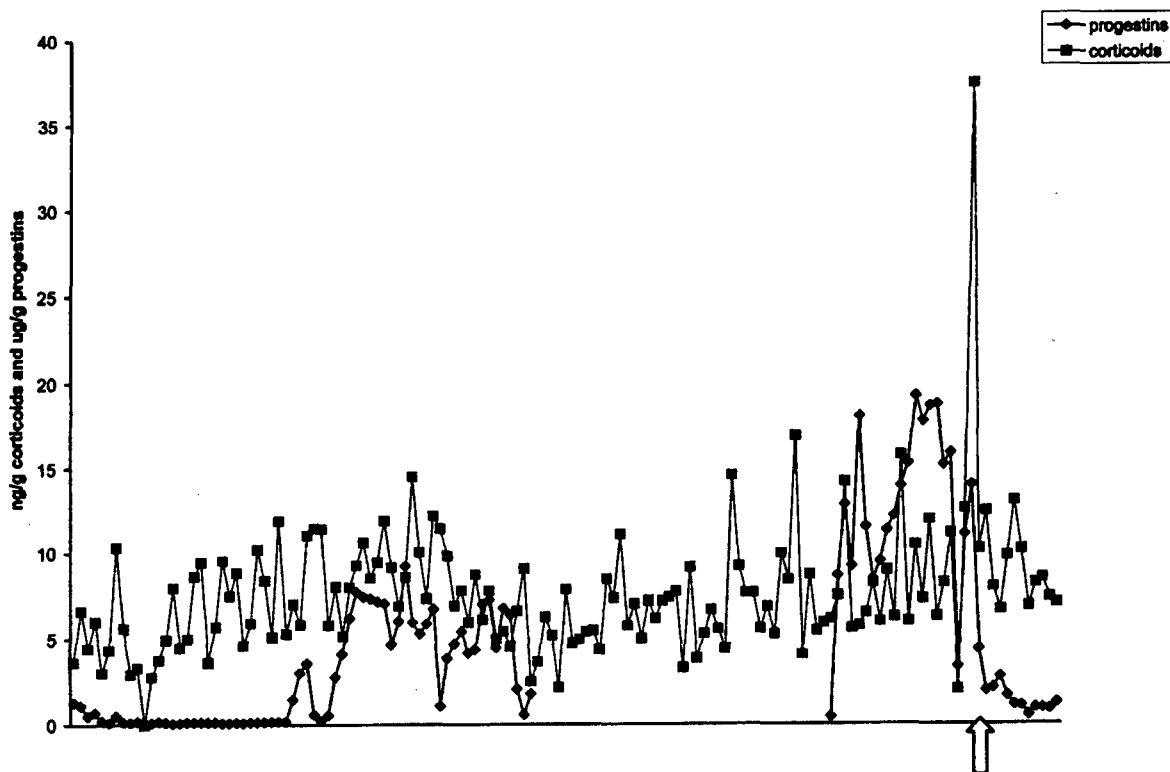


Figure 1. Fecal corticoids and progesterone (ng/g) throughout pregnancy (Animal 6). Arrow denotes parturition.

Table 1. Mean ($\pm$ SEM) fecal corticosteroid concentrations (ng/g) in nonpregnant and pregnant okapi.^a

	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Animal 6
<i>n</i>	157	119/110	57/41	28/133	43/130	33/56
Nonpregnant	23.5 $\pm$ 1.5 ^a	7.2 $\pm$ 9.3 ^b	8.6 $\pm$ 0.5 ^b	9.5 $\pm$ 0.6 ^b	18 $\pm$ 0.7 ^a	7.1 $\pm$ 0.5 ^b
Pregnant	-	9.3 $\pm$ 0.4 ^b	15 $\pm$ 0.8 ^{b,c}	11 $\pm$ 0.3 ^{b,c}	14 $\pm$ 0.5 ^{a,c}	7.9 $\pm$ 0.4 ^b

^aRow and column means followed by different italicized superscripts^{a,b,c} differ $P < 0.05$.

ORAL ENROFLOXACIN AS A PROMISING ANTIBIOTIC THERAPEUTIC OPTION FOR NONDOMESTIC RUMINANTS

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Abstract

Providing antibiotic therapy to nondomestic ruminants is an ongoing challenge for veterinarians due to handling and administration constraints and lack of pharmacokinetic-pharmacodynamic data. Oral treatment offers the opportunity to reduce the stress and risk of trauma associated with physical restraint or remote drug administration. Enrofloxacin (Baytril®, Bayer Corp., Shawnee, MI 66201 USA) is a synthetic fluoroquinolone antimicrobial. This study evaluated single dose pharmacokinetic-pharmacodynamic parameters of enrofloxacin for three different routes (i.v., s.c., and p.o.) following administration to six alpacas (*Lama pacos*) (weight range 63-78 kg) and for two routes (i.v. and p.o.) following administration to four goral (*Nemorrhhaedus goral arnouxi*) (weight range 25-35 kg). Using a cross-over design, each animal received a single dose of 10 mg/kg p.o., 5 mg/kg i.v., and (alpacas only) 5 mg/kg s.c.. Blood samples from the goral were collected prior to dosing and at 4, 8, 12, and 24 hr postadministration. Samples were collected from the alpacas prior to dosing and at 10 min, 20 min, 30 min, 1, 2, 4, 6, 8, 12, 24, 36, and 48 hr postadministration. Plasma samples were analyzed for enrofloxacin and ciprofloxacin using high performance liquid chromatography (HPLC) and UV detection. Pharmacokinetics were determined and include the following mean calculations (C_{MAX} and AUC calculations include enrofloxacin and the active metabolite ciprofloxacin):

Alpacas

Terminal half-life (i.v., s.c., and oral) = 11.2, 8.7, and 16.1 hr, respectively; Systemic availability (s.c., oral) = 90.17% and 29.31%; Peak concentration (C_{MAX}) (s.c., oral) = 3.79 and 1.81 µg/ml; Area under the curve (AUC) (s.c., oral) = 50.05 and 33.97 µg hr/ml.

Goral

Terminal half-life (i.v. and oral) = 13 and 5.2 hr, respectively; Systemic availability (oral) = 14.6%; C_{MAX} (oral) = 3.33 µg/ml; AUC (oral) = 27 µg hr/ml.

Based on the inherent antibiotic properties of enrofloxacin, the pharmacokinetic values generated for the oral dosing in this study, and the minimum inhibitory concentrations (MIC) of many gram-

positive and gram-negative bacterial pathogens common to ruminants, oral administration of enrofloxacin at a dose rate of 10 mg/kg every 24 hr is an appropriate antimicrobial therapy in alpacas and goral. No adverse gastrointestinal effects were noted during the study, following a 10-day 10 mg/kg every 24 hr dose regimen in the alpacas, or over a 1-mo observational period following the study.

DENTAL DISEASE AND SEROUS ATROPHY OF FAT SYNDROME IN CAPTIVE GIRAFFES (*Giraffa camelopardalis*)

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Abstract

A condition characterized by serous atrophy of fat (SAF) has been reported as leading to peracute mortality in a number of giraffes at several institutions during the last 3 decades. These mortalities were originally attributed to insufficient protein and high fiber diets but were also associated with stress, other nutritional imbalances, and infectious and toxic conditions.^{2,4} A recent report attributes similar giraffe morbidity and mortality to hypoglycemia and chronic energy malnutrition mainly in younger giraffes.¹

In four aging giraffes that died with SAF at the Smithsonian National Zoological Park, dental attrition, and tooth and gingival abnormalities were consistent findings. Inflamed gingival pockets containing fibrous plant material were frequently noted on postmortem examination. High-resolution, postmortem dental films of a recent case showed advanced attritional changes, alveolar bone loss including periapical changes, and pocketing compatible with severe periodontal disease. The dental attrition and periodontal lesions are considered as major factors in the SAF syndrome of these giraffes.

Dental attrition and periodontal bone abnormalities of six captive and 15 wild giraffes were evaluated in skulls from the Smithsonian Institution collection. Only intact skulls from adult giraffes were examined. Markedly more advanced tooth attrition and evidence of greater dental disease was present in captive giraffes than in those from the wild (Table 1).

In the wild, the giraffe, a selective browser, preferentially feeds on young shoots of *Acacia* spp. and other trees. Such shoots are high in digestible protein and have a relatively low fiber content.^{3,6} in zoo settings giraffes are frequently maintained on a combination of hay and low fiber ruminant pellets.⁵ The diets offered to captive giraffes may be suboptimal in substance and/or their physical form may lead to long-term adverse effects on teeth. Once oral disease has developed, mastication is compromised and animals may suffer malnutrition even when offered an adequate diet.

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Table 1. Dental attrition in wild and captive giraffes.

	None	Mild	Moderate	Severe
Wild (<i>n</i> = 15)	7	7	0	1
Captive (<i>n</i> = 6)	0	0	3	3

CRYOPRESERVATION OF SPERMATOZOA FROM ASIAN ELEPHANT (*Elephas maximus*) USING DIRECTIONAL FREEZING

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Abstract

Male infertility and absence of males in a facility are contributing factors to the limited reproduction of Asian elephants in captivity. Subsequent transport for breeding purposes increase social stress, risks of disease transmission and management costs. Recent success in artificial insemination eliminated these obstacles only transporting the semen. However, the transport of fresh semen involves logistic difficulties: access to semen donors, consistent semen quality and preservation of the spermatozoa during transport. The use of cryo-preserved sperm for artificial insemination (AI) can partially overcome these problems and can additionally be used for the establishment of genome resource banks. However, to date, attempts to cryo-preserve Asian elephant spermatozoa have failed due to its sensitivity to freezing. Aims of this study were to identify the temperature range during which spermatozoa are most sensitive to chilling injury, and to use directional freezing (DF) to reduce cell damage during the freezing process. Semen was collected from two Asian elephants by manual stimulation and DF was used for freezing sperm samples. In contrast to conventional freezing methods DF facilitated a fast cooling rate, controlled ice crystal formation and cryopreservation of large volumes. Samples extended with a variety of DMSO extenders showed post thaw motility of 30-40%. DF was able to cryopreserve Asian elephant spermatozoa for the first time. As DF seems to reduce cryo-injury it may become of interest to optimize existing cryopreservation protocols of other endangered species, or to make cryopreservation even possible in species with cryo-sensitive spermatozoa.

THE NATIONAL SURVEILLANCE SYSTEM FOR WEST NILE VIRUS IN ZOOLOGICAL INSTITUTIONS

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Abstract

In June, 2001, the Lincoln Park Zoo in Chicago co-hosted a meeting with the Centers for Disease Control and Prevention (CDC) that brought zoo professionals together with human and veterinary public health experts from local, state and federal agencies in order to discuss the design and implementation of a nation-wide surveillance system for WNV in zoos accredited by the American Zoo and Aquarium Association (AZA). The result of this meeting was a set of guidelines entitled *Surveillance for West Nile Virus in Zoological Institutions* and the implementation of a 1-yr pilot study. Objectives of the system are paradigm-based and twofold. Zoo veterinarians need access to affordable, reliable diagnostic testing in order to assess and protect the health of collection animals that often include endangered or threatened species. Public health officials recognize that zoos are ideal sentinels for monitoring the spread of zoonotic infectious diseases and that reliable data gathered from this system could be a useful addition to national surveillance.

The reporting structure for this system is unique. Participating institutions submit samples to the Cornell Animal Health Diagnostic Laboratory. To preserve confidentiality, test results are reported directly to the submitting institution and to the centralized zoo database at the Lincoln Park Zoo Davee Center of Veterinary Epidemiology. Submitting institutions are required to share results, both positives and negatives, with local public health officials, public health officials code zoo data for confidentiality and enter it as local data. Summary statistics are compiled and reported from the central zoo database at Lincoln Park Zoo.

The objective of phase I was to detect geographic spread of the virus. In phase I, we solicited samples from suspect animals (neurologic, systemically ill or undiagnosed problem in animals exposed to mosquitoes) in institutions along the front edge of the spread of disease in the United States. The objective of phase II was to detect positive animals in zoological institutions that would predate the first positive in the immediate area. Over the winter months of 2002, a retrospective serosurvey of institutions in the endemic area of the Eastern United States was performed. The objective of phase III involves redirecting the system from surveillance, the detection of cases in previously negative areas, to monitoring trends in captive wildlife now that WNV is endemic throughout most of the United States.

