SINGLE DOSE PHARMACOKINETICS OF AZITHROMYCIN IN BALL PYTHONS (*Python regius*)

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Abstract

Azithromycin is a new sub-class of macrolide antibiotics classified as an azalide. This antimicrobial has a similar mechanism of action to the other macrolides (i.e., erythromycin) by binding to the 50S ribosomal subunit.[2] Azithromycin provides broad-spectrum antibiosis against gram-positive and gram-negative bacteria.[2] It also has the ability to obtain sustained drug concentrations in tissues much greater than the corresponding plasma concentration.[1,3] This study determined the pharmacokinetics of azithromycin (Zithromax®, Pfizer Inc., New York, NY 10017 USA) in ball pythons (*Python regius*), a species that is representative of the Boidae family. Snakes were administered azithromycin intravenously (i.v.) to determine distribution and orally (p.o.) to determine bioavailability and absorption. Seven ball pythons (two males, five females), weighing approximately 0.67-0.96 kg, were used in this experiment. Using a crossover design, each snake was given a single 10 mg/kg i.v. dose of azithromycin via cardiocentesis. For the oral study, each snake was dosed at 10 mg/kg using the same i.v. azithromycin preparation. Blood samples were collected prior to dosing and at 1, 3, 6, 12, 24, 48, 72, and 96 hr post-azithromycin administration. Plasma $t_{½}$ for i.v. and p.o. dosing was 17 hr and 51 hr, respectively, and the average oral bioavailability (F) was 77% (± 27%). The recommended dose for azithromycin in ball pythons is 10 mg/kg at 48-72 hr intervals.

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EVALUATION OF REPTILE THERMOREGULATION AND ENCLOSURE DESIGN USING DIGITAL THERMOGRAPHY

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Abstract

One of the most important factors in successfully maintaining healthy captive reptiles is providing them a proper thermal environment. Most reptile species regulate their body temperatures by using external environmental heat sources such as the sun. Many people commonly refer to this as "cold-blooded". In reality reptiles operate at a preferred optimum body temperature (POBT) similar or higher than internal body temperatures of mammals.[5] The correct term for this behavior is poikilothermic. Reptiles have POBT's that must be reached to maintain normal digestion and immune function.[2,3] In the past, body temperatures of reptiles have been difficult to assess without multiple thermometers, invasive probes, and less accurate spot-point heat detectors. The study of thermoregulation has now been made easier with the development of digital thermography.

Infrared thermography has been used widely in human and veterinary medicine to diagnosis inflammation, nerve, and musculoskeletal injury.[1,3,4,6-8] Thermography is the study of infrared radiation that is emitted from all objects both inanimate and living. This energy is released as photons from objects and is translated in images by the Inframetrics PM280 hand-held, high-resolution (65,000 pixels) thermography camera (Flir Inc., 16 Esquire Rd, North Billerica, MA 01862 USA). Images are then displayed as both black and white and color still images, as well as real time video. These images can then be analysis with the thermography software and used to evaluate the thermal gradient of reptile enclosures. Arboreal, terrestrial, and sub-terrestrial reptiles all have different thermal requirements, which have been difficult to replicate in the past. This technology allows for accurate thermal imaging of the entire enclosure, as well as the animals that live within them. An enclosures "thermal" design can then be altered to be suite individual species resulting in optimal husbandry and health.

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CLINICAL APPLICATIONS OF A SUPRAVERTEBRAL (SUBCARAPACIAL) VEIN IN CHELONIA

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Abstract

A variety of sites have been described for the purposes of blood collection and intravenous drug administration in chelonians, including the heart, jugular vein, brachial vein, ventral coccygeal vein, dorsal coccygeal vein, and the post-occipital sinus.[1,2] However, another large vessel that is positioned in a supravertebral position, just below the carapace, is available for blood collection and injection.[3]

This vertebral vessel may be approached with either the head extended from, or retracted into, the coelom. For example, in the red-eared slider (*Trachemys scripta elegans*) a 22-ga, 1-inch needle is positioned dorsal to the head and neck, on the midline close to where the skin attaches to the cranial rim of the carapace. The needle is advanced caudally at an angle (to the horizontal) of 0° to 60° incline and the vessel is located just dorsal to the spine.

This vein is particularly useful for blood collection from, or intravenous injection in:

1. Small or dehydrated individuals where peripheral vessels may be very small.
2. Those species that possess a front plastron hinge (e.g., *Terrapene* spp.).
3. Strong individuals that are capable of resisting head extraction for jugular sampling.
4. Individuals where the jugular or associated areas have been damaged or diseased.
5. Individuals where the jugular vessel is catheterized or is being reserved for catheterization.
6. Blood donor chelonians, where large volumes of blood are required quickly and easily.

Contrast radiography was performed by injecting iohexol (Omnipaque, Sanofi Winthrop Pharmaceuticals, New York, NY 10016 USA) at 500-580 mg/kg i.v. into the aforementioned vessel of two red-eared sliders (*Trachemys scripta elegans*) via the previously described technique. A distinct line of radio-opaque material was noted coursing along the midline in both cases. Iohexol was also injected into the equivalent vessel of an eastern box turtle (*Terrapene carolina*) and fluoroscopic radiography performed. Within 10 sec, the contrast material was identified in the heart. This suggests that the contrast material drained from a vertebral vessel to the junction of the external jugular vein into the heart. Anatomic dissections to further describe the position and utility of this vessel are underway.

This vessel has been utilized by the veterinary coauthors for blood collection and injection of anesthetic drugs, such as propofol, without any untoward effects. Therefore it is recommended as a potentially valuable i.v. access site in chelonians.

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COMPUTED TOMOGRAPHY OF PECTORAL GIRDLE INJURIES IN TWO CHELONIANS

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Abstract

Radiographic evaluation of the pectoral and pelvic girdles in chelonians is often complicated by the superimposition of the shell structures. The following report describes two cases where computed tomography (CT) was used in addition to radiography.

Case 1

A long-term captive, 4.6-kg, adult, 11-yr-old, male, radiated tortoise (*Geochelone radiata*) was admitted for evaluation of a 2-wk history of shifting lameness and muscle weakness. The animal was reported to be in good body condition with decreased appetite. Initial evaluation included physical examination, hematology and plasma chemistry analysis, fecal analysis and culture, and whole body radiography. On physical and radiographic examinations, diffuse swelling of the left pelvic limb was observed. During blood analyses, hyponatremia, hypochloremia, hyperproteinemia and a mild leukocytosis were detected. During radiography, moderate-to-severe intestinal distension was detected. Therefore, contrast radiography was performed (60% w/v barium sulfate suspension p.o.). Transit time through the stomach and the small intestine was clinically normal. Transit time through the large intestine presumptively was delayed. This finding was attributed to enteritis or partial intestinal obstruction; however, no filling-defect was observed and the contrast material passed completely. If abdominal pain were also present, then the paresis might have been a secondary problem. The left pelvic limb swelling was attributed to resolving cellulitis or trauma. At this time, treatment included fluid therapy (Lactated Ringer's Injection USP, Baxter Healthcare Corporation, Deerfield, IL 60015 USA) at 15 ml/kg s.c., s.i.d., vitamin B complex based on a dosage of thiamin (The Butler Company, Columbus, OH 43228 USA) at 10 mg/kg s.c., s.i.d., and vitamin E (Schering-Plough Animal Health Corp., Union, NJ 07083 USA) at 50 IU once. The tortoise also received ceftazidime (Tazicef®, SmithKline Beecham Pharmaceuticals, Philadelphia, PA 19101 USA) at 20 mg/kg i.m. every 72 hr, metoclopramide (Faulding Pharmaceutical Co., Elizabeth, NJ 07207 USA) at 0.06 mg/kg once, sucralfate (Martec Pharmaceutical, Inc., Kansas City, MO 64120 USA) at 500 mg once, and warm water enemas (60 ml) s.i.d. for 5 days. Three weeks later, improved appetite and regular defecation were noted. However, the lameness and muscle weakness persisted. Six weeks after the onset of therapy, the keepers noted persistent non-weight-bearing right thoracic limb lameness. The results of routine radiography were negative. The animal was transported to Cornell University College of Veterinary Medicine and whole-body CT was performed in transverse planes with contiguous 2-mm slices using a bone-window and algorithm.

The right humerus was displaced caudodorsally and the normal right shoulder joint space was not visible (Figs. 1-3). No degenerative bone or joint changes were observed. Therefore, a diagnosis of caudodorsal right shoulder luxation was made. The limb was maintained in a flexed position against the body with a bandage. Physical therapy was performed at each bandage change, about 3-4 times per wk, which involved a short period of massaging the muscles of the shoulder and arm, isometric and resistive movements, stretching, joint approximation, and assisted walking. Significant improvement in weight bearing and ambulating was noted within 3-4 wk. Within 2 mo, the tortoise was returned to its exhibit.

Case 2

A free-living, adult, snapping turtle (*Chelydra serpentina*) was admitted to the Cornell University Wildlife Clinic for treatment of a fractured carapace secondary to vehicular trauma. On physical examination, the turtle was depressed, weak, anemic (PCV - 10%; normal - 25%), and had poor withdrawal reflexes on the left side. In the carapace, a fracture line extended approximately 7.5 cm caudally from the left cranial margin. At the cranial margin of the fracture, the shell was displaced about 6.0 mm. Radiographic evaluation revealed that the left scapula also was fractured. Computed tomography evaluation confirmed the scapular fracture and better illustrated the extent (12 mm) of displacement (Fig. 4). The fracture wound was treated for 20 days and then stabilized with fiberglass and epoxy resin. The fracture was allowed to heal by second intention. Additional treatment consisted of half-strength Lactated Ringer's solution and 2.5% dextrose (Baxter Healthcare Corporation, Deerfield, IL 60015 USA) at 10.0 ml/kg i.p., s.i.d. for 7 days, cefazolin (Ancef, SmithKline Beecham Pharmaceuticals, Philadelphia, PA 19101 USA) at 22.0 mg/kg s.c., s.i.d. for 14 days, and enrofloxacin (Baytril, Bayer Corporation, Shawnee Mission, KS 66201 USA) at 2.5 mg/kg s.c., s.i.d. for 7 days followed by 5.0 mg/kg s.c., s.i.d. for the next 14 days. The anemia was treated with injections of iron dextran (The Butler Company, Columbus, OH 43228 USA) at 12.0 mg/kg i.m. once weekly for 5 wk, and vitamin B complex (The Butler Company, Columbus, OH 43228 USA) at 2.5 mg/kg of thiamin s.c., s.i.d. for 7 days. The turtle was kept out of the water until the shell was repaired but daily soaking in a shallow pool was allowed. Forty-two days after the initial examination, the turtle was eating, beginning to use the left thoracic limb, and released to a wildlife rehabilitator for further care. The turtle was released to the wild 9 mo later.

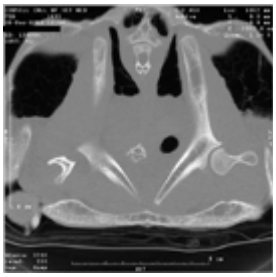

Figure 1. Male radiated tortoise - transverse CT scan (bone window) at the level of the shoulder. The left shoulder joint appears normal and the head of the humerus articulates with the scapula and coracoid.

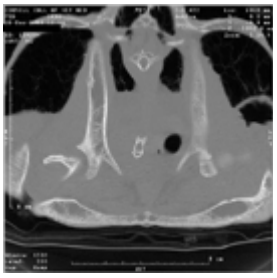

Figure 2. Male radiated tortoise - transverse CT scan (bone window) at a level immediate caudal to the left shoulder. The right scapula is visible but the head of the right humerus is not.

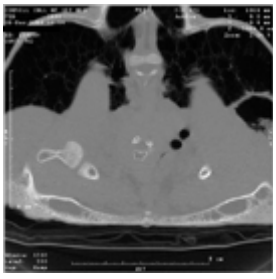

Figure 3. Male radiated tortoise - transverse CT scan (bone window) at a level caudal to both scapulae and shoulder joints. The head of the right humerus is visible, and is displaced caudodorsally from the right scapula.

Figure 4. Male snapping turtle - 3-dimension CT reconstruction of the fractured left scapula.

RENAL DIAGNOSTICS IN THE GREEN IGUANA (*Iguana iguana*)

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Abstract

Renal failure is a major cause of morbidity and mortality in iguanas.[2] Conventional mammalian diagnostics are not applicable for the diagnoses of renal failure in lizards. Urinalysis is unrewarding because of the inability of the reptilian kidney to concentrate urine (metanephric kidneys) and contamination of urine passing through the cloaca before storage in the bladder. Blood urea nitrogen and creatinine concentrations do not reflect renal failure in reptiles since they are uricotelic.[3] Radiographic or ultrasound imaging of kidneys is unrewarding in iguanid lizards due to the kidneys' location deep within the pelvic girdle, and the lack of functional information. Excretory urograms are of limited value for patients with poor renal function because of the poor contrast localization within a diseased kidney. Additionally, preliminary excretory urogram studies in clinically healthy Herman's tortoises (*Testudo hermanni boettgeri*) had only a 50% success rate of kidney visualization.[6]

Renal failure is currently diagnosed in reptiles by increased plasma uric acid concentrations and plasma calcium:phosphorus ratios < 1. These are not specific or reliable indicators, and occur late in disease.[1] Renal biopsy is the definitive test for diagnosing renal failure; however, indications to perform a biopsy, as previously stated, are not usually apparent until late in disease, if at all.

The goal of this project was to evaluate potential early diagnostic indicators of renal failure in iguanas. We have two hypotheses for early diagnostic indicators of renal failure. Hypothesis one assumes nuclear scintigraphy can be used to evaluate functional renal mass in iguanas as it can for mammals. Hypothesis two evaluates the theory that secondary renal hyperparathyroidism occurs in reptiles with renal failure. We established normal values for these two hypotheses using 10 healthy, adult, non-folliculogenic iguanas (0.91 to 2.77 kg; seven male, three female) fed a standardized diet and maintained with full spectrum lighting. Renal biopsies confirmed that this population did not have renal failure.

Hypothesis one was evaluated using ^{99m}Tc-dimercaptosuccinic acid (^{99m}Tc-DMSA) as a renal imaging agent. Dynamic images were acquired for 6 min with static imaging out to 20 hr following an injection of 1.83 ± 0.27 mCi in the ventral tail vein. ^{99m}Tc-DMSA is a cortical tubular agent and can be used as an indicator of functional renal mass. Animals in renal failure will have diminished ^{99m}Tc-DMSA uptake.[4] ^{99m}Tc-DMSA has also been used to evaluate renal morphology in both man and dogs. The uptake of ^{99m}Tc-DMSA at 20 hr was 14.3 ± 2.9% as measured on the ventral view and 12.7 ± 2.8% on the lateral view.

There are no commercial assays available that can detect changes in iguana parathyroid hormone directly. Therefore, hypothesis two was evaluated by analyzing total and ionized calcium, phosphorous, total and ionized magnesium, vitamin D_3, uric acid, and concomitant evaluations of standard radiographs and dual energy x-ray absorptiometry (DEXA) for bone density assessment. The use of DEXA technology in human medicine is most noted in osteoporosis research but has expanded to include research in renal disease.[7]

The complete blood count and serum chemistry values were similar to previously published reports.[5] The other mean plasma values of interest were: ionized magnesium (0.82 ± 0.12 mmol/L); total magnesium (2.16 ± 0.35 mEq/L); ionized calcium (1.68 ± 0.11 mmol/L ; total calcium 13.6 ± 2.59 mg/dl); vitamin D_3 (154.9 ± 80 nmol/L). DEXA scans determined the mean total bone mineral density of healthy iguanas to be 0.56 ± 0.06 g/cm^2; and the total bone calcium to be 1.34 ± 0.04 g calcium expressed as a percentage of body weight.

This study standardized values of DMSA uptake, bone quality (DEXA), and plasma values relevant to renal secondary hyperparathyroidism, in a population of iguanas with healthy kidneys. DMSA appears to be a promising indicator for kidney health in iguanas. Further studies need to be performed to evaluate all of these parameters in a population of renal failure iguanas.

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ANATOMIC STUDY BY MEANS OF ULTRASONOGRAPHY IN TUATARA (*Sphenodon punctatus*)

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Abstract

The only recent species of the order Rhynochocephalia is the tuatara, *Sphenodon punctatus*, which is native to some islands of New Zealand. It is a unique species–a living fossil–fitting in its ecologic niche with an interesting strategy for 200 million yr.