As of December, 2002, 120 of the institutions accredited by the American Zoo and Aquarium Association were full participants in the system. Participants were distributed over 41 states and the District of Colombia. Over 5,200 animals were tested at the Cornell University Veterinary Diagnostic Laboratory; of these, 77% were avian, 3% were equine, 19% were other mammals and 1% were reptile/amphibians. Over 754 different species were tested, and 11.5% of all animals tested were free-range wild animals (90% avian) found on zoo grounds. Animal histories were coded into six categories (serosurvey, suspect-live, found dead, other clinical signs, no information, vaccine titer check). Serosurvey (45%), and live or dead clinical suspects (25%) accounted for the majority of samples. A poorly designed submission form probably accounted for a high percentage of samples without histories (25%), and an increase in extra-label vaccine use in avian species accounted for 5% of the samples submitted.

Preliminary results as of December, 2002 included 152 virus positives confirmed by RT-PCR and virus isolation. Seventy-four (49%) positives were wild animals (mostly crows) found on zoo grounds from seven institutions. Of the 78 collection animals from 14 institutions, 74 (95%) were avian representing 32 spp., and three were mammals (one gray squirrel, one addax, one alpaca). One reptile (crocodile monitor) was positive representing the first WNV confirmed positive, clinically ill reptile with neurologic signs at that time. No zoo animal case was the first found in a nonendemic area (objective I) and no positive animal from a zoo predated confirmed viral activity in any endemic area (objective II). We are currently redesigning the system to accommodate objective III.

In the September 2000 GAO report, "West Nile Virus Outbreak – Lessons for Public Health Preparedness" (GAO/HEHS-00-180), investigators concluded that the WNV events illustrated "the value of communication between public and animal health communities, the latter including those dealing with domestic animals, wildlife, and other animals such as zoo animals." However, they noted that although zoo animals had served as important sentinels for WNV, zoos were, by and large, "left out of the animal and public health paradigm." This project was launched in an effort to bridge the gap between the zoo and public health communities and enhance national surveillance for WNV.

This program has: created/strengthened working relationships between zoos and local/state health officials for the detection/reporting of a zoonotic disease threat; provided data to the public health system by tapping into the strengths of the zoo community (veterinary expertise; routine pathology programs; medical records; serum/tissue banks; commitment to active disease surveillance year round); and provided a framework that may be applied to other biologic threats of concern.

DOXYCYCLINE IN DRINKING WATER FOR TREATMENT OF *Chlamydophila psittaci* IN FRUIT DOVES

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Abstract

Chlamydophila psittaci (formerly *Chlamydia psittaci*) infections have been described in a large number of avian species, but the disease is most commonly associated with psittacines, turkeys and pigeons.^{3,8} Clinical signs are variable and nonspecific, but a large number of birds show no clinical signs while actively shedding the organism.¹ These subclinical carriers may serve as reservoirs of the disease in aviary flock situations. Management of the disease has been complicated by the zoonotic nature of the organism, the inherent difficulties in its diagnosis, and the lack of practical treatment modalities.

Tetracyclines are effective antibiotics in treating the obligate intracellular bacterium *C. psittaci*, and are the treatment recommended by animal and public health regulatory agencies.² Doxycycline has greater tissue penetration when compared to other tetracyclines and absorption in mammals is less affected by food ingestion.⁷ Being a bacteriostatic antibiotic, therapy is most efficacious during active bacterial replication, and specific treatment for *C. psittaci* should last 30-45 days to address the possibility of intracellular dormancy in host cells.^{3,5} However, daily handling of birds for purposes of medicating is not ideal for delicate species, or in large collections where the added labor of treating individual birds is impractical or cost prohibitive.

The purpose of this study was to establish a group treatment protocol that produced therapeutic levels of doxycycline for *C. psittaci* infections. Similar studies done in selected psittacine birds have considered plasma doxycycline concentrations greater than 1 µg/ml as adequate for inhibiting *Chlamydophila* replication.^{4,6} During routine screening of Columbiformes at the Saint Louis Zoo, we identified *C. psittaci* in cloacal swabs of 60% of a small group of fruit doves. Since these birds were housed in areas shared with other fruit doves and the disease is difficult and costly to diagnose, we decided to treat all potentially exposed birds. Treatment of selected individuals was monitored for this study.

Eighteen doves of four species (black-naped fruit doves, beautiful fruit doves, Jambu fruit doves and ring-necked doves) were offered a 500 mg/L solution of doxycycline hyclate as the only source of drinking water ad lib for 45 days. Blood was collected from jugular or basilic veins six times during treatment to measure plasma doxycycline concentrations and complete blood counts. Weight was

monitored weekly in all birds, and birds were monitored for clinical signs of doxycycline toxicity. Birds were retested for *C. psittaci* upon completion of therapy.

During the course of the study, birds experienced no significant changes in weight or body condition. No significant differences were seen in blood counts monitored throughout the study. Preliminary results show that plasma concentrations were more erratic than those measured in similarly dosed psittacine birds.^{4,6} Some birds failed to maintain doxycycline concentrations above 1 µg/ml for the entire treatment period, but the majority of birds reached or exceeded these levels at some point during treatment. However, since few studies have been done to establish blood concentrations that inhibit replication of *C. psittaci*, it is possible that many of the birds below 1 µg/ml were reaching therapeutic levels. PCR swabs collected upon completion of therapy showed no evidence of *C. psittaci* in the collection.

This study provides preliminary evidence that doxycycline administered in drinking water ad lib results in therapeutic plasma levels for some species of fruit doves without adverse effects at concentrations of 500 mg/L. For birds where therapeutic levels were not achieved, the effects of calcium content in the diet, water consumption and other factors need to be explored to maximize the efficacy of this protocol in aviary situations. Although doxycycline plasma levels varied significantly between individual birds, this treatment protocol has significant potential for the management of *C. psittaci* in aviaries and zoological institutions, and has application towards the conservation of rare or endangered fruit doves.

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ENDOSCOPIC TECHNIQUES FOR THE COLLECTION OF LYMPHOID TISSUE FROM PIGEONS: EVALUATION OF SPLENIC AND BURSA OF FABRICUS BIOPSY TECHNIQUE AND HISTOPATHOLOGIC QUALITY

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Abstract

As part of a large ongoing investigation into pigeon circovirus, endoscopic techniques to biopsy lymphoid tissue from 71 juvenile and adult white Carneaux pigeons (*Columba livia*) were investigated under an Institutional Animal Care and Use Committee (IACUC) license at the College of Veterinary Medicine, University of Georgia. Each pigeon was premedicated using butorphanol. Anesthesia was induced by face mask using isoflurane and oxygen. Each bird was intubated and maintained on oxygen and isoflurane adjusted to individual patient requirements. Cloacoscopy was performed with the pigeon in dorsal recumbency using a 2.7 mm telescope housed within a 14.5-Fr sheath. Sterile saline warmed to 100 °F was used for irrigation. The dorsal wall of the urodeum just caudal to the urodeal fold was closely examined for the opening of the bursa of fabricus. If located, 5-Fr biopsy forceps were introduced and one or two biopsies were collected from within the bursa. Then each pigeon was placed into right lateral recumbency. The left flank was aseptically prepared, and a standard approach was made to the coelom behind the last rib. The telescope, sheath and instruments were cold sterilized using 2% glutaraldehyde solution between every procedure. From within the left abdominal air sac, the spleen was located just caudal to the isthmus of the proventriculus and ventriculus. On occasion, it proved necessary to incise the abdominal air sac membrane using 3-Fr scissors to gain clear access to the spleen. Biopsy forceps (5-Fr) were used to harvest one or two biopsies from the spleen. Single biopsies were submitted for circovirus DNA detection, and where two biopsies were collected the second was submitted for histopathologic examination. All birds except one recovered uneventfully from the procedures. One bird died immediately after the endoscopic procedure due to perforation of the bursa and flooding of the air sac system as a consequence of bursal biopsy. A second bird died 10 days later from severe coelomitis. Post endoscopy infection cannot be ruled out as none of the birds received postoperative antibiotics. Of 62 birds examined by cloacoscopy, it was only possible to visualize and biopsy the bursa of 28 individuals (45.2%). Of these 28 birds, 10 were submitted for histopathology (the remainder were submitted for circovirus DNA detection), and only three possessed lymphoid tissue of diagnostic quality (4.8%). Splenic biopsies were successfully obtained from 61 of 71 birds (85.9%). All 10 cases in which the spleen could not be visualized involved female birds exhibiting extensive reproductive activity. Histologically, splenic biopsies were judged as good to excellent

for cell preservation and tissue orientation. In conclusion, endoscopic splenic biopsy appears to be a safe and rewarding diagnostic procedure in pigeons. However, per cloacal endoscopic biopsy of the bursa of Fabricius is problematic and not recommended in the pigeon due to the lack of lymphoid tissue obtained and the potential risks of cloacal perforation.

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VETERINARY CARE OF SHOEBILLS (*Balaeniceps rex*) IN EUROPEAN ZOOS WITH SPECIAL EMPHASIS ON THE ZURICH ZOO

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Abstract

Information on the veterinary management of shoebills (*Balaeniceps rex*) is scarce. This is regrettable since this species has never successfully been bred in captivity and zoos depend on imports from the wild. This certainly is a sign that their captive management is not yet satisfactory. This presentation is intended to improve the available data on shoebills by summarizing physiologic data measured in clinically healthy captive shoebills and by presenting successfully used medications, thus hopefully making a contribution to a improved captive management.

The International Species Information System (ISIS) lists 18 animals that are kept in the United States of America (Cincinnati Zoo and San Diego Wild Animal Park), Japan (Ueno Zoological Gardens), and Europe (Frankfurt Zoo, Wuppertal Zoo, and Zurich Zoo).¹ A single record of egg production outside its natural habitat exists from 1994 in the Stuttgart Zoo.

Data presented originates from European Zoos that keep a total of four male and two female shoebills and of the Stuttgart Zoo, which at this time does not keep shoebills. Due to the fact that the only record of egg production originates from the Stuttgart Zoo and the important number of animals previously kept there, information from that institution has been included in this abstract.

The birds currently living in European zoos are between 4-23 yr of age and they weigh between 5-6.5 kg. All shoebills are apparently clinically healthy. Blood was repeatedly taken from a pair of shoebills at the Zurich Zoo and values have been summarized in Table 1. Regarding hematology it was noted that the white blood cell count including basophile count was high compared to other storks and that lymphocytes were comparatively low.^{2,3} Further blood analyses will be needed to ascertain this observation and allow for interpretation. Elevated uric acid values with large standard deviation as found in the studied shoebills are consistent with carnivory.

Regarding blood values it was recently published, that the shoebill, which phylogenetically still is an enigma, was found to contain as its dominant biliary bile acid 16-alpha-hydroxychenodeoxycholic acid, a heretofore undescribed bile acid. A search for this novel bile acid in other Ciconiiformes showed that it constituted > 92% of biliary bile acids in five of nine herons in the Ardeidae, but was absent in all other families (Ciconiidae, Threskiornithidae, Scopidae, Phoenicopteridae). The presence of this biochemical trait in the shoebill and certain herons suggests

that these birds are closely related.⁴ Two animals at the Zurich Zoo underwent surgical sexing under isoflurane anesthesia without apparent problems.

Veterinary problems in the study population are mainly related to the legs with mild to severe pododermatitis and hyperkeratosis. From observations in the study population it appears that offering soft flooring is the best prophylaxis and treatment. At the Zurich Zoo two animals developed hyperkeratosis on the tibiotarsus and tarsometatarsus. The problem resolved within 2 mo under weekly treatment with an ointment that contained salicylic acid. In all institutions superficial trauma occurred as a result of interspecific aggression. Dressing of the wounds was the method of choice and was always successful. As with other birds shoebills are very susceptible to aspergillosis. Furthermore four cases of arteriosclerosis have been described.⁵ Medications which repeatedly have been successfully used in shoebills in European zoos are listed in Table 2.

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Table 1. Average blood values of a pair of adult shoebills (*Balaeniceps rex*) kept at the Zurich Zoo.

Parameters	Units	Mean	SD	n ^a
Erythrocytes	× 10 ¹² /L	1.7	0.28	8
White blood cells	× 10 ⁹ /L	12	7.22	7
PCV	%	38.1	4.81	7
Hemoglobin	g/dl	10.4	2.09	7
Heterophils	% of WBC	66.8	13.81	7
Lymphocytes	% of WBC	17.9	12.18	7
Monocytes	% of WBC	4.9	2.78	7
Eosinophils	% of WBC	2	3.46	7
Basophiles	% of WBC	8.4	4.16	7
Glucose	mol/L	13.5	2.76	4
Total protein	g/L	43.3	10.63	4
Uric acid	μmol/L	1659.7	3244.69	6
Calcium	mol/L	2.5	0.26	6
Inorganic Phosphate	mol/L	1.6	0.54	6
GOT	U/L	318.3	183.33	6

^aNumber of samples.