The tuatara has an unexpectedly low optimum temperature of about 12° C. Also unique is the anatomy of this rare and endangered species. Our study gave a closer view to some very special anatomic structures that could not be found in any other species in the world. We examined nine young animals of both sexes frequently for 3 yr by transcutaneous ultrasonography. A special particularity is a relict structure from a very early step of evolution, the parietal eye. Its situation could be imaged by ultrasonography, as well as the peculiar construction of the skull with its osseous temple bridges. Further, the organs of the gastrointestinal tract and urogenital tract could be visualized.

Ultrasonography was, one more time, a fine tool to investigate covered structures in a not well discovered species. It is one of the best fitting methods of examination in order to get detailed information about anatomy in a non-invasive way even in such rare species.

BILATERAL PHACOFRAGMENTATION IN A KOMODO DRAGON (*Varanus komodoensis*)

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Abstract

Although not an uncommon clinical entity in reptiles, there is a paucity of published information on the etiology, pathogenesis, clinical manifestations and treatment of cataracts in reptiles. Lenticular degeneration with opacification, or cataract formation, probably occurs in reptiles for similar reasons as in mammals and birds. Cataracts are usually classified by age of onset (congenital, juvenile, senile), anatomic location, cause, degree of opacification (incipient, immature, mature, hypermature), and shape. Cataracts may be inherited (particularly in dogs) but other etiologies include diabetes mellitus, malnutrition, radiation, inflammation, and trauma. Cataracts may be unilateral or bilateral depending on the etiology, and the rate of cataract formation may be different in each eye. Most cataracts result in impaired vision to a greater or lesser degree. Vision may be regained in young animals when cataracts undergo sufficient spontaneous resorption, and congenital nuclear cataracts in young animals may reduce in size with the growth of the lens, restoring vision as the animal grows.[1,6]

Lenticular sclerosis characterized by variable degrees of central fiber compaction in the substance of the crystalline lens has been reported in reptiles.[2] This causes the lens to appear slightly silver-grey but does not seem to impair vision. The etiology of cataracts in reptiles is often obscure. In those few cases where an etiology could be surmised, it was usually secondary to uveitis or trauma. Nutritional and environmental factors have also been implicated in some cases. They have been reported and observed in a wide range of reptile species.[4] Lens opacity may vary from small focal nuclear or cortical opacities, to dense cataracts. Cataracts have been reported in reptiles at various ages with some being evident at birth.[4] Juvenile cataracts have been reported in young varanid lizards.[2] The etiology is unclear, although a genetic predisposition has been suggested. Senile cataracts in aged captive reptiles have been reported. Post hibernation blindness in European tortoises due to cataract development, vitreal opacification, and central nervous system, deficits is a well recognized syndrome.[2,3]

At present the only definitive therapy for cataracts is surgical removal of the lens. Early cataract removal using current surgical techniques (phacofragmentation) has high success rates in other species.[5] There are no reports in the readily available literature of cataract extraction in a reptile. This paper reports successful bilateral phacofragmentation with restoration of vision in an adult male Komodo dragon (*Varanus komodoensis*).

Case Report

An 18-yr-old, 85.6-kg, male Komodo dragon (*Varanus komodoensis*) was noted to have progressive loss of vision in the right eye over a period of approximately 3 mo. Ophthalmic examination included slit lamp biomicroscopy and indirect ophthalmoscopy. The right eye had a mature cataract. There was no menace response, but the eye was light perceptive. The lens of the left eye had mild opacity on the anterior and posterior lens capsule. Fundic examination was normal for the left eye. Vision from this eye was not impaired. The animal was physically restrained and anesthetized using isoflurane in oxygen administered via a face mask, intubated with a size 10 endotracheal tube, and maintained on isoflurane in oxygen using intermittent positive pressure ventilation at four breaths per min. An attempt to dilate the pupil with intracameral pancuronium (Pavulon, Organon Teknika, Baulkam Hills, N.S.W., Australia) was unsuccessful.

A 3.2-mm incision was made at the dorsal limbus using a 45°, matte finish, bevel-up keratome blade (Becton Dickinson and Co., Franklin Lakes, NJ 07417-1881 USA). Air was injected into the anterior chamber thereby displacing the aqueous fluid. A capsule-specific dye (Vision Blue, Dutch Ophthalmic Research Centre, Zuidland, The Netherlands) was used to stain the lens capsule and facilitate the lens capsule removal. A continuous curvilinear capsulorhexis was then performed (4.0 mm diameter). The lens nucleus and cortex were then removed almost exclusively by aspiration via the phacofragmentation unit (Phacoplus, Allergan Medical Optics, Gordon, N.S.W., Australia). The corneal incision was sutured using 8/0 Ethilon on a spatula needle (Ethicon, Johnson and Johnson, Somerville, NJ 08876-0151 USA) in a single interrupted pattern. The globe was reinflated with an air bubble.

Post operative treatment included carprofen (Rimadyl, Pfizer Animal Health, West Ryde, N.S.W., Australia) at 3.5 mg/kg s.i.d., s.c, doxycycline (Vibravet 100 Paste, Pfizer Animal Health, West Ryde, N.S.W., Australia) at 10.0 mg/kg s.i.d., p.o., chloramphenicol plus polymixin B (Opticin eye ointment, Ilium Veterinary Products, Smithfield, N.S.W., Australia) topically t.i.d., and dexamethasone (Maxidex eye drops, Alcon Laboratories, Frenchs Forest, N.S.W., Australia) topically t.i.d. Four days post surgery the cornea was generally clear with a small area of local corneal edema around the suture line. A small amount of white flocculent material was present in the anterior chamber. The doxycycline, topical antibiotics, and dexamethasone were continued for 2 wk post surgery and carprofen was continued for 3 wk. At 16 days post surgery the eye was open, the cornea clear, and the white flocculent material in the anterior chamber had disappeared. It did seem however that the animal was irritated by the eye as he was rubbing it, resulting in ulceration of the skin over the eye brow ridge. Although there was no apparent cause for the irritation, a mild uveitis was suspected. Oral doxycycline was recommenced and continued for a further 7 days. At 22 days post surgery all treatment ceased and by 30 days the eye appeared normal. There was no apparent irritation and vision in the eye appeared to have been restored.

One and a half months after surgery on the right eye, the lens of the left eye had become completely opaque and vision was impaired. Four months after the first surgery the animal was again anesthetized and the left lens removed as previously described. Post operative treatment included

carprofen at 4.0 mg/kg i.v. once, then 4.0 mg/kg s.i.d., p.o. for 5 days, then 2.0 mg/kg s.i.d., p.o. for 9 days, then 2.0 mg/kg every 48 hr for 7 days, and enrofloxacin (Baytril, Bayer, Pymble, N.S.W., Australia) at 5mg/kg s.i.d., p.o. for 7 days. A combination antibiotic and corticosteroid (neomycin, polymixin B, sulfacetamide, prednisolone) ointment (Amacin, Jurox, Rutherford, N.S.W., Australia) was used topically b.i.d. in the eye for 5 days. At 1 wk post surgery some white flocculent material was noted in the anterior chamber but no other abnormalities were detected. Recovery was more rapid after the second surgery and the eye appeared normal and vision restored by 3 wk post surgery. One year after the procedures the animal is normal and appears to have vision comparable to that prior to the development of cataracts.

Discussion

This patient represented a unique opportunity to use a well-established surgical technique to restore vision in a non-mammalian species. Limitations of cataract surgery in this species mainly involved the inability to establish mydriasis using previously reported techniques. Although not specifically reported in this species, the assumption made was that like other reptiles, the iris would be made of skeletal muscle. The lack of response to skeletal muscle blockade using pancuronium is hard to explain. However, in the author's experience with other reptiles there does not appear to be a uniform response to intracameral skeletal muscle blockers. The possibility certainly exists that there could be a smooth muscle component to the iris musculature in Komodo dragons.

Some interesting observations can be made in comparing the surgical procedure between reptiles and mammals. Ocular physiologic phenomenon between mammalian and reptilian eyes has been previously investigated.[7] The surgical procedure itself was remarkably easy compared to similar aged domestic mammals. Of particular note is that the lens itself was incredibly soft, and required little if any phacofragmentation time. In fact, the lens removal was similar in nature to that of a juvenile cataract in a mammal requiring aspiration only. Also, in spite of the age of the animal, the lens capsule itself was very elastic, with no capsular fibrosis. There was also no need for capsular vacuuming after lens extraction, as there were no residual lens epithelial cells seen through the operating microscope. These differences could be accounted for by intrinsic anatomic differences, or the fact that aging proceeds at a rate more typical of humans.

In hindsight, a clear corneal incision for entry into the anterior chamber would have been preferable, as closure of the limbal incision was difficult owing to the presence of scleral ossicles. Hyaline cartilage is present in the sclera of lizards and chelonians from the equator to the posterior pole, with scleral ossicles extending anteriorly from the equator to the limbus.[3] This meant that the needle used was quickly dulled and difficult to pass.

Post operative care was aimed at controlling uveitis. The presence of some flocculent material postoperatively may have been lens material inadvertently missed (especially in light of the lack of mydriasis), or more likely a sterile inflammatory reaction. It did not seem to have a detrimental effect on the final outcome. The use of topical and systemic anti-inflammatory drugs no doubt greatly helped in control of post operative inflammation. The success of bilateral

phacofragmentation in this Komodo dragon suggests that cataract removal in other reptilian species is a viable option.

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CONTROL AND ERADICATION OF EXOTIC TICK INFESTATIONS ON REPTILES

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Abstract

Background

In 1997 the exotic African tortoise tick, *Amblyomma marmoreum,* was identified in Florida outside importation facilities on a reptile-breeding premises where it had become established.[1] Subsequent investigations found that at least 11 exotic tick species had been imported into Florida on reptiles, with at least seven species disseminated beyond importation facilities.[4,10] These findings suggested that there was an unregulated flow of exotic ticks into the United States. This was a cause for concern given that the international trade in live reptiles is increasing and that some of the imported ticks are associated with diseases of veterinary or public health significance.[3] This concern was heightened when *A. marmoreum* was confirmed as a capable vector of heartwater,[8] a fatal disease of domestic and wild ruminants, and when exotic large reptile ticks (*Amblyomma sparsum*) infesting a shipment of leopard tortoises (*Geochelone pardalis*) imported into Florida from Africa were found to be infected with *Cowdria ruminantium*,[5] the causative agent of heartwater.

These findings prompted immediate studies to identify an acaricide that would kill tick infestations on reptiles in a safe and efficacious manner and to develop methods for eradication of tick infestations from premises housing reptiles. The results of these studies are summarized in this abstract.

Identification of Acaricide to Control Exotic Tick Infestations

Since no acaricide was registered in the United States for use on reptiles at the onset of this study, those acaricides registered for use on other domestic animals were chosen for investigation. They were amitraz, carbaryl, chlorpyrifos, cyfluthrin, fipronil, lindane, permethrin and pyrethrins. All acaricides were tested for activity against *A. marmoreum* ticks, and five (amitraz, carbaryl, chlorpyrifos, cyfluthrin and permethrin) were tested for toxicity on leopard tortoises. Only cyfluthrin and permethrin produced 100% tick mortality at a dilution of 1% in acetone, with both producing high mortality (>85%) when used in dilutions up to 0.01%. The only side effect of any duration was eye irritation which was seen only with carbaryl and chlorpyrifos.

This study demonstrated that cyfluthrin and permethrin were acaricides effective in killing *A. marmoreum* ticks and both were safe when used on leopard tortoises. However, cyfluthrin, unlike permethrin, has been found to be toxic for snakes and lizards even at very low doses.[6] Consequently, permethrin was the acaricide chosen for further safety testing, and Provent-a-Mite™

(Pro Products, Mahopac, NY), a formulation of permethrin specifically manufactured for use on reptiles,[9] was the product selected. Provent-a-Mite™ was tested for use on African spurred tortoises (*Geochelone sulcata*), rosy boas (*Lichanura trivirgata*) and green iguanas (*Iguana iguana*), using 10 times the recommended dosage every fifth day for a total of six applications. For each treatment, Provent-a-Mite™ was sprayed directly on the animal (for tortoises) or directly on the substrate (for snakes and lizards). No signs of ill health were detected in any of the tortoises, snakes or lizards exposed repeatedly to excessive amounts of Provent-a-Mite.™

Development of Methods for Eradication of Exotic Tick Infestations

During our investigations of exotic tick infestations in Florida,[3] it became evident that numerous shipments of infested reptiles had been imported and that at least two exotic tick species, *A. marmoreum* and *A. sparsum*, had already become established as breeding populations. Consequently, there was an urgent need to develop methods to eradicate these tick infestations in order to minimize the risk that such exotic vectors of heartwater would spread to native fauna and thus become established as indigenous tick species, as has happened in past years in Florida with the iguana tick *Amblyomma dissimile*[2] and the rotund toad tick *Amblyomma rotundatum*.[7]

We developed a protocol whereby all reptiles on the infested premises were treated with permethrin (Provent-a-Mite™) and removed to a tick-free area. Then the premises were sprayed by a licensed pest control company with a cyfluthrin product specifically formulated for premises treatment (Tempo®, Bayer Corporation, Kansas City, MO) on two occasions 2 wk apart, ensuring that all surface areas including housing and burrows were treated. Finally 1 wk after the second premises treatment, sentinel tortoises, such as Hermann's tortoises (*Testudo hermanni*) which roam freely and whose skin area is easy to inspect, were placed on the treated premises for 10 days. If the sentinel tortoises remained free of ticks, the infestation was considered to have been eradicated from the premises. Using this protocol, we have successfully eradicated both *A. marmoreum* and *A. sparsum* infestations from reptile premises in Florida.

Conclusions

A permethrin product specifically manufactured for use on reptiles (Provent-a-Mite™) has been found to provide excellent control of exotic ticks on tortoises, snakes and lizards. Use of this permethrin product on reptiles, together with spraying of the environment with a cyfluthrin product specifically formulated for premises treatment (Tempo®), have been shown to be effective in the eradication of exotic ticks from infested reptile premises. The recommended method of administration of Provent-a-Mite™ differs by reptilian species. For tortoises, the product is sprayed directly onto the animal from a distance of a few inches (10 cm), using one 1-sec burst of spray into each leg opening for small tortoises and two 1-sec bursts for large tortoises. For snakes and lizards, the animals are removed from their containers and the product is sprayed from a distance of about 12-15 in (30-40 cm) directly onto the substrate at the rate of one 1-sec burst per 1 ft^2 (30 cm^2), with the snakes and lizards returned to the treated container only after the spray has completely dried and all vapors have dissipated.

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CHEMOTHERAPEUTIC TREATMENT OF LYMPHOMA IN A KING COBRA (*Ophiophagus hannah*)

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Abstract

Reports of lymphoma or lymphosarcoma with or without lymphoid leukemia are becoming common in the reptile literature.[2-5,7,11,14,16] The vast majority of these reports consist primarily of necropsy and histopathologic findings. Increasingly however, treatment modalities are being attempted for reptile neoplasias.[1,9,10,12,13,15] This report describes an attempt at chemotherapy for lymphoma in a king cobra (*Ophiophagus hannah*) at the Gladys Porter Zoo.

In November 1996, a captive-born, 2.8-m, adult, 2.0-kg, female cobra was presented for a chronic wound of the right thoracic area. Previous history included one fecal sample positive for strongyles and a mild dystocia in 1991. A keeper recalled that the snake was bitten in that area by a Philippine crocodile (*Crocodylus novaeguineae mindorensis*) approximately 6 mo earlier, and that the wound never healed.

A physical examination was performed using manual restraint. The wound was located 30 cm distal to the head and appeared pink, oblong (2.5 × 5.0 cm), roughly marginated, and non-scaled. It moved easily with the skin and did not appear to be associated with any deeper structures. The tissue was very friable but did not appear necrotic. The ventral scutes at that area also seemed abnormal, appearing involuted. A blood sample was obtained via cardiocentesis and a full-thickness punch biopsy of the site was taken. Differential diagnoses included granulation tissue or a neoplastic process.

Hematology indicated leukocytosis and a lymphocytosis; lymphocytes composed 88% of the total white blood cell count (Table 1). The only abnormality on the plasma biochemistry was hyperglobulinemia (5.1 g/dl; normal value: 3.9 g/dl).[6] The biopsy result indicated severe, granulomatous cellulitis with atypical lymphocytes. The atypical lymphocytes suggested a pre-neoplastic change and a wide surgical excision of the lesion was recommended.

In December 1996, the snake was intubated using manual restraint and anesthetized with isoflurane (Aerrane, Fort Dodge, Overland Park, KS 66210 USA) for surgical removal of the mass. By February 1997, the surgical site was well-healed, the sutures were removed, and the snake was

eating well on its own.

By April 1997, the mass had returned at the primary site, with a second lesion present at the site of the involuted ventral scutes identified in November 1996. At both sites, the surrounding scales appeared edematous and exuded clear, serous liquid. Samples were collected for hematology, plasma biochemistry, fluid cytology, bacterial culture, and histology.

Abnormalities on hematology and plasma biochemistry included leukocytosis and lymphocytosis along with an increased total solids (9.0 mg/dl; normal value: 5.7 mg/dl) and hyperglycemia (108 mg/dl; normal value: 58 mg/dl).[6] Cytology of the fluid from the lesions indicated a secondary bacterial infection, and coagulase negative *Staphylococcus* sp. and *Acinetobacter calcoaceticus* were identified from the culture of the lesions. The biopsy results indicated lymphosarcoma at both sites. The snake was placed on amikacin (Amiglyde-V, Fort Dodge, Overland Park, KS 66210 USA) at 2.5 mg/kg s.c. q 72 hr for five treatments, concurrent fluid administration (two parts 2.5% dextrose in 0.45% NaCl and one part Ringer's) at 25 ml/kg s.c. q 72 hr, and the masses were cleaned with dilute chlorhexidine (Vedco, Incorporated, St. Joseph, MO 64504 USA) q 72 hr.

In May 1997, the snake was again anesthetized with isoflurane and both masses were surgically excised. This surgery resulted in extensive tissue loss, down to the level of the peritoneum on the ventral aspect of the site, and the ribs were compressed ventrally with suture to provide protection for the coelomic cavity. The surgical site was dressed with Duoderm (Bristol-Myers Squibb, Company [Conva-Tec], Princetown, NJ 08543-5254 USA), wrapped with petrolatum gauze (Sherwood Medical, St. Louis, MO 63103 USA), and covered with Tegaderm (3-M, St. Paul, MN 55144-1000 USA). The petrolatum gauze kept the wound moist as well as inhibited the snake from "crawling out" of its bandage. The snake was started on enrofloxacin (Baytril, Bayer Corporation, Shawnee Mission, KS 66201 USA) at 10 mg/kg s.c. q 24 hr for 10 treatments. By June 1997, the snake had shed and was eating well; the surgical site continued to heal slowly. However by July 1997, the masses regrew at both surgical sites.

In August 1997, the two original surgical sites were still being dressed and two new skin lesions appeared, one 60 cm distal to the head on the left side, and one 17 cm cranial to the vent on the left side. A chemotherapy regimen was instituted using L-asparagine aminohydrolase (FloridaInfusion, Palm Harbor, FL 34683 USA), vincristine (FloridaInfusion), and prednisone (Meticorten, Schering-Plough Animal Health, Union, NJ 07033 USA) (Table 1). Periodic hematology and plasma biochemistry, and size and appearance of the skin lesions were used to monitor the efficacy of the chemotherapy protocol.

The snake tolerated handling and administration of drugs reasonably well but generally required forced feeding. Clinical impressions by the keeper staff were that the snake was more aggressive than usual, seemed hungry but didn't want to eat, had lighter overall skin color, occasionally made uncoordinated movements, was possibly in pain as it was more likely to bite than hood, and had poor muscle tone.

Within 1 wk, the skin lesions appeared to respond to the chemotherapy, becoming smaller and less "angry" looking, with areas of necrosis. Hematology parameters showed a decrease in the total white blood cell count, lymphocyte count, and the percentage of lymphocytes (Table 1). In addition, other changes in the hematology and plasma biochemistry included a decrease in packed cell volume (16%; normal value: 28.7%), hyperglycemia (110 mg/dl; normal value: 58 mg/dl), hypoproteinemia (4.3 g/dl; normal value: 6.8 g/dl), and hypoalbunemia (1.5 g/dl; normal value: 2.8 g/dl).[6]

Because the lesions were not regressing as anticipated, a second round of chemotherapy was initiated in early September 1997 (Table 1). But by the end of September 1997, the tumors had once again begun to grow and metastasize to new sites on the body. The snake developed ventral edema and was started on enrofloxacin at the previous dose. The chemotherapy regime was discontinued.

In October 1997, the snake was having trouble shedding and had many unhealthy areas of skin on its body, however, its attitude and appetite were excellent. At this time, a different chemotherapeutic regimen (Table 1) was initiated using prednisone and chlorambucil (FloridaInfusion). By November 1997, the lesions had not progressed but the snake was again being force fed. By December 1997, the lesions were once again growing. The snake was found dead on 8 February 1998, 15 mo after presentation.

At necropsy, the snake weighed 1.6 kg and was in poor body condition. There were four cutaneous lesions as previously described, with the original two sites coalescing into a single mass. The thymus was grossly enlarged (11 × 3 cm) and there were several vertebral swellings noted. Samples of tissues were collected in 10% buffered formalin and submitted for histopathology. Histopathologic lesions included multicentric, intermediate to high grade lymphoma involving most of the visceral organs and bone marrow. Appreciable treatment effect could not be confirmed. In addition, there was mild gastroenteritis, mild glomerulosclerosis of the kidney, moderate atrophy of the fat bodies, and moderate, degenerative osteoarthritis of the vertebrae. Thymus prepared for electron microscopy failed to demonstrate any viral structures; no virus was isolated from thymus tissue passaged on viper heart cell line.[8,17,19] Additional efforts to further characterize the lymphoma are ongoing.[18]

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Table 1. Chemotherapy protocol and response to treatment of lymphoma in a king cobra (*Ophiophagus hannah*) at the Gladys Porter Zoo.

Date	Treatment	Dosage[a]	WBC[b]	Lymphocyte[c]
11 Nov 96	Presentation		16,200	14,260
26 Nov 96	Recheck		14,800	12,880
5 Dec 96	Surgical excision			
25 Apr 97	Recheck		15,600	13,100
15 May 97	Surgical excision			
12 Aug 97	Recheck		6,800	3,944
28 Aug 97	L-asparginase	10,000 IU/m^2 s.c. once	5,200	19,150
	Prednisone	40 mg/m^2 s.c. q 48 hr		
3 Sep 97	Vincristine	0.5 mg/m^2 i.v. once[d]	9,400	6,110
	Prednisone	40 mg/m^2 s.c. q 48 hr		
11 Sep 97	L-asparginase	10,000 IU/m^2 s.c. once	9,300	6,882
	Prednisone	40 mg/m^2 s.c. q 48 hr		
17 Sep 97	Vincristine	0.5 mg/m^2 i.v. once	18,200	13,100
	Prednisone	40 mg/m^2 s.c. q 48 hr		
25 Sep 97	Discontinued			
8 Oct 97	Chlorambucil	2.0mg/m^2 p.o. q 24 hr × 30 T[e]		
	Prednisone	40 mg/m^2 p.o. q 24 hr × 30 T		
23 Oct 97	Recheck		3,500	2,485
7 Nov 97	Recheck		4,200	2,730
7 Feb 98	Death			

[a]Animal calculated to be 0.2 m^2.
[b]White blood cell count; ISIS normal = 7.614 ± 6.993 × 10^3/µl.[6]
[c]ISIS normal = 4.063 ± 5.515 × 10^3/µl.[6]
[d]Administered intracardiac.
[e]Treatment.

VENOMOID SURGERIES IN SNAKES

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Abstract

Venomoid surgery is a surgical technique developed to render a venomous snake functionally nonvenomous. The first descriptions of this surgery involved an approach to the venom duct through the scales on the side of the snake's face, transecting and double-ligating the venom ducts; the venom gland remained in situ. This technique resulted in facial scars and a significant number of patients developed a granulomatous response at the surgical site.

An intraoral approach was developed by the author. An incision is made through the oral mucosa between the maxillary teeth and the lip margin, exposing the venom gland and its duct. The entire venom gland and venom duct are then removed. A silicone prosthesis is then placed into the site to help preserve the cosmetic appearance of the head postoperatively. A segment of surgical stainless steel suture, or a transponder microchip, is placed inside the prosthesis to facilitate future identification of the snake. The incision is closed with absorbable suture in a continuous pattern. The patient is not fed for 3-4 wk postoperatively and is fed only dead prey thereafter. Legally, most jurisdictions still consider a snake in which the venom gland has been surgically removed to be a venomous species although it is no longer functionally venomous.

DIODE LASER SURGERY IN REPTILES AND AMPHIBIANS

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Abstract

Since Albert Einstein theorized the concept of lasers in 1916, considerable development has resulted in an array of equipment available to the human and veterinary surgeon. The term LASER stands for "light amplification by the stimulated emission of radiation" and relies upon the production of electromagnetic radiation in response to photon emission by the lasing medium. The characteristics of a laser are directly attributed to the lasing medium. A class IV gallium-aluminum-arsenide diode laser creates a wavelength in the range of 790-830 nm, which is transmitted from laser unit to surgical site by a solid quartz-core, fiber-optic cable. A visible light beam is combined with the invisible laser beam to facilitate aiming. Laser fibers come in a variety of sizes (400-1000 μm) and shapes, including flat, conical, or orb tips. The fibers may be used through the instrument channel of a variety of rigid and flexible endoscopes, or in hand-pieces for open surgical use. Operator safety necessitates the use of protective glasses by all personnel.

The diode laser (Diomed, Inc., 30-31 Union Warf, Boston, MA 02109 USA) can be used in direct contact with tissue (contact mode) or at a distance from tissue (non-contact mode). In contact mode, the fiber tip is coated with a thin layer of carbon, which is heated as it absorbs laser energy. The heated tip can then be used to incise, excise, and coagulate tissue resulting in a 300-600 μm zone of thermocoagulation, which provides excellent hemostasis of vessels up to 2mm in diameter. In non-contact mode the laser is aimed at the tissue from a short distance. The degree of tissue penetration, coagulation, and vaporization depends upon laser power, duration of exposure, and tissue characteristic, particularly pigmentation.

The diode laser has been successfully used for various procedures in reptiles and amphibians including coeliotomy, ovariosalpingectomy, orchidectomy, cystotomy, enterotomy, abscess excision, tail/limb/digit amputation, oral surgery, enucleation and transendoscopic ablation of lesions within the respiratory tract and coelom. For general surgery, the 1000 μm fibers are preferred, but when performing microsurgery or endosurgery the 400-600 μm fibers with micro-conical tips provide greater accuracy and finer control. These procedures were performed using power settings between 3.5 and 7.5 watts.

Examples of these procedures are described to illustrate the versatility of the diode laser in herpetologic surgery and, as trials continue, it is expected that the indications for such techniques

will increase.

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FENBENDAZOLE OVERDOSE IN FOUR FEA'S VIPERS *(Azemiops feae)*

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Abstract

Fenbendazole is a widely used anthelmintic drug in veterinary medicine that has a wide range of activity, high degree of efficacy and generally, a wide margin of safety in most species.[6,8,9] However, fenbendazole and albendazole have been associated with toxicoses in some species. Toxic changes include radiomimetic lesions in the bone marrow and small intestine in birds, [7,9,11] elevated liver enzymes in quail,[3] bone marrow suppression in dogs, cats and birds,[7,13] teratogenic effects in rodents and sea urchins,[1,4] and tumor promoter effects in rats.[10] Fenbendazole is widely prescribed as an anthelminthic in reptiles, and to date, no toxic effects have been documented in reptiles in regard to this drug. This report describes clinical presentation and pathology associated with fenbendazole overdose in four Fea's vipers (*Azemiops feae*).

Two adult male and two adult female Fea's vipers entered quarantine at the Dallas Zoo within 2-3 wk of collection in Asia. Fea's vipers have historically been difficult to maintain in captivity and previous mortalities have shown moderate to severe intestinal nematodiasis. The broker that shipped these animals administered a single oral dose of 25 mg fenbendazole (Panacur, 100 mg/ml, Hoechst Roussel Vet, Warren, NJ 07059 USA) prior to shipment. On entry to quarantine, the two males weighed 37 g and 48 g and the two females weighed 24 g and 58 g respectively. Snakes were assist-fed, but largely inappetent on their own. All but the largest female lost weight during the time period. The minimal fecal material available for analysis was negative for parasitic ova. Males died 31 days and 32 days post-entry to quarantine and the females died 40 days and 71 days post-entry.

Necropsy of all snakes revealed good body condition and segmental thickening and reddening of the entire small intestine. No parasites were found. The most significant histopathologic changes were in segments of the small intestine of all snakes. The effected intestine had moderate to severe necrosis of mucosal epithelium generally along the entire length of the villous, and particularly severe at the base of the villous. Complete ulceration and stromal collapse occurred in some areas. Mildly increased numbers of lymphocytes, plasma cells, histiocytes, and eosinophilic granulocytes were in the lamina propria, accompanied by extensive congestion and edema. Few areas of mucosal epithelial regeneration were detected. The lesions were associated with bacterial overgrowth. All snakes also had mild to moderate fibrinous rhinitis with intralesional bacteria. Changes consistent with sepsis were seen in all snakes. Electron microscopy performed on formalin preserved gut mucosa from the first snake that died revealed degeneration and necrosis of enterocytes. No viral particles were seen.

Reptiles and amphibians have intestinal mucosal morphologic features that differ from those of mammals and birds. The latter have crypts of rapidly dividing cells that replace the sloughed enterocytes of the villi. Reptiles do not have crypts, but have long villi and villous folds,[5] and appear to replace sloughed enterocytes directly from underlying basal cells of the villi. The radiomimetic intestinal lesions seen in birds with fenbendazole toxicosis manifest predominantly in the crypts, where cell turnover is highest. A radiomimetic insult to enterocytes in a reptile would be expected to have a more generalized distribution in the villous enterocytes, as seen in these cases. Secondary intestinal changes are similar to those of affected birds.

The recommended dose for fenbendazole in reptiles is 50-100 mg/kg p.o. once, repeated in 2 wk[2,12] or 50 mg/kg p.o. q 24 hr for 3-5 days.[2] The estimated oral dose of fenbendazole administered to these vipers was 25 mg. The calculated dosage was 685 mg/kg and 526 mg/kg for the two males and 1064 mg/kg and 428 mg/kg for the two females.

Little is know about this snake species and they have proven to be a challenge to maintain in captivity. The estimated dose of fenbendazole administered to these snakes was considerably higher than the recommended dose. The size and nature of these snakes precluded extensive diagnostic testing. Fenbendazole is regarded as a safe anthelmintic, which can affect how it is administered to individual animals. These findings document the effects seen with a fenbendazole overdose in Fea's vipers.

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ISOLATION OF VIRUSES FROM BOA CONSTRICTORS (*Boa constrictor* spp.) WITH INCLUSION BODY DISEASE IN EUROPE

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Abstract

Inclusion body disease (IBD) is characterized by the formation of intracytoplasmatic inclusions in neurons and in epithelial cells of various organs. It generally affects boid snakes. Clinically, it is commonly associated with central nervous system (CNS) disorders as well as regurgitation, stomatitis, and pneumonia. The disease is believed to be of viral etiology, and retroviruses have been implicated as a possible factor.[8,16] However, boa constrictors (*Boa constrictor* spp.) have also been shown to harbor endogenous retroviruses,[13] making a definitive connection between retroviruses found in IBD positive snakes, and disease difficult.

In the course of this study, tissues from 12 boa constrictors from Germany found to be IBD positive, were tested for cytopathogenic viruses (Table 1). These animals stemmed from three different captive populations and were all captive-bred in Germany. Two IBD-negative animals from populations with no history of IBD, were also included in the study (Table 1). Virus isolation was attempted on viper heart cells (VH2, ATCC CCL-140), terrapene heart cells (TH-1, ATCC CCL-50), and chick embryo fibroblasts (CEF)[15] in animals from population A, and from primary cell cultures and on VH2, in animals from populations B and C and the two negative controls. A polymerase chain reaction (PCR) for retrovirus sequences was performed on tissues from the animals in the study as indicated in Table 1. The degenerated oligonucleotide primers 5'-GT(T/G) TTI (G/T)TI GA(T/C) ACI GGI (G/T)C-3' and 5'-ATI AGI A(G/T)(G/A) TC(A/G) TCI AC(A/G) T-3'[13,18] were used. These are specific for conserved motives in retroviral protease and reverse transcriptase genes. The same PCR was used to test the isolated cytopathogenic agents, as well as uninfected VH2.

Cytopathogenic viruses were isolated in VH2 from a total of five different animals from populations A, B, and C as shown in Table 2. Three of the isolates were identified as reoviruses on the basis of their cytopathic effect (CPE), chloroform stability, lack of inhibition by 5-iodo-2'-desoxyuridin (IUDR), which inhibits DNA-polymerase, and morphology by electron microscopy. Two of the isolates were identified as adenoviruses by the same means. The growth of these isolates was inhibited by the addition of IUDR to the cell culture medium. The PCR for the detection of retroviral sequences was positive in several cases (Table 3). It was not positive on any of the cell cultures tested.