Table 2. Medications used successfully in shoebills (*Balaeniceps rex*) in European zoos.

Medication	Dose / Frequency / Duration	Route
Neomycin	115 mg/animal s.i.d. 7 days	p.o.
Doxycycline	500 mg/animal s.i.d. 7 days	s.c.
Enrofloxacin	50 mg/animal s.i.d. 7 days	i.m.
Fenbendazole	25 mg/animal s.i.d. 4 days	p.o.
Ketokonazole	100 mg/animal s.i.d. 30 days	p.o.

SERUM LEVELS OF IONIZED CALCIUM, VITAMIN D₃, AND PARATHYROID HORMONE OF CAPTIVE THICK-BILLED PARROTS (*Rhynchopsitta pachyrhyncha*)

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Abstract

Serum collected from 68 thick-billed parrots (TBP, *Rhynchopsitta pachyrhyncha*) was analyzed for ionized calcium (iCa), total calcium (tCa), phosphorus (P), total protein (TP), albumin (Alb), parathyroid hormone (PTH), and vitamin D₃ (25[OH]D₃). Ranges established were: iCa (0.82-1.3 mmol/L), tCa (5.5-8.4 mg/dl), P (1.08-5.43 mg/dl), TP (2.1-3.9 g/dl), Alb (0.9-1.3 g/dl), PTH (0-65.68 pmol/L), 25[OH]D₃ (5.2-51 nmol/L). Sixty percent (+/-7.5%) of total calcium was ionized. Female TBP had significantly higher mean iCa (1.11 mmol/L), compared to males (1.05 mmol/L). Total calcium and iCa values in TBP were lower than reported reference values for other avian species. A significant positive linear relationship existed between Alb/TP and iCa/tCa ratios. There was also a significant inverse linear relationship between the tCa/Phos ratio and PTH. These findings are consistent with known domestic avian calcium physiology, and provide a base of reference for clinical calcium physiology in psittacines. This study also establishes reference values for iCa, tCa, P, TP, Alb, PTH, and 25[OH]D₃ in captive thick-billed parrots.

OCULAR ABNORMALITIES IN THE OKARITO BROWN KIWI (*Apteryx mantelli* "okarito")

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Abstract

The Okarito brown kiwi (*Apteryx mantelli* "okarito") is a unique population (subspecies) of kiwi limited to the Okarito forest, southern Westland, South Island, New Zealand. A retrospective review of field notes over a 9-yr period, from 10 March 1994 through 8 May 2002, identified 58 kiwi with ocular lesions in one or both eyes out of a monitored population of approximately 160 birds. The nature of ocular abnormalities in this subspecies of kiwi was characterized by clinical ophthalmic examination with refraction of 11 free ranging and four captive Okarito brown kiwi from 17 June through 21 June 2002. All adults were of unknown age though minimal ages of 4-20 yr were known. Juvenile birds were considered to be less than 4 yr of age. All (four) juveniles and three adults had normal eye exams. Ocular abnormalities (n = eyes affected) included: buphthalmia (1), phthisis bulbi (2), corneal edema (4), corneal vascularization (2) nuclear sclerosis (8), cataracts (11), subluxated and luxated cataractous lenses (3) and vitreal opacity (1). Three birds in good physical condition had chronic ocular lesions associated with severe visual dysfunction. A high frequency of ocular lesions exists in this discreet population of kiwi. The nature and frequency of ocular lesions are suggestive of this population being aged. This is also compatible with a population model with a high incidence of chick and juvenile mortality associated with predation by introduced mammals. Studies have been initiated to investigate the impact of visual dysfunction on breeding success.

FATAL DISSEMINATED YEAST INFECTION IN ORINOCO GEESE (*Neochen jubata*) AND BROWN PELICANS (*Pelecanus fuscus*)

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Abstract

This report describes systemic yeast infection occurring in three captive Orinoco geese and two captive brown pelicans from the San Francisco Zoo during 1996 and 1999. Case 1 was a 2-yr-old female captive bred Orinoco goose (295001), and had a history of recurrent lameness of 4 mo duration. The bird was hospitalized during a 5-day period of lameness in September, 1996. The goose received supportive care but died. Case 2 was a 2-yr-old male Orinoco goose (295004) that had a history of recurrent lameness of 4 mo duration. The bird had been hospitalized during a 7-day period of lameness in September, 1996. The goose received supportive care but died. Cases 3 (298079/99-0010) and 4 (298045) were adult female brown pelicans that were found dead in a lake on the zoo grounds in January, 1999. Case 5 was a male Orinoco goose (298064/1999-0086) that was found dead on the zoo grounds in 1999.

Histologically in all birds, intracytoplasmic organisms were detected in the endothelial cells or luminal macrophages of veins, venules and capillaries of the lung with associated mild to moderate perivascular lymphoid cuffs and interstitial lymphohistiocytic pneumonia. The organisms were also detected in endothelial cells of vessels in peripheral nerves of the leg in cases 1 and 2, and occasionally in low numbers in other tissues. The organisms in the endothelial cells were ovoid and approximately 1-3 μm in greatest dimension. The organisms stained positive with PAS and GMS, and did not react with Brown and Brenn or Fite's acid fast stains. Electron microscopy revealed that the organisms were $1.10 \times 2.04 \mu\text{m}$, had a single central to slightly eccentric nucleus, trilaminar cell wall, thin outer capsule, and replicated by budding from the apical or apico-lateral regions of the cell wall. The light microscopic, ultrastructural, and tinctorial properties of the organisms were features consistent with yeasts. Despite extensive culturing for fungal organisms from tissues of case 3, no significant fungal growth was obtained.

Disseminated yeast infection has been documented in birds, primarily those belonging to the order Anseriformes, particularly from Northwest North America.¹⁻³ The identity of these organisms is based on light and electron microscopic morphology and tinctorial properties. The etiologic agent or agents of this condition have not been named or further characterized because culture attempts have been unsuccessful. The organisms typically infect endothelial cells and macrophages and death

is attributed to associated inflammation and vascular damage.

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SURVEY RESULTS FOR WEST NILE AND ST. LOUIS ENCEPHALITIS VIRUSES AT LION COUNTRY SAFARI

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Abstract

Overview

The Florida Department of Health (DOH) and the Florida Division of Animal Industry (DAI) documented the presence of West Nile virus (WNV) in 56 Florida counties during 2002.² The DOH tested 3,370 birds that year.¹ Positive WNV samples were obtained from 450 dead birds in 47 counties.² Positive WNV samples were obtained from 499 ill horses in 44 counties.² In addition, 1058 sentinel chickens in 29 counties were WNV seropositive.² Six other animals in three counties also had positive WNV samples.² Statewide, 28 human cases of West Nile meningoencephalitis with two fatalities occurred, including a transfusion recipient and a transplant recipient.² Seven human cases of West Nile fever, a milder form of the disease, were also reported including illness in a transplant recipient.²

In support of nationwide multiple-institution efforts to understand the prevalence and pathogenicity of West Nile virus (WNV), biologic sampling of collection animals and wildlife is an ongoing project at Lion Country Safari (LCS), Loxahatchee, Florida. This zoological park exhibits nearly 1000 animals. Eighty-two percent of LCS collection animals are mammals including over 700 hoofstock. The park also exhibits 56 primates including 33 chimpanzees (*Pan troglodytes*), plus 15 African lions (*Panthera leo*), and 4 African elephants (*Loxodonta africana*). Thirteen percent of LCS collection animals are birds including 44 psittacines, 30 Ciconiiformes, and some 20 ratites. Five percent of LCS collection animals are reptiles including 10 American alligators (*Alligator mississippiensis*), 4 Aldabra tortoises (*Geochelone gigantea*), plus other chelonians, iguanas, and snakes.

At the time of this writing, over 10% of collection animals were sampled for WNV and St. Louis encephalitis virus (SLE) since September 2002. The vast majority of sampling involved serologic tests performed at Cornell University Diagnostic Laboratory. Polymerase chain reaction (PCR) testing of tissues from expired collection animals and wildlife found dead on LCS property were also performed at Cornell. Thirty percent of LCS animals sampled were seropositive for WNV. Seropositive animals were asymptomatic with the exception of a chimpanzee that presented for hemiparesis associated with cerebrovascular thrombosis and severe myocardial disease. No positive WNV samples were obtained from deceased collection animals or wildlife from LCS property.

Wildlife has been tested at LCS since year 2001.

Avian Results

East African crowned cranes (*Balearica gibbericeps*) and Marabou storks (*Leptoptilos crumeniferus*) have some of the highest WNV titers of any LCS animal to date. The WNV titers from two cranes were 1:320 and >1:640. Both of these cranes had negative SLE titers. Two marabou storks had WNV titers of 1:160 and >1:640 and SLE titers in these storks were 1:20 and negative, respectively. Such results are strong evidence that WNV has been present at LCS. East African crowned cranes and Marabou storks at LCS have mounted significant antibody response to WNV without overt signs of illness. These birds are exhibited on a completely open outdoor island during the day and remain there at night.

To date, LCS psittacines tested have not shown serologic evidence of WNV exposure. 18 lorries (*Trichoglossus* sp.) tested negative. These birds are housed outside during the day and indoor at night. Macaws (*Ara* sp.) and Amazon parrots (*Amazona* sp.) tested negative. These parrots are exhibited in a completely open outdoor exhibit during the day and screened welded wire mesh outdoor pens at night.

Since flamingoes (*Phoenicopterus* sp.) have been species of concern regarding sensitivity to WNV, it is of interest to note that two untested Chilean and five untested Caribbean flamingoes have not shown signs of illness throughout 2002. These birds are housed day and night in an outdoor netted aviary located within some 200 yards of the East African crowned cranes and Marabou storks.

Reptile Results

A geriatric iguana (*Iguana iguana*) ill with chronic metastatic mineralization and a juvenile alligator tested negative for WNV. An outbreak of WNV among farmed American alligators at a central Florida location was diagnosed in late 2002.³ Although husbandry, environmental circumstances, and disease susceptibility of farmed alligators may differ from that of LCS alligators, this disease outbreak has prompted serologic testing of the LCS alligator population, results of which are pending.

Mammal Results

Nearly all LCS mammals are housed outdoor day and night. Exotic hoofstock are maintained on large preserves. Petting zoo animals have outdoor enclosures. Other housing arrangements relevant to issues of mosquito exposure are included in species summaries below.

Exotic hoofstock requiring immobilization for sampling were tested when anesthesia was required for procedures such as neutering or lameness evaluation. Among hoofstock, approximately 70 individuals from 12 species have been tested to date. Aoudad (*Ammotragis lervia*), nilgai (*Boselaphus tragocamelus*), and zebra (*Equus burchelli*) had > 50% WNV seropositivity of their

respective sample populations. Two of three aoudad had WNV titers of 1:40 with negative SLE results. Nine of 13 nilgai tested were seropositive for WNV with titers ranging from 1:20 to 1:80. These nilgai were seronegative for SLE with the exception of one nilgai reported with a WNV titer of 1:80 and an SLE titer of 1:160. Zebra had titers to WNV ranging from 1:40 to 1:320. These zebra were seronegative for SLE except for one animal with an SLE titer of 1:320. Other species tested included blackbuck (*Antilope cervicapra*), eland (*Taurotragus oryx*), fallow deer (*Dama dama*), gemsbok (*Oryx gazella*), impala (*Aepyceros melampus*), llama (*Lama glama*), mouflon (*Ovis musimon*), pygmy goat (*Capra hircus*) and waterbuck (*Kobus ellipsiprymnus*).

No positive WNV samples were obtained from six squirrel monkeys (*Saimiri sciureus*) housed day and night in an outdoor welded wire mesh enclosure located some 200 yards from WNV seropositive East African crowned cranes and Marabou storks. Available chimpanzee results show an individual with a WNV titer of 1:80 and an SLE titer of > 1:640. This animal presented with left sided hemiparesis and subsequently died, showing severe myocardial fibrosis with thromboembolic disease on histopathology. Further testing on samples from this individual as well as other members of the chimpanzee population are in progress. LCS chimps are housed day and night on outdoor islands.