Reoviruses[1,2,11,12,14,19] and adenoviruses[3,4-7,9,10,17] have frequently been reported in reptiles. Reoviruses have also been associated with CNS disorders in reptiles[2,19] as have adenoviruses.[3,7] The significance of the viruses isolated in this study for the IBD seen in the examined animals is unclear. One or both of the viruses found could play a role in the etiology of this disease. It is possible however, that these viruses were opportunistic pathogens, and were only found because of an immune suppression caused by the IBD. Further studies are planned to clarify these questions.

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Table 1. Animals tested.

Animal No.	Age	Sex	Tissues[a]	PCR[b]	Population	Symptoms
1	mature	f	lu, li, int, br, blood, sp, pancr, kidn	li, pancr	A	CNS, enteritis, stomatitis, euthanatized
2	4 mo	m	lu, li, int, br, kidn	-	A	died
3	4 mo	m	lu, li, int, br, kidn	li	A	CNS, euthanatized
4	4 mo	m	lu, li, int, br, kidn, blood	li	A	gaseous intestine, euthanatized
6	4 mo	m	lu, li, int, br, kidn, blood	li	A	died
8	4 mo		blood	-	A	
12	4 mo	m	lu, li, int, br, kidn, blood	li, pancr	A	CNS, euthanatized
18	4 mo		blood	-	A	
20	4 mo		blood	-	A	
21	4 mo		blood	-	A	
26	juvenile	f	li, kidn, bm, blood	li, blood cells	B	CNS, euthanatized
27	juvenile	m	li, kidn, bm, blood	li, blood cells	C	CNS, euthanatized
31	juvenile		li, kidn, bm, blood	li, blood cells	D	neg. control
32	juvenile		li, kidn, bm, blood	li, blood cells	E	neg. control

[a]Tissues collected for virus isolation: lu = lung, li = liver, int = intestine, br = brain, sp = spleen, pancr = pancreas, kidn = kidney, bm = bone marrow.
[b]Not done.

Table 2. Virus isolates.

Animal No.	Tissue	Virus
1	blood	reo
8	blood	reo
12	li, kidn	adeno
27	li, kidn, bm, blood	adeno
26	li	reo

Table 3. Results of the PCR for retroviral sequences.

Animal No.	Tissues pos.
1	li, pancr
3	none
4	li
6	none
26	li
27	li, blood cells
31	none
32	none
VH2 infected	neg
VH2 not infected	neg

NUTRITIONAL SECONDARY HYPERPARATHYROIDISM IN THE RAMSEY CANYON LEOPARD FROG (*Rana subaquavocalis*)

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Abstract

Nutritional secondary hyperparathyroidism (NSHP) is one of the most common nutritional diseases affecting captive amphibians. Ramsey Canyon leopard froglets (*Rana subaquavocalis*) head-started at the Phoenix Zoo, developed signs of NSHP that was associated with low calcium hardness (calcium carbonate) in the enclosure water of the tadpoles. Reversal of the clinical signs was achieved by adding vitamin D_3 and calcium gluconate to the enclosure water of the froglets.

Introduction

The Phoenix Zoo has been involved in head-starting programs for native ranid frogs for several years. In 2000, an egg mass of the endangered Ramsey Canyon leopard frog (*Rana subaquavocalis*) was brought in for the head-start program. In previous years, water quality parameters were not monitored. In 2000, significant changes were made to the husbandry practices that included a different filtration system and monitoring of the temperature, pH, ammonia, nitrite, nitrate, total hardness, and calcium hardness on a regular basis. As in previous years, the majority of the husbandry was performed by the Tadpole Task Force, a group composed entirely of volunteers. (See www.tadpoletaskforce.homestead.com for more information on this process.)

Clinical Cases

Due to the number of tadpoles involved, it is difficult to monitor the individual progress of any individual specimen. Lacking water quality data from previous years, attempts were made to maintain the water quality parameters at "neutral" levels. For calcium hardness, levels were maintained below 200 ppm most of the time. At the time of metamorphosis, several froglets developed hydrocoelom, gastrointestinal bloating, or tetany following exercise. Some froglets died without signs. These signs are consistent with hypocalcemia so a sample of affected froglets were evaluated radiographically. Portions of the urostyles and lateral processes of the vertebrae were radiolucent and overall bone density was lower than normal. The vertebral bodies, endolymphatic sacs that accumulate calcium, were radiolucent. The presumptive diagnosis was nutritional secondary hyperparathyroidism (NSHP). Nutritional secondary hyperparathyroidism is the most common nutritional disease of amphibians reported in one pathology survey.[1] A thorough review of previous years' husbandry practices revealed that calcium carbonate and mineral blocks had been added to the tank water—although the calcium hardness of the water had not been measured in prior years, the tadpoles were frequently seen resting on the calcium blocks. This had not occurred in 2000.

Due to the number of froglets involved, it was not practical to individually administer oral calcium and vitamin D_3. Calcium and mineral blocks formulated for tropical fish were added to the tank water. In addition, since these are highly aquatic frogs, the treatment plan consisted of a baths of approximately 2.3% calcium gluconate solution and 2-3 IU/ml of vitamin D_3 (High-D 2X Dispersible Vitamin D_3 Liquid Concentrate, 4,000,000 IU/oz, Russell Company, Laboratories, Longmont, CO 80501 USA). The undiluted vitamin D_3 product may cause hypervitaminosis D in humans through skin contact, and anyone handling the product wore disposable latex or vinyl gloves. The gloves were thrown away after use. Once the product has been diluted in water, it is safe to touch the medicated water with bare hands. Initial treatments were performed in a separate bucket when the froglets were removed from their enclosures for cleaning, approximately every 3 days. They were maintained up to 4 hr in the calcium and vitamin D_3 solution. This regimen was changed to a continuous bath after no change was detected radiographically at 6 wk, although signs of hypocalcemia had abated. This solution was freshened whenever their tanks were cleaned, a minimum of three times per wk. Over the following 16 wk, the frogs showed gradual increase in bone density and were radiographically normal at the end of the season.

Discussion

Unfortunately, NSHP continues to be a common problem in captive amphibians. Although this is traditionally associated with problems of prey nutrient composition (i.e., inverse calcium:phosphorus ratios, low total calcium, lack of vitamin D_3), this experience suggests that Ramsey Canyon leopard frogs obtain some of their calcium requirements from their aqueous environment. Data obtained from natural ponds in 2001 revealed a calcium hardness of 250-300 ppm (27 March 2001, Bernstein Pond), approximately 50-100 ppm higher than the average calcium hardness maintained throughout 2000, prior to the presumptive diagnosis of NSHP. Although amphibian skin is permeable to calcium and fat-soluble vitamins, there is no way to know if the treatment administered was absorbed topically, ingested or a combination of both.

This case report illustrates the importance of obtaining water quality data on natural breeding sites of amphibians in order to provide optimum conditions for captive propagation and husbandry of offspring.

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CUTANEOUS FUNGAL MICROFLORA OF HEALTHY SQUAMATE REPTILES AND PREVALENCE OF THE *Chrysosporium* ANAMORPH OF *Nannizziopsi vriesii*

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Abstract

A survey was conducted to investigate the presence of a fungus, the *Chrysosporium* anamorph of *Nannizziopsi vriesii*, on the skin of healthy squamate reptiles. Skin was collected as aseptically as possible from actively shedding animals or from freshly shed exuvia. Initial results of culture from skin samples of 31 different lizards and 48 snakes, belonging to 29 zoologic and veterinary institutions, demonstrate a varied cutaneous fungal microflora. Fungi belonging to 41 genera were identified from these 79 reptiles. *Aspergillus* spp., *Penicillium* spp., and *Paecilomyces lilacinus* were most frequently retrieved, however *N. vriesii* was not recovered from any of these reptiles. *Nannizziopsi vriesii*, increasingly incriminated in fungal disease in reptiles, does not appear to be a common constituent of the microflora of healthy reptiles. This study therefore provides some basis to suggest that mycosis is not an infrequent outcome of exposure of reptiles to *N. vriesii* spores, at least under certain conditions.

Introduction

The fungus *Nannizziopsis vriesii* has been implicated in several outbreaks of skin disease in reptiles.[1-3] In documented outbreaks, animals were wild-caught specimens and the stress of capture and shipping likely was contributory to the onset of mycosis, yet the source of the fungus was never clearly identified.[1,2] Whether *N. vriesii* is a common environmental fungus found in soil or in cage substrate, or is part of the normal reptile skin microflora opportunistically causing disease in stressed animals, or whether it is a true transmissible or contagious pathogen is presently unknown. The main objective was to determine the prevalence of *N. vriesii* and other fungi on the skin of healthy captive reptiles. Initial colonies of *N. vriessi* are white and powdery and the hyphae produce small club-shaped conidia and arthroconidia. We paid particular attention to the presence of *Chrysosporium* species and to any dermatophyte-like fungi, but other fungi were determined at least to genus and often to species.

Procedure

In October 2000, sample submission request packages were forwarded to institutions in the United States and Canada. Each solicited institution was asked to submit duplicate 2.5 × 2.5-cm samples of shed skin from three different individual squamate reptiles. These were to be collected as

aseptically as possible from an actively shedding lizard or snake, or from a freshly shed snake exuvia. Samples were sent to two universities for culturing. Skin samples were cut in small sections, and these were plated onto Mycosel agar (Becton Dickinson, and Company, Cockeysville, MD 21030 USA) containing chloramphenicol (0.0005%), and cycloheximide (0.04%). Fungi were identified on the basis of morphology and growth pattern of colonies and on microscopic observation of conidiation and fruiting bodies.

Results

To date, over 35 institutions have participated in the study. Preliminary results presented here are based on skin samples from 31 lizards and 48 snakes. From the 79 samples (158 culture plates), 451 fungi were keyed to a total of 41 genera. Twenty *Chrysosporium* isolates were identified from 19 of the 79 reptiles (24%). Sixteen of these were *C. zonatum*, three were typical of the *Chrysosporium* anamorph of *Aphanoascus fulvescens*, and one was *C. evolceanui*. None of the *Chrysosporium* isolates met the *N. vriesii*-anamorph criteria. Initial results of this survey strongly suggest that *N. vriesii* is not a common constituent of the cutaneous microflora of healthy reptiles.

The most prevalent fungal genera were *Aspergillus*, *Penicillium*, and *Paecilomyces*. *Penicillium* was isolated from 61 (77 %), *Aspergillus* from 56 (71%) and *Paecilomyces* from 28 (35%) of the 79 reptiles. Remaining isolates belonged to 25 other genera. We cultured an average of 4.4 different genera of fungi per reptile (range of 0 to 15), often with more than one species per genus of fungus (e.g., two species of *Penicillium* or *Aspergillus*). Whether an animal was arboreal, terrestrial or fossorial did not seem to be predictor of the number or types of isolates cultured from its skin. There was a weak but discernible trend for reptile skins from the same institution to yield similar microfungi.

Discussion

Since it has now been implicated in several outbreaks of dermatomycosis in reptiles, and since data provided here suggests a low prevalence in the environment of captive reptiles, it appears logical to infer that *N. vriesii* carries a definite pathogenic potential for reptiles exposed to its spores, and infection needs only be triggered by a specific combination of environmental and host factors, such as faulty husbandry or stress. Further studies are necessary to determine the conditions and factors that govern infection of reptiles by this fungus.

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PAIN MANAGEMENT CONSIDERATIONS AND PAIN-ASSOCIATED BEHAVIORS IN REPTILES AND AMPHIBIANS

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Abstract

Veterinarians that treat exotic species are becoming more aware of the need for analgesia, however, analgesics are often not provided or considered in primary treatment for reptiles and amphibians. These species have the neurologic components, antinociceptive mechanisms and behavioral responses to pain that are similar to those seen in domestic animals and humans. Preemptive analgesia, multimodal therapy and contraindications for use of certain analgesics are addressed as well as behaviors and disease processes that can be associated with pain in reptiles and amphibians.

Introduction

Pain management has become an integral part in providing optimal medical and surgical care for all patients and should be considered in the primary treatment of reptiles and amphibians as well. Many practitioners do not provide pain management in theses species because of the lack of familiarity of drug choices and practical guidelines for their administration. The fear of potential side effects and the inability to recognize the behaviors associated with pain also play a major role in why analgesia is often not a part of routine therapy.

Reptiles and amphibians have the same or similar neurologic components to perceive pain that is found in dogs, cats, and other domestic species.[16] Antinociceptive mechanisms to modulate pain are also present and they display appropriate behavior in response to stimuli that is known to be painful in other species.[16]

The physiologic "stress response" to pain in reptiles and amphibians is also similar to other species. It can include vasoconstriction, increased heart rate and stroke volume, decreased gastrointestinal and urinary tone, and endocrine responses leading to a catabolic state with decreased kidney function.[17] Nociceptive stimulation of the brain also enhances reflex sympathetic responses, which creates increased blood viscosity, prolonged clotting time, fibrinolysis, and platelet aggregation.[17]

Clinically, in all exotic species the response to pain can include immune suppression, impaired wound healing, decreased food and water intake,[14] and secondary medical problems including gastric ulcers, gastrointestinal stasis, shock, and even death. These responses make it important for practitioners to reevaluate the need for analgesia in reptiles and amphibians that are experiencing trauma, disease, surgery, or other invasive procedures in which pain is a known component.

Recommendations Concerning Analgesics for Reptiles and Amphibians

If possible, analgesics should be provided before a painful stimulus occurs. Providing preemptive analgesia helps to decrease postoperative pain including that caused by both neuropathic pain and inflammatory pain.[14] Neuropathic pain occurs when pulling and stretching of tissues during surgery triggers nociceptive nerve endings causing central nervous system changes to occur that enhance pain postoperatively. Handling and crushing of tissues causes inflammation that also increases postoperative pain. By providing preemptive analgesia the dose needed for maintenance anesthesia is decreased. Therefore when using injectable anesthesia the anesthetic doses should be adjusted accordingly.

Combining different classes of analgesics to manage pain, known as multimodal therapy, is also recommended. In each painful situation numerous pain pathways would stimulate different neurotransmitters. Providing multiple analgesics, that would address the different pathways, can have a synergistic effect and the analgesics can be used at lower doses when used together often with less side effects.[14] Each patient should be treated as an individual when deciding what analgesics will be used and evaluated for the need to add to or alter treatment based on its clinical response to the regimen provided.

Care should be taken when using analgesics under certain circumstances in all species. For instance, when bleeding disorders, enteritis, gastritis, gastric ulcers, hepatic impairment, renal impairment, hypotension, hypovolemia or a potential for hypotension during surgery exists, nonsteroidal anti-inflammatory drugs (NSAIDs) should not be used or used judiciously.[14] As with other species, two or more NSAIDs should not be used concurrently.[13] Avoid prolonged use of NSAIDs and it has been suggested to administer sucralfate (especially in herbivores) to those patients kept on NSAIDs for chronic pain.[3]

Opiod analgesics have a relatively short duration of action, which should be considered when determining the frequency of administration. They should also be avoided in hypotensive patients and the use of opiods decreases the dose needed for isoflurane anesthesia. If injectable anesthesia is used it is recommended to administer opiods when the patient is at a lighter plane of anesthesia to prevent overdose.

Behaviors Associated With Pain

Practitioners need to become familiar with normal behaviors of each species that they treat in order to recognize painful behaviors as early as possible. Identification of pain in all prey species is made more difficult because they exhibit less overt pain-associated behaviors in order to decrease their chances of being recognized as ill by predators.[10] Also, due to the same survival instincts, immobility is a common behavior seen in prey species that are presented for examination making it more difficult to determine if pain is present.[6] Nocturnal species present more of a challenge as the diurnal observation of their behaviors when presented for examination may result in inaccurate assessment.[6]

Many of the behaviors presented below can also be seen in any patient that is ill, but pain should be considered as a potential component in the disease process if these behaviors are present. Also, never discount the pet owner's assessment of pain and anxiety in their pet, as they may be the most in tuned to what normal behavior is for that animal.

Behaviors Associated with Pain in Reptiles and Amphibians

Reptiles - Lizards

Anorexia
Hunched posture
Closed eyes
Flight response
Startle easily
Dysphagia
Color changes
Withdrawal
Immobility
Lethargy
Remain standing for extended periods of time
Scratching or flicking foot at affected area
Aggression in normally passive animal
Aerophagia leading to dilated esophagus
Rigid posture on palpation of abdomen
Head elevated and extended
Avoidance of handling
Lameness and ataxia
Absence of normal behaviors

Reptiles - Snakes

Anorexia
Stinting on palpation
Avoidance of handling
Withdrawal
Restless
Agitated
Lethargy
Hold body less coiled at site of pain
Tucked up and writhing in affected area
Aggression in normally passive animal
Easily startled
Flight response
Immobility
Absence of normal behaviors

Reptiles - Chelonians

Anorexia
Hunched posture
Avoidance
Closed eyes
Immobility
Lethargy
Stinting on palpation
Lameness or ataxia
Withdrawal
Flight response
Biting at affected area
Absence of normal behaviors
Intermittently pulling head into shell and then extending the neck out and up

Amphibians

Immobility	Flick foot or bite at affected area
Lethargy	Aggression in normally passive animal
Closed eyes	Lameness and ataxia
Vocalization	Startle response
Anorexia	Flight Response
Color changes	Rapid respiration

Absence of normal behavior

Painful Disease Processes

Trauma	Ophthalmic disease	Gastrointestinal stasis
Gout	Dystocia	Urethral obstruction
Peritonitis	Pododermatitis	Metabolic bone disease
Pneumonia	Enteritis	Limb amputation
Abscesses	Hepatitis	Intestinal foreign body
Frostbite	Otitis	Post surgery
Cystitis	Burn wounds	Osteomyelitis
Bite wounds	Enteritis	Pyelonephritis
Osteoarthritis	Cellulitis	Dental disease
Visceral pain	Meningitis	Self-mutilation
Cystic calculi	Stomatitis	Neoplasia
Fecoliths	Pancreatitis	

Cooling or freezing reptiles and amphibians to produce immobility, anesthesia or euthanasia is not acceptable or humane as it creates decreased nociceptive threshold and increased sensitivity to pain.[1,5,11]

Analgesic Dosages for Reptiles and Amphibians

Formularies that provide analgesic dosages for exotic species attempt to provide dosages that have been found to be safe for those species, some have pharmacokinetic origins but most are derived empirically. Individual reptile species need different analgesic dosages – lizards, snakes, chelonians, and amphibians have different needs from each other. No one dose could satisfy the idiosyncrasies of each species let alone each patient. Relative health or debilitation of the patient also must be taken into consideration when deciding on a dose. The dosages provided in this paper include broad ranges to reflect those individualities. It is, therefore, important for practitioners to have multiple exotic animal reference formularies available that allow them to make the best decisions for each patient that they treat.

Analgesics for Reptiles and Amphibians

Reptiles

NSAIDs

Carprofen – 2-4 mg/kg initially followed by 1-2 mg/kg q 24-72 hr i.v., i.m., p.o., s.c.
Flunixin meglamine - 0.1-1.0 mg/kg q 12-24 hr i.v., i.m. (for 2 to 3 days)
Ketoprofen – 2 mg/kg i.m., s.c. q 24-48 hr
Meloxicam - 0.1-0.2 mg/kg q 24 hr p.o.

Opiods

Buprenorphine HCl - 0.01 mg/kg q12 hr i.m.
Butorphanol tartrate - 0.05-1mg/kg q 12 hr i.m., i.v., p.o., s.c. (up to 25 mg/kg in tortoises)
Morphine – 0.3-4.0 mg/kg q12-24 hr i.l. (intracoelomic), i.m., s.c.

Amphibians

Opiods

Buprenorphine HCl – 35-75 mg/kg q 12 hr i.m.
Butorphanol tartrate - 0.05-1.0 mg/kg q 12 hr i.m., i.v., p.o., s.c.
Morphine – 20-160 mg/kg s.c., i.m., topical

References are available for formularies containing dosages and recommendations for specific species as doses vary greatly between different species.[2,4,7-9,12,13,15]

Alternative Approaches to Pain Management

Little work has been done on alternative approaches to pain management in reptiles and amphibians. Other therapies exist that aid in treatment of both acute and chronic pain including acupuncture, massage therapy, magnetic therapy, chiropractic, and physiotherapy.

Conclusions

As with all species, a return to normal behavior indicates a positive response to analgesic treatment. For reptiles and amphibians this includes eating, drinking, and defecating as well as the resumption of normal postures and behaviors. Until further studies are done to elucidate pain perception and response in reptiles and amphibians, any disease or process that has pain as a known component in other species should be considered painful in these species as well and treated accordingly.

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AMPHIBIANS AS MONITORS OF ECOSYSTEM HEALTH

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Abstract

Amphibians have often been described as sentinels of environmental damage on many scales. On a worldwide scale, many populations of amphibians have experienced marked declines. Many potential causes of these declines have been suggested including:

- habitat degradation, fragmentation, and destruction;
- increased UV irradiation due to ozone thinning;
- global climate change;
- introductions of exotic species;
- infectious diseases such as chytridiomycosis, *Ranavirus* infections, and severe parasitism;
- anthropogenic chemicals such as pesticides, acid-rain, endocrine disruptors, and others.

Because amphibian populations, community structure, and health are influenced by environmental factors that operate on multiple spatial scales, identification of the inciting cause for every population decline is impossible. In particular, anuran amphibians living within damaged ecosystems may be at increased risks of decreased reproductive success, or increased direct mortality due to stress, water quality problems, toxicoses, infectious diseases, and predation.

Our research group has recently demonstrated that herbicide exposure in microcosms or mesocosms reduces size at metamorphosis, causes hypoxia, and may accelerate predation.[4] Additionally, in our field studies, macroinvertebrate richness and anuran species richness were negatively correlated with increased agricultural uses of land. High edge contrasts decreased habitat value for selected species of anurans. However, woodlands near agricultural land seemed to increase habitat value to amphibians, as they were associated with increased populations of some anuran species.[5]

Adult cricket frogs (*Acris crepitans*) in the upper Midwest during breeding season, and recent metamorphs in late summer, were consistently found in areas where there was a gentle slope into the water; and greater numbers were present when aquatic macrophytes were abundant. In the same study, herbicide-induced damage to macrophytes was associated with increased intensities of echinostome infections, a trematode larvae that encysts in renal tissue.[1] Others have described malformations (including missing or supernumerary limbs), and increased death losses, as a

consequence of larval trematode (*Ribeiroia* sp.) infections in laboratory and field studies. Although northern leopard frogs (*Rana pipiens)* at our study sites had a malformation rate of about 1.2 % over 3 yr (3,188 frogs examined in the field), and four ponds had a prevalence of *Ribeiroia* sp. from 6.3-100%, none of malformed frogs examined was found to be infected with *Ribeiroia* sp. metacercariae. We are currently investigating factors potentially related to the prevalence and intensity of trematode infections in frogs.

In another study of Illinois cricket frogs, coplanar polychlorinated biphenyls and polychlorinated dibenzo-furans at three out of three sites had high numbers of juvenile males relative to females (ratio of males to females was 2:1). While at three of three control sites, the male female sex ratio was consistent with historic reports (1:2) suggesting endocrine disruption.[8] Also, our laboratory group recently examined historic specimens of Illinois cricket frogs from museums across the United States, and demonstrated a rate of intersex gonads of approximately 2.5% from 1850-1929, 15.5% from 1930-1942, 16.5% from 1946-1959 and 11% from 1960-1979. Intersex gonads were found in about 3% of cricket frogs collected in Illinois by our group in the 1990s. The greatest percentage of intersex individuals was in the northeastern region of the state. We are currently investigating the possible contributions of environmental contaminants, or weather, to the dramatic increase in the percentage of individuals with ovotestes from the 1930s to the end of the 1950s.

Chytridiomycosis has been implicated in mass die-offs of population of montane anurans in several regions, including the western United States, Panama, and Australia.[2,3,6] Although the fungal organism *Batrachochytrium dendrobatidis* has been demonstrated to be highly lethal in captive as well as field situations, this fungus has been observed in the skin of some species of Midwestern anurans, including Illinois cricket frogs, for several years and it is not always associated with apparent die-offs.[7]

Our research group is endeavoring to determine habitat characteristics that maximize "healthy" outcomes for amphibians. By identifying the characteristics of healthy and unhealthy ecosystems that give rise to diverse amphibian communities, we expect to be able to offer recommendations for habitat restoration programs to help maintain healthier and more abundant frog populations.

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STUDY OF THE SAFETY AND SEROLOGIC RESPONSE OF JAPANESE ENCEPHALITIS VIRUS VACCINE FOR PROTECTION AGAINST WEST NILE VIRUS IN AVIAN MODEL SPECIES

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Abstract

West Nile (WN) virus caused the death of more than 15 birds in the avian collection at the Wildlife Conservation Society (WCS) - Bronx Zoo within a 3-wk period associated with high mosquito activity in late summer 1999. On preliminary evaluation, more than 50 birds in the WCS collection had serologic evidence of exposure to WN virus. Multiple and varied avian species appeared to be susceptible, with both mortality and morbidity, to this virus. The return of mosquito vectors after the winter quiescent period was considered a risk factor for initiation of a second outbreak of WN virus for collection birds, as well as, local indigenous birds.

Japanese encephalitis (JE) virus is in the same group of flaviviruses as WN virus and shares significant nucleotide sequence homology with WN virus; previous studies in non-human primates have shown cross-protection between JE and WN virus vaccines after challenge.[1] We were aware of no studies that had been conducted in birds and considered that JE virus immunization could prove useful to protect endangered species and collection birds, if safe and efficacious. We asked two research questions: (1) Is the inactivated JE virus vaccine (JE-VAX) safe for avian species with considerations of mortality, adverse systemic reactions, local complications, and general behavior? and (2) Does JE-VAX induce a serologic antibody response (neutralizing) to WN virus in vaccinates?

Tragopans (*Tragopan caboti* × *temminckii)*, ring neck doves (*Streptopelia risoria*), and laughing gulls (*Larus atricilla*), as well as, previously free-ranging pigeons (*Columba livia*) were utilized for the study. Birds were selected as model avian species for those avian species with high conservation value and for avian species which suffered high morbidity or mortality associated with WN virus in WCS collection birds.

Briefly, on days 0, 7, and 30, all birds in the study were manually restrained for physical examination, observation of the pectoral muscles (inoculation site), blood collection, and subcutaneous injection of saline or the JE virus vaccine (JE-VAX® aka BIKEN®, manufactured by The Research Foundation for Microbial Diseases, Osaka University, Suita, Osaka, Japan; distributed by Pasteur Merieux Connaught, Swiftwater, PA 18370 USA). Birds in the experimental

group were inoculated subcutaneously with 0.25 ml JE-VAX (tragopans and pigeons) or 0.10 ml JE-VAX (gulls and doves), while birds in the control group were administered a 0.25 ml or 0.10 ml saline placebo subcutaneously, respectively. On day 60, all birds in the study and control groups were manually restrained for physical examination, observation of the inoculation site, and blood collection. Blood samples were processed for complete blood count, biochemical evaluation, and storage for batch processing for WN serology. Frozen plasma and serum samples were delivered to the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) for evaluation of neutralizing antibody titers to JE and WN viruses.

The inactivated JE virus vaccine (JE-VAX) was safe for all avian species in the project. No mortality or morbidity was noted throughout the study period. Statistic analysis and trend identification for the complete blood counts and plasma biochemical panels are in progress.

JE-VAX did not induce a serologic antibody response (neutralizing) to WN virus in previously-WN negative vaccinates. JE-VAX appeared to induce a serologic antibody response (neutralizing) to WN virus in pigeons that already had low WN serologic titers when vaccinated (Table 1). JE-VAX did induce serologic antibody response (neutralizing) to JE virus in some birds (Table 1). Collection birds at WCS were not vaccinated with JE-VAX during the 2001 mosquito season for protection against WN virus, based upon the results of the project.

Further vaccine trials and research are warranted. JE-VAX protection from lethal infection with WNV in American crows (*Corvus brachyrhynchos*) is under evaluation at the National Wildlife Health Center, Madison, Wisconsin (RG McLean, personal communication). Pending financial investment, other investigators plan avian trials of a recently developed WN vaccine that has proved effective in horses (T.S. McNamara, personal communication).

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Table 1. Serologic antibody titers in selected birds to Japanese encephalitis (JE) virus and West Nile (WN) virus at 0, 7, 30, and 60 days post-inoculation with JE virus vaccine or saline placebo.

Bird	Identity	Trial group	JE 0 day	JE 7 day	JE 30 day	JE 60 day	WN 0 day	WN 7 day	WN 30 day	WN 60 day
Tragopan	962243	Vaccinate	<10	<10	20	160	<10	<10	<10	10
	992104	Control	<10	<10	<10	<10	<10	<10	<10	<10
	992105	Vaccinate	<10	<10	10	320+	<10	<10	<10	<10
	992129	Vaccinate	<10	<10	<10	40	<10	<10	<10	<10
	992130	Vaccinate	<10	<10	20	160	<10	<10	<10	<10
	992131	Vaccinate	<10	<10	10	80	<10	<10	<10	<10
Gull	992007	Control	<10	<10	<10	<10	<10	<10	<10	<10
	992008	Vaccinate	<10	<10	<10	<10	<10	<10	<10	<10
	992011	Vaccinate	<10	<10	<10	<10	<10	<10	<10	<10
	992012	Vaccinate	<10	<10	10	40	<10	<10	<10	<10
	992014	Vaccinate	<10	<10	<10	<10	<10	<10	<10	<10
	992015	Vaccinate	<10	<10	<10	<10	<10	<10	<10	<10
	992020	Vaccinate	<10	<10	<10	<10	<10	<10	<10	<10
Dove	972323	Vaccinate	<10	10	<10	320	<10	<10	<10	<10
	972324	Vaccinate	<10	<10	10	320	<10	<10	<10	<10
	982156	Vaccinate	<10	10	10	10	<10	<10	<10	<10
	982295	Vaccinate	<10	<10	<10	20	<10	<10	<10	<10
	992055	Vaccinate	<10	<10	<10	<10	<10	<10	<10	<10
	992114	Vaccinate	20	160	20	80	<10	<10	<10	<10
Pigeon	J1	Vaccinate	<10	<10	<10	20	<10	<10	<10	10
	J2	Vaccinate	20	160	40	160	40	640	320	640
	J3	Vaccinate	<10	10	<10	<10	<10	<10	<10	<10
	J4	Control	10	<10	<10	10	<10	<10	<10	<10
	J5	Vaccinate	80	320	320	160	160	640	640	640
	J6	Control	<10	<10	<10	<10	<10	<10	<10	<10
	J7	Vaccinate	<10	<10	<10	<10	<10	<10	<10	<10
	J8	Vaccinate	<10	<10	<10	<10	<10	<10	<10	<10
	J9	Vaccinate	40	20	20	40	160	640	640	640
	J10	Vaccinate	<10	<10	10	10	<10	<10	<10	<10

Atoxoplasma spp. IN THE BALI MYNAH: SURVEY RESULTS AND MANAGEMENT-ASSOCIATED RISK-FACTOR ANALYSIS

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Abstract

The Bali mynah (*Leucopsar rothschildi*) is an endangered bird endemic to the island of Bali, Indonesia. The wild population is estimated to be approximately nine (T. Norton, personal communication), while the North American Species Survival Plan (SSP) population consists of 232 individuals distributed among 62 institutions as of September 2000.[11] Atoxoplasmosis and hemochromatosis are considered to be the primary medical problems in the captive population.[9] Although a great deal of work has been done on describing the agent, clinical signs, diagnostic methodology and pathology of atoxoplasmosis, no American Zoo and Aquarium Association (AZA) wide effort has been made to identify and assess potential risk factors associated with captive management of the Bali mynah. This study was designed to survey the AZA facilities holding Bali mynahs in order to derive an institutional prevalence for both atoxoplasmosis and hemochromatosis, and to elicit management-related information to identify risk factors for atoxoplasmosis in the Bali mynah.