Three African elephants have been tested, one of which was WNV seronegative. Another elephant had a WNV titer of 1:40 and was seronegative for SLE. A third elephant had preliminary test results showing a WNV titer of 1:320 and an SLE titer of > 1:640. A fourfold difference in titer between WNV and SLE is necessary to determine which of these arboviruses is responsible for a positive titer. Thus, further titrations must be performed on this sample to determine if exposure to WNV or SLE occurred. Two African lions tested reside entirely outdoors and were seronegative for WNV and SLE.

WNV Vaccination at LCS for Year 2002

White rhinoceros (*Ceratotherium simum*) and Brazilian tapirs (*Tapirus terrestris*) were vaccinated for WNV in 2002 using a product marketed for domestic horses (*Equus caballus*) (West Nile Virus Vaccine, Fort Dodge, Fort Dodge, IA 50501 USA). No adverse reactions to the vaccine occurred. These species were the only LCS collection animals vaccinated for WNV that year. The decision to vaccinate 11 white rhinoceros was made following a sudden fatal illness in a 6-yr-old male white rhinoceros that involved neurologic signs. Results of PCR for WNV from tissues of the deceased rhinoceros returned as negative.

ACKNOWLEDGMENTS

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PROBLEMS ASSOCIATED WITH THE 'FREE-DIPPING FISTULA TECHNIQUE' AS A METHOD OF BILE EXTRACTION FROM ASIATIC BLACK BEARS (*Ursus thibetanus*)

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Abstract

The Asiatic black bear (*Ursus thibetanus*) is the only species of bear currently legally allowed to be extracted for bile within China. The "free-dipping fistula technique" is the only method allowed under current regulations. Officials within China believe that this technique "can ensure the health of the bears and avoid their maltreatment." Cholecystectomies performed on Asiatic black bears rescued from bear farms that have had the "free-dipping fistula technique" performed indicates that cholecystitis, cholelithiasis, polyp formation and the presence of foreign bodies within the gall-bladder are common problems found in these bears, which would lead to significant health problems and a reduced lifespan.

ACKNOWLEDGMENTS

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HEMATOLOGIC AND BIOCHEMICAL REFERENCE RANGES FOR THE CALIFORNIA CONDOR (*Gymnogyps californianus*)

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Abstract

Introduction

The California condor (*Gymnogyps californianus*) was at the point of extinction with only 22 individuals in the wild 2 decades ago.⁸ Condors were taken into captivity for captive propagation and subsequent reintroduction with the last wild condor being captured in 1987. Today there are approximately 200 condors; 130 condors in captivity and 70 successfully reintroduced in the wild (J. Zuba, personal communication). In order to provide proper medical care for this endangered species, veterinarians need accurate hematologic and biochemical reference ranges on which to base medical evaluations and treatments.

Methods

Blood samples from 61 captive California condors housed at the San Diego Wild Animal Park were evaluated by sex and age to determine serum biochemical and hematologic reference ranges. Chloride, cholesterol, and total plasma protein concentrations were higher in males than females ($n = 23$ males, 21 females; $P < 0.05$). The condors were grouped by age for analysis as follows: group 1 included birds 30 days of age or less; group 2 included birds from 30 days to 6 mo of age; group 3 included birds from 6 mo to 5 yr of age; group 4 included all birds older than 5 yr.

Results and Discussion

When comparing biochemical values by age, significant differences in glucose, potassium, phosphorus, calcium, albumin, globulin, cholesterol, and bile acid concentrations, as well as, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, and creatine phosphokinase activities were identified. When comparing hematologic values by age, differences in white blood cell count, hemoglobin, hematocrit, total plasma protein concentration, heterophils, lymphocytes, and eosinophils were identified. A steady increase in glucose and a decrease in alkaline phosphatase and lactate dehydrogenase activities, as well as cholesterol, bile acid, calcium, and phosphorus concentrations were correlated with age. Condors from age group 1 and age group 2 were identified as unique compared to other age groups. Due to these differences two reference ranges (condors under 6 mo of age and condors over 6 mo of age) have been established by calculating a 90%

confidence interval. Reference ranges obtained from other published avian data including those for psittaciformes, ratites, galliformes, anseriformes, and raptors were similar to the condors in this study.^{1-7,9,10}

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IMMOBILIZATION OF RHEBOK (*Pelea capreolus*) WITH CARFENTANIL-XYLAZINE AND ETORPHINE-XYLAZINE

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Abstract

The purpose of this study was to compare the cardiopulmonary, metabolic and anesthetic effects of carfentanil-xylazine and etorphine-xylazine combinations in rhebok (*Pelea capreolus*), a species of South African antelope that is rarely maintained in captivity. Twelve adult rhebok were immobilized using a combination of 0.4 mg/kg xylazine and either 0.01 mg/kg of carfentanil ($n = 6$) or 0.01 mg/kg etorphine ($n = 6$), delivered intramuscularly using a remote injection system. Induction and recovery times, heart rate, respiratory rate, rectal temperature, oxygen saturation (SPO₂), endtidal CO₂, anesthetic depth, indirect blood pressures, and arterial blood gases were recorded. Rhebok were not intubated, but nasal oxygen was administered. Anesthesia was antagonized with naltrexone (100 mg naltrexone/ 1 mg opioid) and yohimbine (0.25 mg/kg), one half dose administered intravenously and one half dose administered subcutaneously, approximately 40 min after induction. Mean initial heart rates were significantly higher in the carfentanil group than in the etorphine group. During the first 7-10 min of anesthesia in both groups, arterial pH decreased, while SPO₂, pO₂ and pCO₂ (pCO₂) increased. These findings were consistent with respiratory acidosis associated with decreased ventilation. Initially, rhebok in the carfentanil group were more responsive to stimuli than rhebok in the etorphine group. Time to first arousal was significantly shorter in the etorphine group (1.3 min), than in the carfentanil group (7.6 min). Although there were few statistical differences between quantitative data of the two groups, qualitative aspects of the two regimens demonstrated notable differences. Rhebok in the carfentanil group were at a comparatively lighter degree of immobilization, and some required additional manual and chemical restraint. In conclusion, 0.01 mg/kg of etorphine and 0.4 mg/kg of xylazine is recommended over 0.01 mg/kg carfentanil and 0.4 mg/kg xylazine due to qualitatively better anesthetic episodes and shorter recovery times.

CEREBRAL CHROMOMYCOSIS IN A JUVENILE SNOW LEOPARD (*Uncia uncia*)

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Abstract

Chromomycosis is a chronic, cutaneous and subcutaneous fungal infection.³ This disease is caused by pigmented fungi, which belong to the Ascomycete order Chaetothyriales.¹ These opportunistic fungi are common in soil and rotting plant material, with a worldwide distribution.^{1,3} Occasional infection involving the central nervous system (CNS) has been reported in man.^{2,3} Brain abscesses due to fungal infections in domestic animals are deficient in the literature.⁴ Reports of fungal infections of the CNS in zoo animals are also lacking. This report describes a cerebral chromomycosis infection in a juvenile snow leopard at the Tulsa Zoo.

A 22-wk-old hand reared snow leopard cub suffered an acute illness characterized by lethargy, bilaterally exposed nictitans membranes and over 3 days progressed to a mild ataxia. The snow leopard cub was anesthetized with medetomidine (Domitor®, Pfizer Animal Health; 0.048mg/kg) and ketamine (Ketaset®, Fort Dodge Animal Health; 3.7mg/kg) by intramuscular injection for diagnostic investigation. The animal was intubated and maintained on isoflurane (Isoflo®, Abbott Laboratories). Atipamezole (Antisedan®, Pfizer Animal Health; 0.2mg/kg) was administered intramuscularly to reverse the cardio-respiratory effects of medetomidine. Physical examination revealed mild pyrexia and flea dirt. Fundic examination was negative for retinal lesions and all other clinical findings were normal. A tapeworm proglottid was seen in the cub's feces; fecal flotation was negative for other gastrointestinal parasites. Blood was obtained for hematology, biochemistry profile and serologic analyses. The animal never recovered from anesthesia and died 12 hr post anesthetic induction.

Hematology revealed a mature neutrophilic, monocytic leukocytosis (20,900/ μ l) with a mild anemia (hematocrit = 29.6%). The biochemistry profile was normal and serologic tests for feline leukemia virus antigen, feline immunodeficiency and infectious peritonitis virus antibodies, and *Toxoplasma gondii* antibodies were negative. The gross necropsy examination was unremarkable except for partial cerebellar herniation through the foramen magnum with a large, necrotic area in the right cerebral hemisphere that surrounded a dark pigmented lesion. Histopathologic diagnoses of the brain lesion were chromomycosis with acute suppurative meningitis and acute vascular degeneration. At the time of this report, the sibling cub remained in good health and had no clinical signs of a similar illness.

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FATAL DYSTOCIA DUE TO CHONDROSARCOMA OF THE PELVIS IN A GIRAFFE (*Giraffa camelopardalis*)

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Abstract

A 13-yr-old giraffe presented with dystocia. Previous history revealed she had given birth uneventfully to three calves and presented 2.5 yr before with a pelvic mass and a history of trauma in the same location a few months before. Cytology and biopsies were nondiagnostic at that time. The mass grew for 2 yr. Radiographically, the outer portion of the mass consisted of a thick radiopaque capsule and irregular radiodensities within a radiolucent tissue. Deeper biopsies were obtained. Cytologically, they consisted of peripheral blood, round to oval, occasionally binucleate cells with an eccentric nucleus and moderate to abundant basophilic cytoplasm with small clear vacuoles, accompanied by a eosinophilic granular background and a radiating basophilic or eosinophilic filamentous material. Histopathology revealed well-differentiated cartilage with mild to moderate anisokariosis and anisocytosis of chondrocytes. A diagnosis of low-grade chondrosarcoma or chondroma was made. She was suspected to be pregnant and treatment was postponed.

During dystocia, the fetus was manually positioned in a proper birth canal presentation because one front limb and the head were flexed transversely and backwards, respectively; it was pulled out but it had died. Shortly thereafter the dam fell down in shock with marked hypotension and died. Grossly, she had a 40-cm, white to blue-grayish, soft to gelatinous mass extending from the right ilion to the pelvic canal, with a thick bony capsule and multifocal mineralization and/or ossification. The rumen and abomasum were displaced to the right, and left and up, respectively; ruminal contents were watery. The calf had atelectasia of the right lung but the left lung was expanded; the trachea and bronchi had yellowish fluid and froth. Microscopically, the tumor was a chondrosarcoma. The calf had amniotic fluid squames within airways and hepatocellular lipidosis. Dam's death was attributed to possible amniotic fluid embolism and contributing factors included ruminal fluid loss, retroperitoneal hemorrhage, exhaustion, stress, and compression of the thorax by abdominal viscera. To our knowledge, this is the first report of chondrosarcoma and of its etiologic role in dystocia in giraffes.² Chondrosarcomas have predilection for flat bones.¹

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DIAGNOSIS AND MANAGEMENT OF SYSTEMIC HYPERTENSION IN A GIANT PANDA (*Ailuropoda melanoleuca*)

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Abstract

Introduction

The behavioral husbandry program for the two giant pandas (*Ailuropoda melanoleuca*) housed at Ocean Park, Hong Kong has been developed since 1999, and allows veterinary procedures such as physical examination, blood collection, radiography, and ultrasonography. Since systemic hypertension and its consequences are increasingly recognized in veterinary medicine, particularly in geriatric animals, the two giant pandas, a 16-yr-old male and a 24-yr-old female were trained to allow measurement of arterial blood pressure by non invasive oscillometry.

Methods

The animals were desensitized to placing their forelimb in a metallic holder, and an oscillometric monitor (Vet/BP Plus 6500 NIBP, Heska Corporation, Waukesha, WI USA) connected to a 15-cm wide cuff (Dura cuff ref 2772, adult long, 23-33 cm, Criticon USA) placed around the forelimb. Blood pressure was measured twice each week over a period of 10 wk. Systolic blood pressure, diastolic blood pressure, and mean arterial pressure in the female giant panda was on average 50-75% higher than in the male panda. Average values for the female were 241/ 135 mm Hg and mean arterial pressure was 176 mm Hg. Based on comparison with the male panda housed at the same facility, with normal values of domestic species³ and anecdotal data from another geriatric panda, a tentative diagnosis of systemic hypertension was made in the female giant panda.