Introduction

Atoxoplasmosis, the actual disease process caused by *Atoxoplasma* spp., recently classified as *Isospora rothschildi*, is a coccidian parasite of passerine birds with a prolonged life cycle that involves the reticuloendothelial system as well as the intestinal epithelium.[10] Clinical signs associated with atoxoplasmosis are often nonspecific and may include diarrhea, decreased appetite, weight loss and ruffled feathers,[10] however infestation of fledgling birds between 3 and 8 wk may result in acute death.[7] Hemochromatosis (iron storage disease) is caused by excessive iron accumulation in numerous organs throughout the body, especially the liver. This process commonly leads to end stage liver disease in the Bali mynah as well as other birds. Twenty-one percent (15/70) of postmortems performed by the Bali mynah SSP pathology advisor showed some degree of this disease.[8] A complete review of these diseases, including clinical signs, diagnostic protocols and methodology is available from the SSP veterinary advisor.[8]

Atoxoplasmosis was first reported in North American Bali mynahs at the National Zoo in 1989.[7] Retrospective studies showed that the organism had been present in Bali mynahs at the National Zoo since 1975.[10] Atoxoplasmosis has been described previously in numerous other species of birds

(sparrows, canaries, evening grosbeaks, rose breasted grosbeaks, common mynahs, Indian hill mynahs).[7] Several reports exist in the literature regarding atoxoplasmosis in Bali mynahs in North American institutions. One report mentioned that 8/9 (89%) U.S. institutions screened were positive,[7] while Greiner et al. found at least one positive Bali mynah in 34/36 (84%) facilities and a minimum prevalence of 62% (152/245 examined) based upon the presence of oocysts in the feces and/or zoites in mononuclear phagocytes.[5] A study of atoxoplasmosis in captive Bali mynahs in the U.K. showed an 18.5 % bird prevalence based on fecal smears and an 47% (9/19) institutional prevalence.[3] Though oocysts have been found in the feces of wild Bali mynahs, it is not yet known the extent to which atoxoplasmosis has contributed to the wild population's decline.[9,2]

Methods

A survey designed to retrospectively collect both medical and husbandry information was distributed to 55 AZA-accredited zoologic institutions currently or previously holding Bali mynahs. Veterinarians and animal management staff were asked to complete the survey either on paper or on the 31/2" floppy disc provided and return it to the Lincoln Park Zoo in Chicago. Upon receipt, completed surveys were coded in order to ensure confidentiality and data entered into an Access database. Descriptive data were summarized using Microsoft Excel spreadsheet software and statistics (odds ratios [OR], chi-square and confidence intervals) were calculated using the Center for Disease Control's Epi Info 2000 software.[4]

After descriptive summation of results, institutions were labeled as a "case" if they had diagnosed *Atoxoplasma* spp. in their collection at any time. Chi-square tests (Yates corrected chi-square) for significance were performed on each relevant question in the survey. In addition, extended Mantel-Haenszel chi-square for trend analysis was performed where appropriate. For all chi-square tests, *P*-values of less than 5% ($P < 0.05$) were considered significant. Odds ratios comparing cases (case vs. non-case) to specific exposure variables (exposure vs. no exposure) were calculated using 2×2 tables. The odds ratio is an indicator of the degree of association between cases and exposure to a specific variable. Odds ratios less than one represent a decreased relationship between cases and exposure and may be considered protective if biologically plausible; an odds ratio of one means that no relationship exists between cases and exposure; while odds ratios greater than one represent an increased likelihood that exposure increases risk of being a 'case.' Ninety-five percent confidence intervals (Cornfield approximation and exact) surrounding odds ratios were calculated; confidence intervals that included 'one' were considered not significant.[4]

Results

Forty out of 55 surveys were returned for a 73% response rate. Of those that responded, 18 (45%) indicated that they had diagnosed *Atoxoplasma* spp. in at least one Bali mynah. These respondents were classified as cases for the risk factor analysis. Fecal exam (55%) was the most common method used to diagnose *Atoxoplasma* spp., followed by examination of buffy coat smears (35%) and necropsy (25%). *Atoxoplasma* spp. was reported in other avian species by 15% of respondents; the yellow-bellied laughing thrush, tananger, golden breasted starling (suspect), superb starling,

yellow-billed cardinal and warbler were the other positive species listed. Eighty-five percent of the respondents have treated Bali mynahs for atoxoplasmosis, either after diagnosis or for prevention. Only 10 (10%) responded that they performed routine medical exams, once per year or greater; frequency of procedures within those 10 can be found in Table 1. Sixteen institutions (40%) responded that they had diagnosed hemochromatosis in at least one Bali mynah. Necropsy was by far the most common way that hemochromatosis was diagnosed (40%) followed by liver biopsy (20%). One-third (5/16) of the institutions that reported positive diagnoses have attempted treatment. Almost all (90%) respondents submit adults for necropsy, while 72% submit chicks and 10% responded that they do not submit Bali mynahs for necropsy. When comparing cases (respondents which have diagnosed *Atoxoplasma* spp. in at least one bird) to non-cases (respondents which have never diagnosed *Atoxoplasma* ssp. in Bali mynahs), all tests of significance were not statistically significant at the 95% confidence level. Raw data, percent of institutions for each response, odds ratios and 95% confidence intervals for all relevant questions are provided in Table 1.

Discussion

The absence of statistic significance in this study is most likely due to the small sample size ($n \leq 40$); this illustrates the need for high response rates needed when surveying small, endangered populations even with relatively prevalent diseases. Even though all associations did not prove to be statistically significant, overall odds ratio estimates for several factors were quite high, therefore these factors may prove to be biologically significant should the power of the study be increased. Odds ratios represent the degree to which a specific factor is related to 'cases' in the study; in this case, the degree to which the factor is associated with institutions finding *Atoxoplasma* spp. in at least one Bali mynah. Statistically non-significant odds ratios (i.e., with a 95% confidence interval including 1) that are elevated and consistent with "common sense" should still be discussed. When evaluating odds ratios, a score of one (1) indicates no relationship, a score greater than one (>1) indicates an increased association between cases and the risk factor, while a value less than one (<1) may represent a factor that is protective for atoxoplasmosis. Risk factors were not evaluated for association with hemochromatosis. Odds ratios that make biologic sense when discussing risk factors associated with atoxoplasmosis are discussed below.

In general, ORs increased as number of enclosures at an institution increased (Table 1). This may be due to a greater number of birds at these institutions, a greater effort put forth to diagnose disease in larger institutions, or may be a result of the increased amount of labor required to thoroughly clean and disinfect a greater number of enclosures. The following enclosure factors had increased ORs and may represent a biologically plausible increase in risk: indoor enclosures (2.4); concrete (1.2) and wire fencing (1.3) enclosure materials; soil/leaf litter (6.4), gravel (3.2) and concrete (1.4) enclosure substrates (notice that risk decreases as substrate is more easily cleaned). Combination indoor/outdoor enclosures (0.6), glass (0.6), wood (0.6), plastic sheeting (0.4) and polypropylene mesh (0.6) were less associated with cases and may be protective, but the potential for confounding between enclosure substrate and disinfection practices must be further investigated.

Partial lighting situations (natural or artificial) appear to be more strongly associated with cases than full lighting, though again this may be due to the association between partial lighting and indoor enclosures. There also appears to be a correlation between frequency of cleaning of an enclosure and institution positivity, with increased OR associated with decreased frequency. Most disinfectants, however, were positively associated with cases (probably because almost everyone uses them); with bleach and the "other" category being the only ones that may be protective. These results, as in the lighting data, may be due more to enclosure design (i.e., decreased prevalence with outdoor) than with the factor of concern. Chemical pest control products (invertebrate and vertebrate) were protective (OR < 1), while physical methods (invertebrate and vertebrate) were more strongly associated with cases (OR > 1). All water sources represented an increased association (risk) for atoxoplasmosis with the exception of the 'enclosure item' category, which had an odds ratio close to one, however, this may again be due to an association with an outdoor enclosure type.

Mixed species exhibits (3.7) were of greater risk than those that previously or never were mixed (OR < 1). We must be careful not to put too much weight on this factor since no further interaction was investigated, including observed disease in the other species of birds or type of "coccidia" noted on exam. Risks associated with frequency of medical exams made biologic sense; with those that performed exams once per year or more had an OR of less than one, while those performing exams only upon clinical illness or preshipment had an OR of 1.3. There was a positive association in cases where atoxoplasmosis was diagnosed in other species but these data do not indicate disease directionality. There was a decreased risk of institutions being positive associated with treatment for atoxoplasmosis (0.53) and an increased risk with never having treated (1.9). The increased association with having diagnosed hemochromatosis (2.1) may not be valid because we do not know if they were in the same animal or just in the same institution. This holds for the protective association with hemochromatosis treatment (0.79) as well.

Therefore, we feel that these preliminary data, focusing on information gleaned from the intensive survey sent to Bali mynah-holding institutions, provide evidence of the information that can be acquired through active participation in such surveys, and will form the basis for additional epidemiologic investigations focused on this disease of concern in a critically endangered bird species. While much of the data may not be statistically significant, the directionality of results (indicating a trend towards being a risk factor, a protective factor or unimportant in the overall disease state) will assist in the ongoing allocation of effort (and funds) to better understand this disease state. Only through observing and collecting this type of information on a population-wide basis can these types of questions be addressed in a scientifically defensible manner, therefore ongoing participation of zoologic veterinarians and animal care staff are critical to the success of such efforts.

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Table 1. Summary of survey results, calculated odds ratios for risk factors associated with *Atoxoplasma* spp.-positive institutions and 95% confidence intervals.

Question	Category choices	# Responses	%	OR	CI 95%
Have ever diagnosed atoxoplasmosis	Yes	18	45		
	No	22	55		
Number of BM enclosures	1	21	52.5	0.36	0.08-1.57
	2	9	22.5	1	0.16-5.5
	3	4	10	6	0.5-309
	4	2	5	0.6	0.01-12.4
	>4	4	10	4.2	0.3-230
Location of enclosures	indoor	33	82.5	2.4	0.3-27
	outdoors	11	27.5	1	0.2-5
	combo in/out	3	7.5	0.6	0.01-13
Enclosure composition	concrete	38	95	1.21	0.25-6
(materials used)	glass	16	40	0.6	0.13-2.6
	wood	14	35	0.56	0.11-2.5
	wire fence	30	75	1.31	0.25-7.6
	plastic sheeting	3	7.5	0.37	0.01-5.2
	polypropylene mesh	3	7.5	0.59	0.01-12.4
	other	11	27.5	1.7	0.34-8.8
Enclosure substrates used	soil/leaf litter	33	82.5	6.38	0.63-310
	gravel	9	22.5	3.17	0.53-22
	concrete	14	35	1.36	0.31-6.1
	other	13	32.5	0.67	0.14-3.4
Enclosure lighting	natural - open (full)	18	45	0.64	0.15-2.7
	natural - skylight (partial)	21	52.5	1.9	0.45-8
	artificial - full	7	17.5	0.9	0.11-6.3
	artificial - partial	18	45	1.4	0.34-6.1
Enclosure cleaning	daily	28	70	0.93	0.2-4.3
	weekly	7	17.5	1.81	0.26-14
	regularly but <weekly	2	5	0.6	0.01-12.4
	only when necessary	3	7.5	2.63	0.12-162
	never	1	2.5	1.24	0.02-101
Enclosure disinfection	daily	2	5	1.24	0-49
	weekly	11	27.5	1	0.21-5
	regularly but <weekly	10	25	1.3	0.25-6.8
	only when necessary	7	17.5	0.9	0.13-6
	never	10	25	0.76	0.14-4
Disinfection products	quaternary ammonium	5	12.5	2	0.2-26
	phenolics	1	2.5	1.24	0.02-101
	chlorhexadine (nolvasan)	3	7.5	2.63	0.12-162
	iodophores (betadine)	2	5	1.24	0.02-101
	bleach	20	50	0.44	0.1-1.9
	other	15	37.5	0.72	0.16-3
Pest control products	physical invertebrate	12	30	2.16	0.5-11
	chem invertebrate	21	52.5	0.83	0.2-3.5
	physical vertebrate	23	57.5	3.1	0.7-15
	chem vertebrate	17	42.5	0.76	0.18-3.3
	other	7	17.5	0.9	0.11-6.3

Table 1. Summary of survey results, calculated odds ratios for risk factors associated with *Atoxoplasma* spp.-positive institutions and 95% confidence intervals (continued).

Question	Category choices	# Responses	%	OR	CI 95%
Enclosure water source	bowl	31	77.5	3.7	0.5-41
	bottle	0	0	none	
	mister	3	7.5	2.6	0.12-162
	enclosure item	19	47.5	0.8	0.2-3.3
	other	0	0	none	
Mixed species exhibit	currently yes	23	57.5	3.12	0.7-15
	no, but previously	9	22.5	0.53	0.07-3
	never	8	20	0.33	0.03-2.3
Reproductive history	had egg laying	31	77.5	1	0.18-6.25
	no egg laying	9	22.5	none	
Medical management/exam	>1/year	3	7.5	0.6	0.01-12
	1/year	7	17.5	0.9	0.11-6.3
	illness/shipment only	30	75	1.31	0.25-7.6
Routine medical exams	physical exam	10	25		
	body weight	10	25		
	CBC	9	22.5		
	chem panel	9	22.5		
	buffy coat	7	17.5		
	serology (chlamydia)	2	5		
	serology (paramyxovirus)	0	0		
	aspergillus diagnostics	1	2.5		
	fecal (smear and float)	10	25		
	fecal for atox	6	15		
	fecal cytology	2	5		
	fecal culture	3	7.5		
	radiographs	3	7.5		
Atoxoplasmosis diagnostic methods used to detect positives	fecal	22	55	1.57	0.37-6.8
	buffy coat exam	14	35	0.34	0.06-1.6
	biopsy	3	7.5	0.59	0.01-12.4
	necropsy	10	25	0.43	0.06-2.4
Atoxoplasmosis in other species	yes	31	77.5	3.7	0.5-41
	no	6	15	0.56	0.05-4.6
	never mixed	3	7.5		
Atoxoplasmosis treatment	have treated	5	12.5	1.89	0.45-8.17
	never treated	35	87.5		
Have ever diagnosed hemachromatosis	yes	16	40	2.14[a]	0.5-9.5
	no	24	60		
Hemochromatosis diagnostic methods used to detect positives	radiographs	3	7.5		
	biopsy	7	17.5		
	necropsy	16	40		
	other	3	7.5		
Hemochromatosis treatment	yes	5	12.5	0.79[b]	0.06-7.9
	no	35	87.5		
Routine necropsies	adult necropsy	36	90	2.7	0.2-150
	chick necropsy	29	72.5	2.8	0.5-19
	no necropsy	6	15	1.2	0.02-101

[a]OR calculated for hemochromatosis as risk factor for atoxoplasmosis.
[b]OR calculated for hemochromatosis treatment as risk factor for atoxoplasmosis.

HEMATOLOGIC AND BIOCHEMICAL METHODOLOGIES AND THE DEVELOPMENT OF REFERENCE RANGES

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Abstract

"A physician who depends on the laboratory to make his diagnosis is probably inexperienced; one who says that he does not need a laboratory is uninformed. In either instance the patient is in danger." – JA Halsted

According to the Principles of Quality Assurance authored by the American Society of Veterinary Clinical Pathologists, every laboratory should generate reference ranges for appropriate species for each machine in that laboratory.[3] It should be noted that two different machines can and will have different reference ranges. For instance, clinically significant differences are frequently observed between chemistry analyzers that use wet reagents, such as the Hitachi 911, and dry reagents, such as Kodak Ectachem analyzers (Tables 1,2).

A reference range is obtained by sampling a representative population, statistically eliminating outliers, with the resultant limits defining values equivalent to health. In human medicine, a reference range is generated with no less than 120 clinically healthy individuals. This is not always practical in veterinary medicine. Accepted veterinary protocol generally requires no less than 30, though 40 individuals are preferred.

Most values have a Gaussian or normal distribution and can be evaluated using statistics that follow the rules of normality. Exceptions to this generally include total bilirubin, eosinophils, and basophils. Ninety-five percent of the Gaussian distribution is located within 1.96 standard deviations of the mean. Reference ranges are calculated using 1.96 standard deviations or can be calculated using quartiles for populations with lower numbers of individuals. Using this method, 2.5% of healthy animals will have values above the reference range and 2.5% of healthy animals will have values below the reference range. Therefore, there is a 5% chance that a true normal animal would be classified as having a mild abnormality. Clinicians are willing to designate normal animals with extreme values of a particular analyte as being abnormal as a trade-off for not accepting too many abnormal animals as normal. A clinician cannot determine whether a single measured borderline value represents an abnormality in that individual or is a normal state.

Reference ranges may be inappropriately widened by variation within a population. For instance, neonates and young juveniles of most species are known to have increased phosphorous, decreased blood urea nitrogen, and transient anemia. If these were included in the reference range, many abnormal animals would not be detected.

In an ideal setting, one would develop a baseline on every individual animal and compare this to the established reference range of clinically healthy animals in that laboratory. This can be quite costly and again is not always practical. Therefore, clinicians are frequently dependent on reference ranges that are hopefully generated by the laboratory that they are using. Frequently, when dealing with exotic animals, clinicians are dependent on reference ranges in books developed using unknown methods that may or may not generate relevant numbers.

Reference ranges can be impacted by selection of subclinically ill animals, a poor quality sample such as a hemolyzed or lipemic sample, the methodology used, poor laboratory quality control, and sampling a small number of animals in a population with significant variation.