Results and Discussion

Primary or essential systemic hypertension is common in human beings. In domestic cats hypertension is usually secondary to chronic renal disease and hyperthyroidism, and in domestic dogs, renal glomerular and interstitial disease, hypothyroidism, diabetes mellitus and hyperadrenocorticism have been associated with secondary hypertension.^{2,3} The only clinical sign reported in this female possibly related to systemic hypertension is an episode of epistaxis. Ongoing evaluations do not currently demonstrate the presence of any underlying condition in this animal.

Hypertension can irreversibly damage organs with rich arteriolar supply, such as ophthalmic, renal,

cardiovascular and cerebrovascular tissues. It was decided to initiate therapy as this female giant panda presented persistently elevated blood pressure. Amlodipine besylate, a long acting calcium channel blocker has been used successfully as a single agent in hypertensive domestic cats.^{1,2} It is also commonly used as monotherapy in the treatment of essential hypertension in human beings and is considered to have few adverse effects. Ankle edema is the most common adverse effect in human beings.¹ A dose of 7.5 mg of amlodipine (Norvasc, Pfizer, West Ryde, Australia) p.o., (equivalent to 0.093 mg/kg bodyweight) administered as a single daily dose in this female giant panda resulted in a reduced systolic blood pressure, diastolic blood pressure and mean arterial pressure, and no adverse effects were observed. This dose may need to be reviewed as more data obtained in conscious healthy giant pandas becomes available and allows establishment of a reference range for blood pressure values in this species.

ACKNOWLEDGMENTS

The authors wish to thank the giant panda trainers Jason Tang, Paul Ng and Jacky Chui for their dedication and patience, which made this work possible.

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OCCURRENCE AND PREVENTION OF CAPTURE WOUNDS IN SUMATRAN ELEPHANTS (*Elephas maximus sumatranus*)

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Abstract

The capturing of elephants in Indonesia began in 1986 as an attempted solution to human-elephant conflict. The intent was to train "problem" elephants for use in agriculture, logging and tourism. The initial captures were conducted under the guidance of Thai mahouts and Thai koonkie elephants (trained elephants used for capture). A number of the Indonesians that were originally trained in capture techniques still work for the government forestry department (KSDA). The younger pawangs (elephant handlers) that participate in captures have learned from their peers. There is no formal training program.

The actual mortality rate associated with elephant captures in Sumatra is unknown as official reports are lacking. The age structure of the existing ~ 400 captive elephants is young (most under 25) which suggests that smaller, younger elephants are preferentially captured and / or that adult elephants do not survive the capture and training processes. Our personal experiences (Mikota and Hammatt) in Sumatra show that mortality in newly captured elephants is high.

In 2001, with endorsement from the World Wide Fund for Nature-Indonesia (WWF), the Wildlife Conservation Society (WCS), Fauna and Flora International (FFI), and the International Elephant Foundation (IEF), we requested a 2-yr moratorium on elephant captures during which time capture techniques would be improved and alternative conflict mediation techniques evaluated.

A moratorium against placing additional elephants into the Elephant Training Centers has been issued by the central government, however capture for translocation is still sanctioned. Unfortunately, the provincial governments have increasingly acted in their own interests since the government of Indonesia began a de-centralization process a few years ago.

Riau Province is thought to have the largest remaining populations of wild Sumatran elephants. Fifty-seven, human-elephant conflicts occurred in Riau between 1997-2000. Although Riau is a hotbed of conflict, problems are occurring throughout Sumatra and we are aware of conflicts and captures in Bengkulu and North Sumatra.

In October 2002, we were invited by KSDA (the provincial forestry department) to accompany their team into the field as they attempted to capture a large bull that had been raiding a palm oil plantation. This opportunity was invaluable as we were able to observe first hand the techniques

being used and where improvements were needed. As a result of this and other experiences with newly captured elephants we observed:

- Equipment (Palmer) is old, poorly maintained, and used improperly.
- Essential supplies are lacking or homemade substitutes are used.
- The dose of xylazine is very high compared to wild elephant capture doses used in India and Malaysia. The same dose is often used regardless of the size of the elephant.
- The needles are too short to reach muscle; open-ended needles are used which can become plugged with tissue, thus preventing injection.
- Neither the correct charge nor the correct load is selected. We observed that many darts bounced making it difficult to ascertain the amount of drug injected or its depth of penetration. Selection of an inappropriate charge results in unnecessary trauma.
- The preparation and use of darts, needles, and syringes lacks basic hygiene.
- Dart wounds are not treated and antibiotics are not administered.
- There is no understanding of stress or capture myopathy.
- The capture team was not aware that sternal recumbency severely compromises respiration in elephants and that they can quickly die in this position.
- It is believed that elephant restraints must inflict pain to prevent wild elephants from escaping once captured.
- There is no veterinarian on the capture team.

The current capture techniques result in leg wounds from unprotected chains, neck wounds from “kahs” (neck yokes made of wood and wire), and abscesses from inappropriately administered darts. Leg and neck wounds often become maggot infested. Infections from dart wounds are, however, the primary cause of capture-related mortality. These abscesses can drain for several months, even with treatment, and often progress to a necrotizing fasciitis, acute sepsis, and death.

The Riau Province KSDA Team has been receptive to suggested changes to minimize wounds. Provision of heavier chains has alleviated the fear that elephants will escape. Covering the chains with fire hose or heavy plastic minimizes injuries to legs and use of the kah has been discontinued. A basic dart wound treatment protocol has been established.

In June 2003, a comprehensive Elephant Immobilization and Translocation Workshop for Sumatra is planned to retrain all of Sumatra’s field teams and to upgrade equipment.

Sumatra’s wild elephant population probably numbers fewer than 3000 and is under continued threat. With so few elephants left, the preservation of as many viable herds as possible takes on increased urgency.

The moratorium achieved in 2001 has set the groundwork for KSDA to choose translocation of wild elephants rather than capture and placement into already over-crowded and under-resourced Elephant Training Centers. We cannot guarantee that Sumatra will capture elephants only for

translocation, and it is inevitable that many more elephants will end up in captivity. Regardless, all of the elephants that must suffer the interruption of their lives at the hand of man deserve, at the very least, humane treatment.

Translocations are neither simple nor a complete panacea. Identifying suitable translocation areas and insuring that elephants remain there are significant challenges. WWF-Indonesia is continuing its efforts to secure the lowland forest of Tesso Nilo in Riau Province as a “safe haven” for at least some of Sumatra’s wild elephants (see WWF AREAS Program – Riau, Sumatra: http://www.worldwildlife.org/species/attachments/riau_profile.pdf). The identification of interim release sites, together with improved capture techniques, offers the hope that fewer elephants will be removed from the wild.

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ANALYSIS OF TESTOSTERONE AND CORTISOL FECAL METABOLITES IN MALE PALLAS' CATS (*Otocolobus manul*) HOUSED UNDER ARTIFICIAL LIGHTING CONDITIONS

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Abstract

Introduction

The Pallas' cat (*Otocolobus manul*) is a small felid species indigenous to central Asia. Maintenance of this species in captivity is difficult because of chronic infection and reactivation with *Toxoplasmosis gondii* and feline herpes virus 1.³ Pallas' cat kittens in captivity experience high neonatal mortality from toxoplasmosis. Current research on this species at north carolina state university college of veterinary medicine is focused on the creation of specific pathogen free (SPF) kittens. Pallas' cat breeding cycles are photoperiod dependent. Male cats experience significant weight gains, increased testosterone levels, and sperm production levels between December and march when the days begin to lengthen.⁴ Previous studies have demonstrated that artificial lighting conditions can cause abnormal reproductive cycles in captive female Pallas' cats.²

Methods

In order to assess the impact of artificial lighting on reproductive cycling in male Pallas' cats, an analysis of fecal androgen metabolites was conducted on four male Pallas' cats housed under fluorescent light timed to match natural photoperiod. To evaluate baseline cortisol levels in captive male Pallas' cats, assays for fecal cortisol metabolites were also conducted. Fecal samples were collected twice each week from each cat from November through June. Ethanol extraction and enzyme immunoassays (EIA) validated for this species were conducted to quantify testosterone and cortisol metabolites in the samples.¹ Data were analyzed with logarithmic transformation and f tests based on analysis of variance.

Results

The mean testosterone level of each cat was significantly higher from mid-January to mid-March. These results demonstrate that male Pallas' cats maintain normal breeding cycles in artificial lighting conditions. Mean cortisol levels for each cat were evaluated for differences between the breeding season, mid-January to mid-March, and the nonbreeding season. The male paired with the

female had a significantly higher cortisol level during the breeding season. Calculations of Z values for cortisol were conducted to assess the number of samples that differed from the mean cortisol value for each cat. Only 16 of 207 z values from the four cats were greater than ± 2 , with three of those 16 Z values greater than ± 3 .

Discussion

These results demonstrate that male Pallas' cats maintain a normal breeding season, previously established as January-April, when housed under artificial lighting conditions. While it is difficult to interpret the significance of fecal cortisol metabolites in captive wildlife as an indicator of chronic stress without reference values from free-ranging animals, the relative lack of cortisol values that vary from the mean suggests that these four males are adapted to their artificial environment. Further studies are needed to determine whether increases in cortisol in male Pallas' cats paired with females during the breeding season are normal.

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LYMPHOSARCOMA IN A CRAB EATING RACCOON (*Procyon cancrivorus*)

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Abstract

Introduction

Lymphoid tumors are among the most common types of the neoplasms of domestic animals.^{5,7} There are many different cytologic forms of lymphosarcoma. They are variable within and between species, giving rise to their classification as multicentric, thymic, alimentary, splenic and cutaneous lymphosarcoma.⁵ This report describes a cutaneous lymphosarcoma in a crab eating raccoon (*Procyon cancrivorus*).

Methods

A 9-yr-old, captive, female, crab eating raccoon (*Procyon cancrivorus*) was housed with a male in an outside enclosure. The primary manifestation of disease was involvement of the skin. The animal showed a delineated and firm consistency mass, localized in the skin of the left side of the chest. The skin was reddish, thick and the hair coat was thinner than normal. Dexamethasone was used without improvement. The mass was surgically removed and representative tissue samples were fixed in 10% formalin solution for histopathology.

Results and Discussion

Histologically, the mass was composed of large and rounded neoplastic lymphocytes, with large nuclei and rare mitotic figures. There was moderate cellular pleomorphism and the growth was expansive.

There are some significant differences between this case and the lymphosarcomas documented by other authors in North Americans raccoons.^{2,7} This case presented as just a mass. The others cases were diagnostic on postmortem examination. They found infiltration in various tissues including lymph nodes, spleen, liver, pancreas,² kidney, brain, and meninges.⁷ We found large lymphoblastic cells. Some authors found large lymphoblastic cells² and some found well-differentiated small neoplastic lymphocytes.⁷

Neoplasia, with the exception of thyroid tumors, appears to be rare in raccoons.^{2,4} In a retrospective survey of over 400 raccoon (*Procyon lotor*) necropsies, one author found only two cases of neoplasia, an astrocytoma of the brain and a fibroma of the skin.¹ Other reports show eight cases of

adrenal gland adenomas,⁴ two cases of multifocal skin papillomas,³ hepatocellular adenoma and adrenocortical adenoma.⁶

The paucity of reported cases of neoplasia in raccoons may be due to genetic resistance of the raccoon to neoplasia, high turnover in free-ranging populations, or a lack of thorough pathologic examination of sufficient numbers of raccoons.⁴

This report represents the first recorded lymphosarcoma in a crab eating raccoon.

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SUSPECTED IVERMECTIN TOXICITY IN A NENDAY PARAKEET (*Nandayus nenday*)

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Abstract

Twenty birds in an aviary were prophylactically treated with a single dose of ivermectin. The following morning one adult Nenday parakeet (*Nandayus nenday*) was found on the floor of the cage, prostrate with some wing movement and with signs of bradypnea, hypothermia, ataxia, and depression. The animal was placed in a warm place and was treated with intraosseous fluid therapy and a single high dose of corticosteroids. The parakeet did not recover and died 10 hr after the onset of the treatment. No necropsy was performed. The death was supposed to be an adverse reaction to ivermectin.

Introduction

Ivermectin is an antiparasitic drug effective for some nematodes, mites and lice, and is commonly used in avian practice.^{4,10} It has a wide safety margin in a variety of birds^{7,8,10} but increased sensitivity to ivermectin has been described in some species.^{1,3,10} Toxic reactions occurred following i.m. administration in finches and budgerigars,⁹ oral and topical administration in psittacine birds,³ and topical administration in finches.⁹ Toxicity due to the propylene glycol used to dilute ivermectin has also been described.^{5,9} The purpose of this poster is to describe a suspected ivermectin intoxication in a Nenday parakeet.

Clinical Report

Twenty birds in an aviary were prophylactically treated with a single dose of ivermectin (Ivomec, Merial, Spain). The drug was not diluted with propylene glycol and was given in the pectoral muscles at a dose of 0.2 mg/kg. The flock was predominantly macaws, cockatoos, lorries, parakeets, and toucans. The following morning (10 hr after injection) one of the two adult Nenday parakeets (*Nandayus nenday*) was found on the floor of the cage, prostrate, with half-closed eyes and some wing movement, and showing signs of bradypnea, hypothermia, ataxia and depression. The animal was immediately hospitalized and placed in a warm place, with intraosseous fluid therapy. A single high dose of corticosteroids (methylprednisolone [Solu-Moderin, Pharmacia&Upjohn, Spain] 1 mg/kg i.m.) was administered. The animal did not recover and died 10 hr after the onset of the treatment. No necropsy was performed. No other bird in the aviary (not even the other specimen of the same species) showed any kind of symptom.

Discussion

In the present report, this parakeet showed similar signs as chickens treated at doses of 15 mg/kg⁷ and falcons treated at 11 mg/kg.⁸ These doses are 55-75 times higher than the recommended dose for birds (0.2 mg/kg).⁹ This fact suggests an individual increased sensitivity to the toxic effects of ivermectin, rather than toxic effects due to the wrong dosage or to toxicity to other components of the vehicle. This kind of adverse reaction involving neurologic signs after correct ivermectin dosage has been described in collie dogs, Old English sheepdogs, and Murray Grey cattle.⁶ The cause is probably due to some characteristics that allow ivermectin to penetrate the blood-brain barrier more readily than would normally be expected.⁶ In this Nenday parakeet, as in mammals, individual sensitivity could have allowed ivermectin to enter the CNS and stimulate the GABA-ergic neurons, producing prolonged and potentially irreversible toxic effects.²

As a result of the potential for adverse reactions to ivermectin in some avian species, it is recommended to use this antiparasitic drug in those species in which it has been demonstrated to be safe. Administration into the crop or via s.c. is recommended due to toxic episodes when administered i.m., p.o. or topically.

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RAPTOR MITES

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Abstract

Birds of prey host many mites, ranging from harmful to harmless to beneficial species. These mites include feather mites, quill mites, skin and subcutaneous mites and respiratory mites, and they reproduce either on or in the host or in the nest. The mites feed on blood, tissue fluid, skin and feather lipids and debris, keratin, fungi, algae and other mites. The mite fauna of over half the falconiform and strigiform species is completely unknown, but 21 families of mites are associated with falconiforms and 17 families are associated with owls, with nearly 100 total mite species in each case, and up to 18 mite species known from an individual raptor species. Healthy looking raptors without obvious mites may harbor populations of 15,000 feather mites and 4,000 quill mites. Abnormal host transferences can occur with birds in captivity (e.g., crane mites occurring on falcons). Owls tend to have the greatest variety of raptor mites, and both owls and eagles often host large mite populations. Hawks tend to have fewer mites and falcons very few. My website at <http://raptormites.babson.edu> provides a more detailed overview of the varied raptor mites, with photographs, and an up-to-date host/parasite species checklist for mites of the Falconiformes and Strigiformes of the world. Zoo veterinarians have the opportunity to examine many of the raptor species whose mite fauna is poorly known or unknown, and to discover many new raptor mite species and host relationships.

FATAL ANEMIA AND DERMATITIS IN CAPTIVE AGOUTIS (*Dasyprocta mexicana*) INFECTED WITH *Echidnophaga* FLEAS

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Abstract

Two captive adult agoutis (*Dasyprocta mexicana*) (cases 1 and 2) died within 1 mo with severe flea parasitism, pale carcass with tissue hypoperfusion, severe anemia (case 2), severe centrilobular to submassive hepatocellular necrosis, marked erythroid hyperplasia in the bone marrow (case 2), and severe cardiomegaly and mild heterophilic and lymphoplasmacytic perivascular blepharitis (case 2). Case 3 was an adult agouti that had died 2.5 mo before with severe bacterial cellulitis and myositis secondary to a cutaneous penetrating wound associated with fleas, alopecia, crusting, and epidermal hyperplasia and hyperkeratosis with a caudodorsal distribution. At the time of death of case 2, all agoutis were variably parasitized by fleas and one of three animals had anemia. There were also two agoutis with cutaneous lesions similar to those of case 3 and associated with pruritus and self-mutilation in the same area as the wound of case 3 (flank). Skin biopsies from both animals revealed a perivascular superficial dermatitis with multifocal focal dermal fibrosis and epidermal hyperplasia and hyperkeratosis. All agoutis were anesthetized for manual removal of fleas and were treated with propoxur and selamectin, and were moved to another facility. Fleas collected from the necropsy cases and live agoutis were classified in the genus *Echidnophaga* based on the absence of genal and pronotal combs, angular front margin of head, contracted thorax, and the fact that the fleas did not jump.^{2,6} Furthermore, case 2 had severe infection of the eyelids, a finding typical of *Echidnophaga* flea infestation in birds. These findings are similar to the effects of fleas on different animal species, particularly carnivores.^{3,7} The death of cases 1 and 2 was attributed to hypoxia. Cardiomegaly was a contributing factor to the death of case 2 and was attributed to anemia. The cutaneous disease found in three agoutis is suggestive of flea bite hypersensitivity and secondary self trauma. To the authors' knowledge, there are no descriptions of fatal anemia and dermatitis associated with flea ectoparasitism in any rodent species although rodents are commonly infected with different flea species, including *Echidnophaga*.¹ Fleas and rodents are important in the transmission of relevant infectious diseases such as plague (*Yersinia pestis*) and tularemia (*Francisella tularensis*).^{1,3-5}

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LAPAROSCOPIC TUBAL CAUTERIZATION IN TWO AFRICAN LIONS (*Panthera leo*)

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Abstract

Introduction

Historic methods of surgical contraception in felids have included ovariectomy, hysterectomy, tubal ligation, and ovariectomy through a large ventral midline laparotomy incision. Complications associated with these procedures include prolonged anesthetic time, infection, dehiscence, self-mutilation of the incision, and prolonged recovery.^{1,2}

Evaluation of the reproductive tract as well as assisted reproduction and ovariectomy have been documented through the use of laparoscopic instrumentation in felids.^{1,4,6} Advances in human laparoscopic surgical techniques have resulted in decreased anesthetic time, self mutilation, and recovery time.³

Materials and Methods

Two female African lions (*Panthera leo*) were selected for management-related contraception, though permanent sterilization was not desired. One adult, a 5-yr-old, overweight (160 kg) female (Lion 1), and her 18-mo-old female offspring (Lion 2), weighing 111 kg, were immobilized with a combination of 5 mg/kg ketamine hydrochloride (Ketaset, Fort Dodge Laboratories, Inc., Fort Dodge, IA 50501 USA), lyophilized by Congaree Veterinary Pharmacy, Cayce, SC 29033 USA, 0.5 mg/kg xylazine hydrochloride (Rompun, Bayer Corp., Shawnee Mission, KS 66201 USA) and 0.1 mg/kg midazolam hydrochloride (Faulding Pharmaceutical Co. Elizabeth, NJ 07207 USA) i.m. via blowdart (Telinject, Saugus, CA 91350 USA) in Lion 1, and hand-syringe in Lion 2. The lions were placed in dorsal recumbency, intubated, and maintained on isoflurane (Isoflo, Solvay Animal Health, Inc., Mendota Heights, MN 55120 USA). Respirations were controlled with a time-cycled ventilator (Mallard Medical, Inc., Irvine, CA 92714 USA) attached to a circle anesthetic system. Mechanical ventilation was initiated upon abdominal insufflation with carbon dioxide (CO₂).

A cephalic intravenous catheter was placed and 10 ml/kg/hr of lactated Ringer's solution (Abbott Laboratories, North Chicago, IL 60064 USA) administered throughout the procedure. A urinary catheter (Rusch Inc., Duluth, GA 30136 USA) was placed to maintain an empty bladder, facilitating visualization of the reproductive tract. Continuous electrocardiography, direct arterial blood pressure

(BP), capnography (Critikon, Inc. Southington, CT 06489 USA), respiratory excursions, and oxygen saturation (Pulse Oximetry, Nonin Medical Inc. Plymouth, MN 55447-4755 USA), were monitored and recorded every 5 min. A dorsal pedal arterial catheter was placed to facilitate serial blood gas and direct BP analysis throughout surgery (Critikon, Inc.). Indirect BP (Cardell™, CAS Medical Systems, Inc. Branford, CT 06405 USA) values were monitored during the surgical procedures by placing the cuff over the base of the tail. Indirect BP values closely paralleled direct BP measurements. Mean arterial pressure (MAP) in Lion 1 dropped below 80mm Hg, and 0.1mg/kg of ephedrine sulfate (Bedford Laboratories, Bedford, OH 44146 USA) was administered i.v.. MAP rose to acceptable levels within 10 min.

After sterile preparation, a 1.5 cm ventral midline incision was made 6 cm caudal to the umbilicus into the abdominal cavity. A purse-string suture (0 Polydioxanone PDS, Ethicon, Inc., Somerville, NJ 08876-0151 USA) was placed in order to tack the abdominal fascia to the peritoneum. An optical obturator (Optiview Disposable Surgical Trocar, Ethicon Endo-Surgery Inc. Cincinnati, OH 45242 USA) with a 10mm surgical telescope (Olympus Corp., Melville, NY 11747 USA) was used to visualize the subcutaneous tissues, fascia, peritoneum and abdominal cavity. Insufflation with CO₂ to 15 mm Hg of pressure was achieved with an Endoscopic CO₂ insufflator (Olympus Corp., Melville, NY 11747 USA) attached to the optical obturator. In Lion 1, extensive deposits of adipose tissue were observed, at times obscuring the field of view. Once the uterine horn was identified, it was followed cranially to the ovary, whereupon the ovarian tube was identified between the ovary and the mesosalpinx.⁵ The ovarian tube was gently elevated away from adjacent tissues with laparoscopic Kleppinger® forceps and subsequently cauterized with bipolar current (Richard Wolf GmbH Postfach 1164 75434 Knittlingen, Germany). The procedure was repeated for the opposite ovarian pedicle. Extreme caution was used when cauterizing the ovarian tube to minimize contact with adjacent tissues. Once both ovarian tubes were cauterized, the surgical sites were observed for hemorrhage, and upon finding none, the abdominal CO₂ pressure was decreased to less than 5 mm Hg. The trocar was removed, and the incision closed via the original purse-string suture. A subcuticular simple continuous suture (0-PDS, Ethicon, Inc.) was placed and topical formulated cyanoacrylate (Nexaband, Veterinary Products Laboratories, Phoenix, AZ 85013 USA) was applied to the incisions. The incisions were infiltrated with 20 ml of 0.5% bupivacaine hydrochloride with 1:200,000 epinephrine (Marcaine, Abbott Laboratories, North Chicago, IL 60064 USA) for site-specific analgesia.

Both lions recovered without complication and began eating the same day. Lion 1 was observed to occasionally lick the incision, causing dehiscence of the subcuticular layer; it was allowed to heal by second intention. Lion 2 was not observed to debride the incision but dehiscence of the subcuticular layer was observed along with significant seromatous formation. The incision was allowed to heal by second intention. Both lions were returned to the pride the same day as surgery, returned to the exhibit within 10 days of the procedure and recovered without further complications.

Discussion

Megesterol acetate implants have been used historically for contraception in felids. Current

recommendations advise removal of the implant and a return to normal reproductive cyclicity for a period of 1 yr after removal. In this case, space was not available to keep the females separated from the intact male of the pride. Tubal cauterization was elected in these lions for potential reversal in the future. In humans, > 75% of re-anastomoses surgeries are successful, with more than 30-40% of women becoming pregnant. Total surgical time was approximately 35 min and a rapid return to normal function was apparent in both animals. In this case, Lion 1 was imported from Africa, and her genetic lineage could become important when future breeding recommendations are considered.