There are several artifacts that may originate in the animal or be due to sample handling. Lipemia, hemolysis, and icterus can falsely elevate or decrease so many values that moderate to severe change can almost result in random numbers. Severe hyperglycemia can falsely elevate total protein generated on a refractometer and falsely lower potassium on a dry analyte chemistry analyzer. Marked hyperproteinemia can falsely lower electrolytes determined by the flame photometric method. Ketonemia can falsely elevate creatinine and AST. Severe azotemia can falsely elevate total bilirubin, glucose, and total protein on a refractometer. (Further detailed information can be found in references 1 and 7.)

Methodologies may also cause significant variation. Manual white blood cells counts are imprecise and have documented variation of over 30%.[4,8,10] This is currently the only commercially available way to generate a white blood cell count for nucleated species. Unfortunately, variation increases with inflammation and therefore trends in white blood cell counts can be difficult to interpret. New hematology analyzers such as the Cell Dyn 3500 have a new computer design which enable automated white blood cell counts. This has been validated in alligators at the University of Florida. Strict validation procedure and quality control should be performed prior to generating numbers that would be used clinically. In the alligators, we have seen a significant increase in the variation of WBC counts with the anticoagulant heparin. In lambs, Synnove observed an increased variation from 5% using EDTA to 30% using heparin.[9] This means that automated counts will only be worthwhile in species that can be collected in EDTA. This includes pythons, parrots, alligators, and iguanas. Further work is needed to determine which species can be collected in EDTA.

Certain enzymes that are measured to assess organ function must be handled very carefully to insure accurate results. For instance, sorbitol dehydrogenase (SDH) is found in the cytosol of hepatocytes and testicular tissue. It is generally considered liver specific. However, it is very labile in serum especially at increased temperatures. It must be run within 8 hr and preferably sooner. Even when frozen, the serum concentration of this enzyme will decrease by 25% in 1 wk.[2] One can imagine how problems with transport could affect this value. For this reason, it is has been replaced with ALT in small animals. Additionally, the assay must be performed with strict controls and is one of the more difficult assays to run. It is still used in large animals especially at referral institutions. However, the clinician should be aware of its limitations and use discretion when interpreting this value with the rest of the chemistry panel and the clinical presentation. When establishing a

reference range for SDH, one must have intimate knowledge of sample handling and laboratory quality control to insure that the reference range is representative. Ammonia levels are useful to assess liver function and hepatic encephalopathy but have similar quality control issues to SDH.

Bile acids can be measured by either the direct enzymatic method that quantifies total serum 3 alpha-hydroxylated bile acids or a radioimmunoassay (RIA) procedure that quantifies specific bile acid moieties. Different RIA kits quantify different bile acid moieties; the most commonly used RIA procedure used in veterinary laboratories measures nonsulfated conjugated primary bile acids. The enzymatic procedure measures all 3 alpha-hydroxylated bile acids whether they are conjugated or not and is a better estimate of total serum bile acid concentrations. The direct enzymatic method is the procedure that has been clinically validated as a diagnostic test in dogs and cats. Note that the direct enzymatic method and RIA quantitate different compounds. One cannot compare numbers generated by these methods as they are actually measuring different compounds in the serum or plasma. If RIA was to be used regularly in small animals, a reference range for RIA would also have to be established in dogs and cats. One advantage of RIA is that it is less affected by hemolysis however, the assay is only linear up to 50 µmol/L and dilutions are frequently needed. Heparin has been reported to artifactually decrease bile acid specifically using the colorimetric enzymatic reaction.[5] Therefore, the RIA is the method that is used for bile acids in birds at the University of Florida. It has not been validated.

There are several different methods that have been employed to measure different hematologic and biochemical values over the years. These methods go in and out of favor in the same way that treatments fall in and out of favor. When measuring total protein, the biuret method is the most common method used in chemistry laboratories while the refractometer is the most common method used in private practices. In the biuret method, cupric ion reacts with the peptide linkages of protein in a basic solution to form a violet-colored complex with an absorption maximum of 540 nm. This has proven to be the most specific method for measuring total protein and is cost effective. A refractometer measures the scatter of light caused by a solution. Highly refractile non-protein compounds, such as glucose, can significantly change the value observed. This method is not as specific and Lumeij and Debruihne demonstrated that it is unreliable in avian practice. The refractometric method consistently yielded higher results than the biuret method, and the correlation coefficient between these two methods was low.[6] Including values from both of these methods in a single reference range for total protein would be inappropriate.

Albumin is commonly determined chemically by the bromocresol green dye-binding method. Most commercial laboratories use a human standard for total protein and albumin determinations, without validation for specific species. Discrepancies between values obtained by dye binding techniques and those obtained by electrophoresis have been demonstrated for chicken, duck, turkey, and pigeon.[6] Albumin determinations performed with dry methods have not been validated for use in birds or reptiles.

Assuming that sample collection and handling is adequate and the methodologies are consistent, one must then be concerned with quality control in the laboratory. Bulbs on spectrophotometric

machines age and decrease in intensity. Additionally, specific wavelengths may not be emitted. Different assays use different wavelengths and so an aging bulb may affect some tests and not others. The optics in a machine can become dirty and affect accurate readings. Kinetic enzyme chemistries are also dependent on temperature so significant temperature fluctuation in the lab or within the machine can alter results. Daily quality control and regular calibration is required to insure dependability of results. Of course, adequate technical training of personnel is required to insure meaningful results in both chemistries and hematologies.

Attempting to establish reference ranges for the numerous species in the field of zoologic medicine is daunting. Strict quality control at all levels is necessary. This should be followed by statistic analysis to insure that reference ranges are accurate. If practical, each zoologic institution should establish normals within its own laboratory using a specific methodology. Differences between institutions could then be addressed. Continual reassessment of reference ranges is required as methodologies change and more animals are sampled.

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Table 1. Reference values for Hitachi 911 analyzer.

UF-VMTH
CLINICAL PATHOLOGY
FELINE REFERENCE RANGES
HITACHI 911

2 - 43	Alk. Phos (IU/L)
2 - 36	AST (IU/L)
6 - 88	ALT (IU/L)
0.0 - 0.2	T. Bilirubin (mg/dL)
6.5 - 8.6	T. Protein (g/dL)
2.0 - 3.0	Albumin (g/dL)
0.3 - 0.7	A/G Ratio
3.9 - 6.3	Globulins (g/dL)
8.6 - 11.1	Calcium (mg/dL)
2.9 - 6.7	Phosphorus (mg/dL)
1.0 - 2.4	Creatinine (mg/dL)
14 - 34	BUN (mg/dL)
69 - 143	Glucose (mg/dL)
72 - 256	Cholesterol (mg/dL)
0 - 3	GGT (IU/L)
151 - 162	Sodium (mEq/L)
3.7 - 5.5	Potassium (mEq/L)
111 - 128	Chloride (mEq/L)
13 - 21	CO2 (mEq/L)
13 - 35	Anion Gap
1.5 - 2.7	Magnesium (mg/dL)
19 - 146	Triglyceride (mg/dL)

Rev. 12/29/00

UF-VMTH
CLINICAL PATHOLOGY
CANINE REFERENCE RANGES
HITACHI 911

4 - 110	Alk. Phos (IU/L)
14 - 38	AST (IU/L)
10 - 71	ALT (IU/L)
0.0 - 0.4	T. Bilirubin (mg/dL)
5.6 - 7.4	T. Protein (g/dL)
2.8 - 3.8	Albumin (g/dL)
0.7 - 1.4	A/G Ratio
2.3 - 4.2	Globulins (g/dL)
8.9 - 11.5	Calcium (mg/dL)
2.1 - 6.0	Phosphorus (mg/dL)
0.7 - 1.6	Creatinine (mg/dL)
6 - 27	BUN (mg/dL)
79 - 127	Glucose (mg/dL)
139 - 342	Cholesterol (mg/dL)
0 - 8	GGT (IU/L)
147 - 158	Sodium (mEq/L)
3.9 - 5.2	Potassium (mEq/L)
107 - 120	Chloride (mEq/L)
16 - 27	CO2 (mEq/L)
15 - 30	Anion Gap
1.4 - 2.5	Magnesium (mg/dL)
30 - 141	Triglyceride (mg/dL)

Rev. 12/01/00

UF-VMTH
CLINICAL PATHOLOGY
EQUINE REFERENCE RANGES
(INTERIM RANGES FOR HITACHI 911)
Rev. 7/27/00 **(PLASMA)**

68 - 200	Alk. Phos (IU/L)
180 - 365	AST (IU/L)
0.6 - 3.0	T. Bilirubin (mg/dL)
5.5 - 8.0	T. Protein (g/dL)
3.0 - 4.5	Albumin (g/dL)
0.7 - 2.0	A/G Ratio
2.5 - 5.0	Globulins (g/dL)
10.5 - 13.5	Calcium (mg/dL)
2.6 - 5.1	Phosphorus (mg/dL)
1.0 - 2.1	Creatinine (mg/dL)
12 - 25	BUN (mg/dL)
65 - 139	Glucose (mg/dL)
7 - 67	Triglyceride (mg/dL)
1.7 - 2.6	Magnesium (mg/dL)
10 - 40	GGT (IU/L)
100 - 600	CPK (IU/L)
133 - 150	Sodium (mEq/L)
3.0 - 5.6	Potassium (mEq/L)
95 - 109	Chloride (mEq/L)
18 - 25	CO2 (mEq/L)
15 - 30	Anion Gap

Rev. 7/27/00

UF-VMTH
CLINICAL PATHOLOGY
BOVINE REFERENCE RANGES
(INTERIM RANGES FOR HITACHI 911)

19 - 126	Alk. Phos (IU/L)
46 - 185	AST (IU/L)
0.1 - 0.4	T. Bilirubin (mg/dL)
6.5 - 8.0	T. Protein (g/dL)
3.1 - 4.3	Albumin (g/dL)
0.7 - 1.6	A/G Ratio
2.4 - 4.8	Globulins (g/dL)
8.1 - 11.0	Calcium (mg/dL)
4.7 - 8.0	Phosphorus (mg/dL)
0.5 - 1.5	Creatinine (mg/dL)
10 - 22	BUN (mg/dL)
40 - 80	Glucose (mg/dL)
1.8 - 3.0	Magnesium (mg/dL)
11 - 60	GGT (IU/L)
135 - 155	Sodium (mEq/L)
4.0 - 5.5	Potassium (mEq/L)
99 - 110	Chloride (mEq/L)
15 - 34	CO2 (mEq/L)
10 - 30	Anion Gap

Rev. 7/27/00

Table 2. Reference values for Kodak Extachem analyzer.

Veterinary reference ranges using Kodak Ektachem analyzers.

Chemistry	Canine	Feline	Equine	Bovine
CHLORIDE	110-118 mmol/L	115-128 mmol/L	94-102 mmol/L	96-104 mmol/L
CO_2	16-26 mmol/L	16-25 mmol/L	24-31 mmol/L	19-29 mmol/L
POTASSIUM	3.5-5.0 mmol/L	3.5-5.1 mmol/L	2.7-4.9 mmol/L	4.0-5.7 mmol/L
SODIUM	138-148 mmol/L	148-157 mmol/L	132-141 mmol/L	134-144 mmol/L
ALBUMIN	2.3-3.9 g/dL 23-39 g/L	2.7-3.9 g/dL 27-39 g/L	2.5-4.2 g/dL 25-42 g/L	2.4-3.5 g/dL 24-35 g/L
AMMONIA	<32 µmol/L	NA	11-55 µmol/L	NA
BUN/UREA	4.3-30.5 mg/dL 0.7-5.1 mmol/L	15-29 mg/dL 2.5-4.8 mmol/L	8-27 mg/dL 2.9-9.6 mmol/L	6-22 mg/dL 2.1-7.9 mmol/L
CALCIUM	9.7-12.2 mg/dL 2.4-3.0 mmol/L	9.0-11.6 mg/dL 2.3-2.9 mmol/L	10.7-13.4 mg/dL 2.7-3.3 mmol/L	8.2-10.0 mg/dL 2.0-2.5 mmol/L
CHOLESTEROL	126-359 mg/dL 33-93 mmol/L	77-306 mg/dL 20-79 mmol/L	51-109 mg/dL 1.3-2.8 mmol/L	68-199 mg/dL 1.8-75.1 mmol/L
CREA	0.5-1.5 mg/dL 44-133 µmol/L	0.9-2.6 mg/dL 80-229 µmol/L	0.6-1.8 mg/dL 53-159 µmol/L	0.5-1.1 mg/dL 44-97 µmol/L
GLUCOSE	67-147 mg/dL 3.7-8.2 mmol/L	75-199 mg/dL 4.2-11.0 mmol/L	72-114 mg/dL 4.0-6.3 mmol/L	44-78 mg/dL 2.4-4.3 mmol/L
MAGNESIUM	1.7-2.4 mg/dL 0.7-1.0 mmol/L	1.9-2.6 mg/dL 0.8-1.1 mmol/L	1.6-2.5 mg/dL 0.7-1.0 mmol/L	2.0-2.8 mg/dL 0.8-1.2 mmol/L
PHOSPHORUS	2.2-7.9 mg/dL 0.7-2.6 mmol/L	2.6-9.2 mg/dL 0.6-3.0 mmol/L	1.9-5.4 mg/dL 0.6-1.7 mmol/L	4.7-9.0 mg/dL 1.5-2.9 mmol/L
TOTAL BILIRUBIN	0.1-0.7 mg/dL 1.7-12.0 mmol/L	0.1-0.5 mg/dL 1.7-8.4 mmol/L	0.1-1.9 mg/dL 1.7-32.5 mmol/L	0.1-.6 mg/dL 1.7-10.3 mmol/L
BILIRUBIN Conjugated (Bc)	NA NA	0 mg/dL 0 mmol/L	NA NA	NA NA
BILIRUBIN Unconjugated (Bu)	NA NA	<0.2 mg/dL <3.4 mmol/L	0.4-2.3 mg/dL 6.8-39.3 mmol/L	NA NA
TOTAL PROTEIN	4.8-6.6 g/dL 48-66 g/L	5.5-7.1 g/dL 55-71 g/L	4.9-6.9 g/dL 49-69 g/L	5.8-7.5 g/dL 58-75 g/L
TRIGLYCERIDE	21-116 mg/dL 0.24-1.31 mmol/L	21-155 mg/dL 0.24-1.75 mmol/L	11-59 mg/dL .12-.67 mmol/L	NA NA
URIC ACID	<0.4 mg/dL <23.8 µmol/L	<0.3 mg/dL <17.8 µmol/L	<.48 mg/dL <28.6 µmol/L	0.4-1.2 mg/dL 25.6-69.6 µmol/L
ALKP	20-155 U/L	23-107 U/L	109-315 U/L	41-116 U/L
ALT	3-50 U/L	20-107 U/L	3-25 U/L	25-74 U/L
AMYLASE	388-1007 U/L	433-1248 U/L	<30 U/L	30-38 U/L
AST	1-37 U/L	6-44 U/L	205-555 U/L	58-100 U/L
CK	25-467 U/L	49-688 U/L	90-565 U/L	56-1236 U/L
GGT	5-25 U/L	5 U/L	12-45 U/L	22-64 U/L
LDH	105-1683 U/L	161-1051 U/L	520-1480 U/L	2666-4293 U/L
LIPASE	268-1769 U/L	157-1715 U/L	460-870 U/L	32-198 U/L

NA = Not Available

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ATOXOPLASMOSIS: BEYOND BALI MYNAHS

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Abstract

Atoxoplasma sp. are intracellular, coccidian parasites that have been associated with morbidity and mortality in several species of passerine birds.[1,3-5,7,9,10] Infection begins with ingestion of sporulated oocysts and release of sporozoites within the intestinal tract.[2,9] Sporozoites subsequently invade intestinal epithelial cells, lymphocytes and macrophages. Dissemination occurs when infected cells gain access to the blood. Merogony (asexual replication) occurs both in the cells of the reticuloendothelial system and intestinal epithelial cells while gametogony (sexual replication) is limited to intestinal epithelial cells. Clinical disease is not always apparent but can include inappetence, lethargy, diarrhea and weight loss.[8] Splenomegaly and hepatomegaly[9] can be seen at necropsy and lymphohistiocytic inflammation is often associated with the presence of intracellular organisms. Ante-mortem diagnosis can be challenging, but the diagnosis can be made if characteristic 3-5 micron diameter, intraleukocytic sporozoites are identified in peripheral blood or buffy coat smears. Supportive evidence is obtained if feces are found to contain oocysts that show typical *Isospora* morphology (two sporocysts, each with four sporozoites) when sporulated.