Tubal cauterization via laparoscopy is associated with shorter surgical times than laparotomy, facilitating decreased total anesthesia time. Disruption of tubal function may also be accomplished by ligation. This procedure may be more easily reversed but requires more initial operative time and expertise to perform. The shorter incision length (1.5 cm vs. > 10 cm) reduces the risk of dehiscence and may reduce self-mutilation behavior. Although both subcuticular suture lines dehisced in these lions, both subsequently healed via second intention. Additional therapy was not necessary for healing.

In humans, abdominal insufflation activates peritoneal stretch receptors resulting in pain that persists even after the intra-abdominal pressure has been decreased. Residual CO₂ within the abdomen is also associated with discomfort. Neither of these lions demonstrated observable discomfort after the procedure and returned to normal behavior the same day as surgery.

Laparoscopic surgery requires specialized equipment and technical expertise. In addition a ventilator is required to prevent severe hypoventilation and hypercapnia that results from abdominal insufflation with CO₂.

Use of laparoscopic tubal ligation in lions appears to be a valuable tool for long-term contraception with the added benefit of potential reversibility while maintaining reproductive cyclicity.

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SERUM CORTISOL IN FALLOW DEER (*Dama dama*) AFTER CHEMICAL RESTRAINT

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Abstract

Introduction

Stress is one of the major concerns in zoo animal management. There are well-known effects on behavioral patterns,¹⁻⁴ cardiovascular and immune system,^{4,8} hypothalamus-pituitary-gonads axis (HPG)^{1,3,7,8} and ultimately on homeostasis^{4,8} and reproduction^{3,7} in different species. Among wild mammals, the cervids are one of the most stress-sensitive groups, which means that their restraint is a very stressful event and the use of anesthetic or sedative drugs is always advisable in order to reduce the stress level and to enhance the safety of the procedure. Serum cortisol has been used as an indicator of stress level in different species.^{1-3,5,6,8,9} The aim of this project was to analyze levels of serum cortisol during three different protocols currently used at the São Paulo Zoo for chemical restraint of fallow deer (*Dama dama*), reflecting, potentially different degrees of stress.

Materials and Methods

We studied 63 individuals (30 males and 33 females), maintained in The Zoo Safari/São Paulo Zoo, São Paulo, Brazil, divided in three different protocols as follows: a) xylazine hydrochloride (Rompun®, Bayer do Brasil, São Paulo, Brazil; 5 mg/kg i.m.) alone in 10 males 12 females; b) the association of xylazine (3.5 mg/kg i.m.) and fentanyl citrate (Fentanyl®, Janssen USA; 0,01 mg/kg i.m.) in 14 males and 11 females; and c) the association of xylazine (2-3 mg/kg i.m.) and ketamine (Vetaset®, Fort Dodge, São Paulo, Brazil; 5-7 mg/kg i.m.) in six males and 10 females. All of them were injected by the use of blow darts. Right after the immobilization we collected the first blood sample and two more samples within an interval of 15 or 20 min. The serum obtained was frozen and kept in - 20°C until assayed at Department of Animal Reproduction of The Faculty of Veterinary Medicine and Animal Science, University of São Paulo. The levels of serum cortisol were determined by radioimmunoassay (RIA) using the commercial kit (Coat-a-Count® Cortisol, Diagnostic Products Corporation, Los Angeles, California USA). The coefficient of variation inter and intra-assay were lower than 10%. The data obtained were compared by the T test with 95% of confidence. The final value for each animal was obtained by the mean of the results of the three blood samples.

Results

In the three protocols the females showed higher levels of cortisol than the males ($P < 0.05$). The results are presented as mean $\pm$ SD, as follow:

Males - $0.83 \pm 0.66 \mu\text{g/dl}$ and females - $2.22 \pm 1.47 \mu\text{g/dl}$.

Males - $1.03 \pm 0.76 \mu\text{g/dl}$ and females - $2.22 \pm 1.86 \mu\text{g/dl}$.

Males - $1.32 \pm 1.11 \mu\text{g/dl}$ and females - $2.25 \pm 1.04 \mu\text{g/dl}$.

Comparing the different protocols (considering both sexes), the results were: a) $1.61 \pm 1.33 \mu\text{g/dl}$, b) $1.54 \pm 1.45 \mu\text{g/dl}$, and c) $1.87 \pm 1.15 \mu\text{g/dl}$, showing no difference between groups ($P > 0.05$).

Discussion

The results showed a clear difference between sexes in the three tested protocols, been the cortisol higher in the females. The fallow deers live in groups with males been the dominants and often showing aggressive behavior against females. So the females probably have higher levels of cortisol normally. The data found also suggested that there were no significant difference in the level of stress among the tested protocols.

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SALMONELLOSIS AND EXUNGULATION IN A PRZEWALSKI HORSE (*Equus caballus przewalskii*)

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Abstract

Introduction

Within the European Endangered Species Programme (EEP) a 6.5-yr-old Przewalski horse (*Equus caballus przewalskii*) stallion originating from “Tierpark Sababurg” (Germany) was assigned to the Przewalski breeding group of the Royal Zoological Society of Antwerp (RZSA). The horse had been declared fit to travel by the veterinarian in charge. Before departure the horse was immobilized, based on an estimated body weight of 250 kg, with 4.9 mg etorphine hydrochloride and 20 mg acepromazine maleate (Large Animal Immobilon, Vericore Ltd, Marlow, Bucks, SL7 1FJ, United Kingdom). Simultaneously, his hooves were trimmed, blood was taken for routine analysis, an intradermal tuberculin test was performed, a transponder was inserted subcutaneously, he was vaccinated against tetanus and influenza, and also a prophylactic dose of 250 mg vitamin E (DL-alpha tocopherol acetate) and 7.5 mg selenium (sodium selenite) (Etosol Se®, Eurovet, B-3550 Heusden-Zolder, Belgium) was administered intramuscularly. After 45 min the animal was injected with the antidote: 6.52 mg diprenorphine hydrochloride (Revivon, Vericore Ltd, Marlow, Bucks, SL7 1FJ, United Kingdom) intravenously and the same dose intramuscularly, which put him back on his feet in a few minutes. On arrival in Belgium the animal was put in a separate stable. The next day he was dewormed with 60 mg ivermectin (Eqvalan® paste, Merial, B-1170 Brussels, Belgium) and fecal analysis was carried out. Because the horse was very anxious to join the other horses in the outdoor enclosure, the introduction scheme was shortened.

Case Report

First the stallion was introduced to the most docile mare and both supported each other well. Second, three other mares and one gelding were put together with them. Later on, many fights were observed. Especially the gelding, and to a lesser extent one of the mares, reacted very aggressively. Six days later the gelding had to be removed from the group and was translocated to another zoo. A few more days later the stallion had gained total control over his mares.

During the first 3 wk, routine fecal examinations remained negative. Four weeks after arrival an acute heavy diarrhea developed and *Salmonella virchow* was isolated. The stallion was isolated and treated. Table 1 shows the evolution of the disease. This *Salmonella* was resistant against ceftiofur, intermediate resistant against streptomycin, but sensitive for ampicillin, amoxycillin-clavulanic acid, doxycycline, enrofloxacin, gentamycin, kanamycin and trimethoprim-sulphonamide. The stallion was

injected with kanamycin-penicillin during the period of anorexia and then treatment was continued with trimethoprim-sulphadiazin perorally. Although the animal weakened enormously in the beginning, it started to eat hay again on day 6 and survived. Subsequent fecal cultures did not reveal any *Salmonella*.

Four days after the end of the acute illness, the horse started limping on the left hind leg. Flunixin (Finadyne®, Schering-Plough, B-1180 Brussels, Belgium) 1.1 mg/kg was administered perorally for 3 days and limping decreased gradually during 5 days, but reappeared thereafter. Then, 1 g phenylbutazone was given orally daily and 10 days later a small bleeding was observed at the coronary region. Thereafter, no more limping was observed.

Three weeks later the horse suddenly had lost its left hind leg hoof with only minimal bleeding. The animal was put in a stable with a rubber floor with disinfectant. The first day the stallion was biting in its foot, but administering flunixin stopped this behavior. For the next 10 wk 1 g phenylbutazone was given daily perorally combined with vitamins and minerals containing biotin (Equitop® Forte, Boehringer Ingelheim, B-1200 Brussels, Belgium). Additionally, trimethoprim-sulphadiazin was given for 2 wk. Unexpectedly, the stallion started to use its leg again after 9 days and 3 mo later the hoof had grown half way. Five and a half months after exungulation a full new hoof was formed and the animal was released in the outdoor enclosure with the mares.

Discussion and Conclusion

Salmonellosis is a very well known disease in livestock, but reports on *Salmonella virchow* in equines are very scarce.⁵ Severe dysentery is a hallmark in equine salmonellosis and can occur when animals are exposed to stress.³ The stallion was most probably a carrier, that became ill during a very stressful period, although a new infection can not be ruled out, as fecal contamination by birds is a risk factor.² Albeit that, none of the other horses got sick. Treatment of salmonellosis is a standard procedure, involving regular administration of electrolytes.³ In wild animals however, this remains difficult, because they are unapproachable. Anyhow, the oral administration of electrolytes is safer than the intravenous one with regard to a possible hyperkalemia.³ Laminitis can be a result of severe diarrhea and bacterial toxin and has been observed in Przewalski horses before.^{1,3,4} One of the reasons for exungulation is terminal laminitis.³ Only anecdotal reports (personal communications) of exungulation in exotic species exist. There is no standard treatment for sloughing of the hooves,³ but all measures taken in this case contributed to a positive outcome.

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Table 1. Evolution of salmonellosis in a male Przewalski horse.

Day	Symptoms	Treatment ^a
0	Lethargy, anorexia	No
1	Diarrhea, anorexia, drinks	1.5 mg/kg kanamycin + 6000 IU penicillin ^b
2	Diarrhea, anorexia, drinks	1.1 mg/kg flunixin ^c
3-4	Diarrhea, anorexia, drinks	30 mg/kg trimethoprim-sulphadiazin ^d
5	Diarrhea, anorexia, drinks	1.5 mg/kg kanamycin + 6000 IU penicillin + 1.1 mg/kg flunixin
6	Less diarrhea, eats hay, drinks	1.5 mg/kg kanamycin + 6000 IU penicillin + 1.1 mg/kg flunixin
7-8-9	Less diarrhea, eats hay, drinks	30 mg/kg trimethoprim-sulphadiazin
10	No symptoms	30 mg/kg trimethoprim-sulphadiazin

^aElectrolytes were given daily (glucose 20 g, NaCl 3 g, NaHCO₃ 3 g, KCl 1.5 g / L drinking water).

^bKanacillin Trifortis, Continental Pharma, B-1050 Brussels, Belgium.

^cFinadyne®, Schering-Plough, B-1180 Brussels, Belgium.

^dEmdotrim 60% Mix, Ecuphar, B-5377 Zuienkerke, Belgium.

CERVICAL SPINAL CORD VASCULAR ANOMALY IN AN AFRICAN WARTHOG (*Phacochoerus africanus*)

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Abstract

Vascular anomalies of the spinal cord are rare in mammals, with humans being the most frequently recognized species with such lesions. In man, they account for 3-11% of spinal space-occupying lesions. Traditionally, these abnormalities in the spinal vasculature have been classified as cavernous angiomas (cavernomas), arteriovenous malformations, capillary telangiectasias, metameris angiomas (Cobb's Syndrome), disseminated angiomas (Osler-Weber-Rendu Syndrome) and venous angiomas.¹⁻⁴

An 8.5-yr-old female African warthog (*Phacochoerus africanus*) presented in the late afternoon with an acute onset of tetraparesis while on exhibit. This warthog resided in the exhibit with two other warthogs; her 11-mo-old female offspring, and a male conspecific. She was able to partially lift her head, but was lying on her right side unable to rise. Although movement was present in all four limbs, the forelimbs were most severely affected by the paretic state, with withdrawal reflexes present in the hindlimbs. A tail flick was still present, and anal tone was preserved. There was no external evidence of trauma, and there was no salivation or oral frothing to suggest seizure activity. Both pupils were equally dilated but reactive. A cursory examination of cranial nerve function was normal.

The warthog was immobilized with medetomidine (49 µg/kg i.m.; Zalopine, Orion Corporation, Espoo, Finland), butorphanol tartrate (0.20 mg/kg i.m.; Torbugesic, Ayerst Veterinary Laboratories, Guelph, Ontario, Canada N1K 1E4) and midazolam (33 µg/kg i.m.; Versed, Hoffman-La Roche, Mississauga, Ontario, Canada L5N 6L7) and transported to the zoo's animal health centre for further diagnostics. Initial therapy consisted of 100% oxygen via facemask, and placement of an intravenous catheter for blood collection and fluid therapy (Plasmalyte, Baxter Corporation, Mississauga, Ontario, Canada L4Z 3Y4). A complete blood count revealed a mild increase in hematocrit, likely due to excitatory splenic contraction. Abnormalities on the biochemical profile were a moderate stress hyperglycemia, mild hypoalbuminemia, and mild hyperglobulinemia.

Due to the tetraparetic state, with lower motor neuron dysfunction of the forelimbs and upper motor neuron dysfunction of the hindlimbs, a cervicothoracic (C7-T2) spinal cord lesion was suspected. The lack of a Horner's sign suggested that the lesion could further be isolated to the cervical spine.

With the acute onset of clinical signs, differential included trauma (including intervertebral disc disease), ischemia (vascular lesion, fibrocartilaginous emboli), infectious (spinal abscess), and inflammatory disease. Neoplasia, such as lymphosarcoma, was also considered, while metabolic, nutritional, and toxic etiologies were considered less likely. Survey radiographs of the cervical, thoracic and lumbar spine did not reveal any significant abnormalities except for mild bridging spondylosis in the mid-lumbar spine.

The warthog was treated with dexamethasone sodium phosphate (0.25 mg/kg i.v.; Dexamethasone 5, Vetoquinol, Lavaltrie, Quebec, Canada J0K 1H0), vitamin E/selenium (0.025 ml/kg i.m.: Dystosel DS, Pfizer Animal Health, London, Ontario, Canada N6A 1K7), vitamin B complex (0.07 ml/kg i.m.; Vetoquinol, Lavaltrie, Quebec, Canada J0K 1H0), and Florfenicol (20 mg/kg i.m.; Nu-Flor, Schering Plough Animal Health, Pointe Claire, Quebec, Canada H9R 1B4). After being transferred to an indoor hospital holding enclosure, anesthesia was reversed using atipamezole (0.25 mg/kg; Antisedan, Novartis Animal Health Canada, Mississauga, Ontario, Canada, L5N 1V9) and naltrexone (1 mg/kg; Trexonil, Wildlife Laboratories Inc., Fort Collins, CO USA 80524) administered intramuscularly and subcutaneously in equal proportions. Straw bales were used to further confine movement. Over the next 12 hr there was further decline in neurologic status with increasing mental depression and development of profound hypothermia (33 °C). Hyperreflexia was present in the hindlimbs, while there was hyporeflexia in the forelimbs, with extensor rigidity in the right foreleg. Pain perception was still preserved in all four limbs. The warthog had urinated overnight, however no feces were passed. There was no interest in food or water.

Response to warming efforts was sluggish with a rise in rectal temperature to only 34.7 °C. Meloxicam was administered (Metacam, Boehringer-Ingelheim Ltd., Burlington, Ontario, Canada L7L 5H4) at a dosage of 0.1 mg/kg subcutaneously. The warthog was then immobilized using the same anesthetic protocol as before, an intravenous catheter placed for intravenous fluid administration (Plasmalyte with 5% dextrose), and transported to Foothills Hospital for magnetic resonance imaging (MRI), where findings confirmed suspicions of the spinal lesion. At the level of approximately the C7 vertebral body, there was an increase in T1 and T2 signal strength consistent with a focus of intramedullary spinal cord hemorrhage with surrounding edema. Over 50% of the cord was affected on the sagittal view, with approximately 30-40% affected centrally on the transverse view. Blood vessels in the arachnoid space also appeared abnormal. The MRI findings were suggestive of an arteriovenous malformation or a cavernous angioma.

The warthog was transported back to the zoo hospital and recovered from anesthesia through administration of flumazenil (4 µg/kg i.v.; Anexate, Hoffman-La Roche, Mississauga, Ontario, Canada L5N 6L7) and atipamezole (0.25 mg/kg i.m.). The butorphanol was not reversed in order to provide additional analgesia. Mannitol (0.15 mg/kg) was administered intravenously over 40 min. Intravenous fluids were continued at 2 ml/kg/hr. By 2 hr post anesthesia, mentation had improved, the rectal temperature had increased to 35.1 °C, and the warthog consumed a few pieces of chopped vegetables and fruit. No further changes in neurologic status were observed. The warthog spent the next several hours resting comfortably. Approximately 6-7 hr post anesthesia, the warthog expired in a resting position, with a body temperature that had increased to 41 °C.

On necropsy, the entire spinal canal contained a sanguinous fluid, most prominent in the cervical and cranial thoracic segments. Extending from the C6-C7 intervertebral space caudally was a 15 × 10 mm area of bruising and hemorrhage of the spinal cord visible beneath the dura. The dorsal aspect of the cord was most prominently affected. On transverse sectioning, the hemorrhage obliterated the central third to half of the spinal cord, affecting most of the dorsal, intermediolateral and ventral grey columns, as well as the dorsal and ventral funiculi. Cavitation of the dorsal funiculus of the cervical cord, due to hemorrhagic myelomalacia, was apparent. A small 6-8 mm focus of hemorrhage also was present in the left cerebral cortex at the level of the callosomarginal branch of the anterior cerebral artery. Culture of the cerebrospinal fluid yielded scant growth of *Corynebacterium* sp. Other gross findings included moderate to marked pulmonary, hepatic and splenic congestion, patchy white streaking in the myocardium suggestive of mild myocardial fibrosis, multifocal endometriosis of the uterine serosa, and historic vulvar herniation of retroperitoneal fat. Histologic consultation is still pending to further characterize the nature of the spinal cord lesion, however preliminary findings are most suggestive of a cavernous angioma.

Vascular malformations of the spinal cord have not been described in porcine species, and are a rare finding in companion animals.^{4,5} In humans, these malformations represent a major cause of spinal cord myelopathy.³ Magnetic resonance imaging is the screening modality of choice. Prognosis is dependent on the duration and severity of the hemorrhage. Surgical options exist in people, but even with surgery, a return of normal neurologic function is often not possible, even with small lesions.^{1,3} Even if a surgical option were possible in this case, the acute onset of clinical signs, with rapid deterioration of neurologic function due to the severe intraparenchymal hemorrhage, would have precluded successful surgical intervention.

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ASSESSMENT OF IMPACTS OF ANESTHESIA AND BLOOD SAMPLING FOR HEALTH SCREENING ON SURVIVAL OF WILD BIG BROWN BATS (*Eptesicus fuscus*)

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Abstract

We assessed the impact of disease investigative techniques on survival of wild big brown bats (*Eptesicus fuscus*), as a part of a larger ongoing investigation of the ecology of bat rabies in Fort Collins, Colorado.

Introduction

Our objectives were to determine if the health monitoring techniques we employed had an effect on survival, which if true could lead to erroneous overestimates of the impact of disease on the population at risk.

Methods

To estimate impacts of anesthesia and bleeding on short-term (14-day) survival, we randomly assigned adult female and volant juvenile bats captured at maternity roosts in buildings during summer 2002 into two groups within each age category. Techniques employed in group 1 (controls) included: capture, transport, morphologic measurements, oral swabbing for PCR, aseptic wing biopsy and dorsal subcutaneous passive integrated transponder (PIT) tag injection (with tissue glue closure), and re-release at the site of capture within 6 hr of bat collection. In group 2 (treated), inhalant anesthesia and blood sampling from vessels in the tail membrane (targeting samples at < 1% of body weight) were added to the above procedures.

Results and Discussion

Anesthesia was delivered in a capsule specially designed to minimize stress and exposure of personnel to bites from bats. We recorded 876 immobilization times for bats anesthetized and bled in 2002; these averaged 9.1 ± 5.1 (SD) min (range 1-71). Volumes of blood samples averaged 58 ± 12 μ l (range 13-126, $n = 718$), not including wastage. We monitored fate of bats subsequent to release by placing PIT tag hoop readers with computer memory at roost openings of wild colonies in urban areas. Bats were passively verified as alive when they crawled across the hoops during

egress and returns to the roost. Short-term (14-day) survival probabilities were calculated using subroutines within program MARK³, an online software package that estimates numeric maximum likelihood-based survival probabilities from recapture data. In the control groups studied for 14-day survival in 2002, 86 adult females, and 92 volant juveniles (females: males = 47:45) were processed. In the treatment groups, 187 adult females and 87 volant juveniles (44:43) were processed and studied. These data suggested no adverse effect of anesthesia and sampling on 14-day survival (adults: $\chi^2 = 22.22$, *d. f.* = 27, $P > 0.05$; juveniles: $\chi^2 = 9.72$, *d. f.* = 18, $P > 0.05$), when compared to bats undergoing the same procedures minus anesthesia and blood sampling.

We also computed 1-yr return rates of bats PIT tagged in 2001 and not anesthetized and bled, anesthetized and bled once, or anesthetized and bled twice that year. Returns were monitored at roosts with hoop readers in 2002, and confidence intervals for rates calculated on the basis of the binomial distribution. The 1-yr return rates for big brown bats processed in 2001 and monitored in 2002 were comparable among adult bats not anesthetized and bled (81%, $n = 72$, 95% CI = 70-91%), adult bats anesthetized and bled once the previous year (82%, $n = 276$, 95% CI = 81-84%), and adult bats anesthetized and bled twice the previous year (84%, $n = 50$, 95% CI = 74-94%). Lack of effect was also noted in 1-yr return rates of volant juvenile females not bled (55%, $n = 29$, 95% CI = 37-73%), juvenile females anesthetized and bled once the previous year (66%, $n = 113$, 95% CI = 58-75%), and juveniles anesthetized and bled twice (71%, $n = 17$, 95% CI = 49-92%). Comparability of 1-yr return rates among control and treatment groups of females (males are not expected to return to maternity colonies and were not included in the analysis) suggest no long-term effects on survival. These 1-yr return rates also are comparable to or exceed more crudely estimated values in the literature^{1,2} for wild big brown bats that were captured and handled in various studies but without collection of multiple biologic samples, suggesting that the entire suite of sampling techniques has no marked effect on survival. These data clearly show that our techniques for anesthesia and sampling blood do not adversely bias assessment of impacts of disease on either short-term survival or 1-yr return rates in wild big brown bats. Passive integrated transponder technology allowed this assessment to be made with considerable efficiency.

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TISSUE CONTENT OF NOVEL RENAL DISEASE MARKERS IN PIGEONS (*Columba livia*)

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Abstract

Acute renal disease remains challenging to diagnose in avian species due to their ability to hide illness, an absence of specific clinical signs, and a lack of validated sensitive markers of glomerular and tubular dysfunction or damage. Acute avian renal disease occurs in response to a range of nephron damaging events, including: severe dehydration, high protein load, vitamin A deficiency, nephrotoxin exposure, and renal adenocarcinoma.¹

Introduction

Mammalian renal indices are of limited value.¹⁻³ For example, birds make predominately creatine, not creatinine; they are more uricotelic than ureotelic, and BUN when produced is strongly influenced by dietary protein intake and differential handling by functionally discrete nephron populations.^{1,2,4} The purpose of the present study was to determine tissue levels of creatine and N-acetyl-b-D-glucosaminidase (NAG) as a prelude to renal disposition and disease studies.

Methods

A modified two-step creatinine assay procedure (Boehringer-Mannheim, now Roche) was previously adapted and validated and used to measure creatine, using an Hitachi 917 clinical chemistry Autoanalyzer. NAG was similarly assayed using a standard commercial kit (Roche). Selected tissue samples (pancreas, liver, skeletal muscle, kidney and small/large intestine from four captive-bred homing pigeons fed a standard diet and humanely euthanatized as a part of flock culling were rapidly collected, snap frozen, powdered in liquid nitrogen and stored at -80 °C. At the time of assay, aliquots of frozen powdered tissues were resuspended in cold Tris buffer (pH 8.0), vortexed 30 sec, centrifuged at 10,000 rpm, and assayed for creatine and NAG. Analysis employed one-way ANOVA using tissues as factors.

Results

Results indicated that creatine was significantly ($P < 0.0001$) elevated in skeletal muscle as compared

to other tissues examined, and that NAG was significantly ($P < 00001$) elevated in renal tissue as compared to the other tissues.

Discussion

These results were promising in that creatine shares the same relationship with avian muscle that creatinine does in mammals; likewise, NAG appears specific for renal parenchyma. Thus, both these potential acute renal disease markers warrant further investigation as indices of renal dysfunction.

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