The threat of atoxoplasmosis to birds in zoologic collections or captive breeding programs was probably underappreciated prior to the deaths of several Bali mynahs in 1988.[9] Birds housed in mixed species exhibits or naturalistic enclosures that allow exposure to wild birds may be at increased risk of infection. Losses due to infection may have devastating effects in collections of endangered birds or in captive breeding programs. The Zoological Society of San Diego presently maintains 169 species of passerine birds, but prevalence and distribution of atoxoplasmosis in the collection and the pattern of disease associated with the organism have not been described. The goals of this retrospective study were therefore to identify the host range of *Atoxoplasma* sp. in our collection, document the distribution of organisms and histologic lesions associated with infection in individuals within species, and compare the pattern of infection and associated disease between species.

The Department of Pathology at the Zoological Society of San Diego maintains its necropsy and biopsy records in a computerized database. All of the records from 1983-2000 were searched for a diagnosis of atoxoplasmosis. Eighty-one of the 93 cases in which this diagnosis was made were available for review at the time of manuscript preparation. Six of the cases were excluded from the study because they did not contain organisms that were histologically or cytologically consistent with descriptions of *Atoxoplasma* sp.[2,9] The remaining 75 birds represented 33 passerine species (Table 1). Sparrows, starlings, tanagers and thrushes represented 60% of the total number of cases.

Fifty-four of the affected birds (28 species) were housed at the San Diego Zoo (SDZ) and 19 (four species) were housed at the Wild Animal Park (WAP). Two of the affected birds were wild northern house sparrows (one dead, one euthanatized for *Atoxoplasma* sp. screening) found on grounds at the SDZ. Thirty-four of the total number of birds were male, 40 were female and the sex of one bird was undetermined. Sixty-two animals died naturally. Thirty-six of these were found dead and 26 were clinically ill but died despite medical treatment. Humane euthanasia was performed in the remaining 13 cases. Excluding the wild sparrows in which age was not known, the overall mean age of the birds in this study was 25.5 mo (range: 0.17-215.2 mo). Birds (n = 32) in which atoxoplasmosis was implicated as the primary cause of death had a mean age of 23.8 mo. Birds in which atoxoplasmosis was considered a secondary diagnosis or an incidental finding (n = 41) had a mean age of 27.1 mo. Forty-four percent of birds in the former group and 37% of birds in the latter group were less than 6 mo old at the time of death.

The diagnosis of atoxoplasmosis was based on identification of protozoa that were morphologically consistent with *Atoxoplasma* sp.[2,9] in peripheral blood from impression smears of post-mortem tissue samples, in histologic sections or after sporulation of oocyst-containing feces. Both sexual (gamonts) and asexual (zoites) stages of the organism were found in 21 (64%) different species of birds. Zoites were more commonly identified in the cytoplasm of mononuclear leukocytes from impression smears of lung, spleen, and liver than in histologic sections. Infected monocytes in cytologic preparations (modified Wright-Giemsa stain) generally contained a single 2-4 micron diameter, intracytoplasmic, clear to lightly basophilic zoite with central granular, magenta material. The presence of the organism caused indentation of the nucleus in most cells.

In histologic tissue sections, gamonts were present in epithelial cells lining the villi of the small intestines. They were basophilic and varied from 6-12 microns in diameter. Intraleukocytic zoites were generally difficult to find but were most often seen in the lung, liver and spleen. Zoites appeared smaller (1-2 microns in diameter) in tissue sections than in cytologic preparations, which was probably an artifact due to different preparation methods. Inflammation was more often associated with intraleukocytic zoites than with gamonts, and was generally lymphohistiocytic. When intestinal inflammation was present, it was primarily associated with intraleukocytic zoites in the lamina propria rather than with intraepithelial gamonts. The most severe cases had the appearance of an infiltrating leukocytic neoplasm in which small numbers of cells contained intracytoplasmic zoites. Similar descriptions of severe lymphoproliferative disease associated with intracellular protozoa have been reported in goldfinches[7] and warblers.[11]

In contrast to the general pattern of lymphohistiocytic inflammation, all of the Indochinese white-rumped shamas contained aggregates or infiltrates of extremely large, histiocytic cells with abundant eosinophilic cytoplasm and one or two intracytoplasmic, 1-2 micron in diameter, eosinophilic organisms. The organisms were identified as apicomplexan protozoa with transmission electron microscopy and the diagnosis of atoxoplasmosis was based on their presence and the production of oocysts with *Isospora* morphology after fecal sporulation. A description of these cases has been published elsewhere.[6]

The results of this retrospective study expand the list of susceptible passerine species known to become infected with, develop clinical illness and die from atoxoplasmosis. Important questions such as whether the protozoal infections in the present cases are due to a single or multiple species of atoxoplasma and whether interspecies transmission can occur remain to be answered. Polymerase chain reaction (PCR) for *Atoxoplasma* sp. has recently been implemented in the Molecular Diagnostics Laboratory at the Zoological Society of San Diego. Examination of archived tissue and antemortem tissue and fecal samples will be important tools with which to answer these questions.

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Table 1. Passerine species with atoxoplasmosis.

Species	Common Name	Scientific Name
Mynahs	Bali mynah	*Leucopsar rothschildi*
	Sulawesi mynah	*Basilornis celebensis*
Robin Chats	Western white crowned robin chat	*Cossypha albicapilla albicapilla*
	Western snowy crowned robin chat	*Cossypha niveicapilla niveicapilla*
Sparrows	Northern house sparrow	*Passer domesticus domesticus*
	Southern cape sparrow	*Passer melanurus melanurus*
	Sudan golden sparrow	*Auripaser luteus*
Starlings	Eastern golden-breasted starling	*Cosmopsarus regius magnificus*
	Emerald starling	*Lamprotornis iris*
	New Guinea metallic starling	*Alponis metallica metallica*
	Northern superb starling	*Spreo superbus superbus*
	Wattled starling	*Creatophora cinerea*
Tanagers	Northern paradise tanager	*Tangara chilensis paradisea*
	Northern silver throated tanager	*Tangara icterocephala frantzii*
	Northern spotted tanager	*Tangara punctata punctata*
	Northern swallow tanager	*Tersina viridis occidentalis*
	Guianan turquoise tanager	*Tangara mexicana mexicana*
	Western bay headed tanager	*Tangara gyrola catharinae*
	Western golden masked tanager	*Tangara larvata fransicsae*
Thrushes	Eastern white-throated laughing thrush	*Garrulax albogularis eous*
	Nepalese greater necklaced laughing thrush	*Garrulax pectoralis picticollis*
Other species	Blue-backed fairy bluebird	*Irena puella sikkimensis*
	Javan Ruby throated bulbul	*Pycnonotus melanicterus dispar*
	Himalayan grey-headed bullfinch	*Pyrrhula erythaca erythaca*
	Siberian goldfinch	*Carduelis carduelis major*
	Red throated parrot finch	*Erythrura psittacea*
	Tien shan red mantled rosefinch	*Carpodacus rhodochlamys rhodochlamys*
	Fukien niltava	*Niltava davidi*
	Surinam crested oropendola	*Psarocolium decumanus decumanus*
	Red fronted serin	*Serinus pusillus*
	Indochinese white-rumped shama	*Copsychus malabaricus interpositus*
	Northern white headed buffalo weaver	*Dinemellia dinemelli dinemelli*
	Southern capped wheateater	*Oenanthe pileata pileata*

MALARIA IN BIRDS AT THE SMITHSONIAN NATIONAL ZOOLOGICAL PARK

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Abstract

Malaria occurs in birds, humans and other primates, and is caused by protozoal parasites in the genus *Plasmodium*. Sexual reproduction of this parasite occurs in many species of *Anopheles, Aedes* and *Culex* mosquitoes resulting in sporozoites that find their way to the insects' salivary glands and saliva.[4] Several sporozoites are injected into the vertebrate host at every blood meal. The parasites pass through two stages of asexual reproduction within the vertebrate host: exoerythrocytic and intraerythrocytic.[5] Exoerythrocytic replication within the liver occurs first; the merozoites that are produced eventually break out of the hepatocytes and invade erythrocytes. Once in the cytoplasm of an erythrocyte, the merozoites progress through several morphologic stages. In the 'ring' stage, the protozoan contains a centrally located vacuole and a peripheralized, red nucleus. With development into the trophozoite stage, food vacuoles are formed from invagination and pinching off of the host cell's cytoplasm. Digestion of this meal leaves the characteristic, birefringent pigment associated with fulminant *Plasmodium* parasitism. As in the exoerythrocytic stage, the parasite replicates and forms merozoites which lyse the host red blood cell and enter other red blood cells.[1] Serial infection of erythrocytes may occur indefinitely. Eventually, the intraerythrocytic merozoites develop into sexual gametes that are ingested by a mosquito during a blood meal.[5] The incubation time of *Plasmodium* spp. (i.e., the period between initial infection of the vertebrate host by the sporozoites and the manifestation of disease) in birds has not been extensively studied. However, in humans infected with *P. vivax*, the incubation time can range between 12-14 days.[5]

Individual *Plasmodium* species are not always species specific, but their infectivity and vector preference are usually limited. The human malarial pathogen, *P. falciparum* executes its sexual cycle only in *Anopheles* mosquitoes and infects only humans.[8] A less selective species, *Plasmodium relictum* is carried by *Culex, Anopheles, Aedes and Culiseta* mosquitoes and is infective for many birds, including ducks, doves, and passerines.[6]

A retrospective survey of the pathology records at the Smithsonian National Zoological Park (SNZP) revealed a high prevalence of malaria in four species of birds within the captive collection. Because the incubation period of *Plasmodium* is approximately 14 days, only birds that were greater than 14 days old were included in this study. During the period 1975-2000, malaria was diagnosed in 32% (15/47) of Inca terns (*Larosterna inca*), 22% (2/9) of Patagonian crested ducks (*Anas specularioides specularioides*), 11% (2/19) of red-breasted geese (*Branta ruficollis*), and 6.2% (10/161) of common eider ducks (*Somateria mollissima dresseri*) that died.

Manifestation of disease showed a variably seasonal occurrence. Most of the birds affected with malaria died in the fall and winter with 52% (15/29) presented for necropsy in September and October. A smaller, secondary peak of malaria mortality (7/29 or 24%) occurred in June and July. No deaths from malaria occurred in the spring months of March, April or May.

Birds affected with malaria had either a short clinical course or were found dead with no previous disease manifestations. When clinical signs were evident, they were vague and non-specific: lethargy and inactivity. The time between onset of clinical signs and death in these cases was commonly no greater than 24 hr.

The most significant gross pathologic lesion in birds with malaria at SNZP was enlargement and congestion of the liver and spleen. Intraerythrocytic and/or intrahepatocytic stages of *Plasmodium* protozoa were readily identified on cytologic impressions of these organs. Many of the birds, especially the eider ducks, were affected concurrently with pulmonary or systemic aspergillosis. These birds had multiple fungal plaques within the airsacs and associated with lungs or liver. Both affected red-breasted geese also had amyloidosis affecting several organs including the liver, spleen and kidneys. These contributing disease factors may have been sequelae to the primary *Plasmodium* insult.

Histologically, there was periportal lymphocytic and plasmacytic inflammation in the livers. Kupffer cells and macrophages within the livers contained varying amounts of malaria pigment. Malaria pigment was also present within splenic histiocytes and was accompanied by moderate plasmacytosis. Protozoa could occasionally be seen within the red blood cells on histologic sections of various organs. However, tissue fixation and processing for histology causes shrinkage of blood cells, often making it difficult to detect the intraerythrocytic malarial organisms by this method.

Cytologic preparations of blood smears (ante mortem) or impressions of liver and spleen at necropsy were the most ideal methods for confirming the presence of malarial infections. Wright's or Giemsa stains were used to visualize the intraerythrocytic and intrahepatocytic forms of the protozoa.

Speciation of *Plasmodium* organisms required examination of the shape of the meront and schizont stages and determining the degree of displacement of the host cell nucleus.[6] In some cases at SNZP, fixed blood smears and/or tissue imprints were sent to the International Reference Centre for Avian Haematozoa in Newfoundland for positive identification. *Plasmodium cathemurium* was detected in one Inca tern and *P. relictum* was found in an Inca tern and a common eider. Both *P. cathemurium* and *P. relictum* are found in the common house sparrow. *Plasmodium hegneri*, a rare species previously only found in the green-winged teal (*Anas crecca*) in Taiwan, was found in both Patagonian crested ducks.

Inca terns and Patagonian crested ducks are native to the western coast of South America.[3] Inca terns are permanent residents of offshore islands and the rocky coasts of Peru and Chile. Crested ducks are similarly non-migratory and live on the coasts of Chile and Argentina and on the Falkland Islands. Red-breasted geese and common eiders exist in aquatic environments of the Northern

Hemisphere.[3] Common eider ducks are pelagic birds of the North Atlantic Ocean and nest along the Atlantic North American coast. Red-breasted geese nest near lakes and reservoirs in the Siberian tundra and spend winters near the Black or Caspian Seas.

Mosquitoes require shallow, still, fresh water in a temperate or tropical environment for breeding.[7] Cold coastal waters and a tundra climate are not ideal for the insects' life cycle. Thus, it is unlikely that these birds evolved with the presence of mosquitoes or malarial organisms; this may explain the increased susceptibility of these birds to malaria, compared to birds from more temperate climates. Black-footed penguins (*Spheniscus demersus*), like the species of birds that we studied, evolved in a marine environment which does not support the mosquito life cycle. Studies have shown that these penguins are highly susceptible to *P. relictum* and *P. elongatum*.[2]

The increased susceptibility to malaria exhibited by these species warrants strenuous efforts on the part of zoos and wild animal parks aimed at mosquito control and detection and treatment of disease. Control programs may include removal of mosquito breeding areas, utilization of mosquito-feeding fish in standing ponds, mosquito trapping, and/or selective use of pesticides. Enclosed exhibits can minimize access of mosquitoes to malaria-susceptible bird species. In areas where mosquito control is inadequate or not feasible, cytologic screening of stained blood smears from susceptible species in summer and fall may identify affected animals. Early detection and treatment with chloroquine and/or pyrimethamine may minimize mortality due to *Plasmodium* infections.

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MOLECULAR DISEASE DIAGNOSTICS IN PSITTACINE BIRDS

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Abstract

New molecular technologies provide a powerful means to diagnose and manage infectious disease agents in psittacine birds. The underlying theory and application to Psittacine Beak & Feather disease, avian polyomavirus, Pacheco's disease virus, and chlamydiophyla is discussed. Submission recommendations and test interpretations are provided.

Introduction

Historically, infectious diseases of avian species have been some of the most difficult to confirm by diagnostic testing and difficult to manage in an avian population. Advances in the field of molecular biology have allowed for the development of extremely sensitive and specific DNA based testing to aid these diagnoses. The first commercial application to avian disease testing was in the diagnosis of Psittacine Beak & Feather Disease (PBFD) and Avian Polyomavirus (APV) infections (Research Associates Laboratory, Inc., 1992).[1]

DNA amplification techniques coupled with internal nucleic acid probes provide diagnostic tests of extreme accuracy. Practitioners should possess a good understanding of the principles involved in this new technology to best utilize it's potential. Additionally, a thorough evaluation of the available laboratory services and tests being offered is essential to identify those best suited to provide valid test results.

Molecular Test Technology

Molecular based testing comprises several distinct steps. The first is the isolation of nucleic acid (DNA or RNA) from the diagnostic sample. Samples may include blood, serum, tissue and environmental swabs, and feces. Strict attention to sample collection, storage, and transport is essential to avoid sample contamination and inaccurate (false positive) test results. Laboratories must also adhere to strict methods of sample handling. Improper sample handling can result in sample, cross contamination and contaminated work environments, leading to inaccurate test results.

The next step in the testing process involves the enzymatic amplification of the target DNA sequence. This is generally accomplished through a process known as the polymerase chain reaction (PCR). A thermal stable DNA polymerase is used to synthesize a complimentary DNA strand from the target sequence. Target strand amplification occurs between two sets of nucleic acid primers. These are synthetic oligonucleotides, complementary to a particular sequence of DNA, found in the bacteria, virus or protozoa being tested. It is important to remember that the particular sequence of

these primers confers specificity to the DNA amplification process. The design and location of these primers is usually determined by the individual laboratory and is crucial to the accuracy of the PCR test. If a laboratory designs primers in a variable or non-conserved region, the PCR test may not give a consistent positive result among various strains of the target organism. A (false) negative test result can occur in an actual positive test sample. The recent experience of practitioners screening birds for the Psittacine Beak and Feather Disease virus confirms this occurrence.

Throughout the PCR process, samples are ramped through a cycle of temperature settings in an instrument called a thermal cycler. Each cycle of the reaction consists of three distinct phases. A denaturing step in which the double stranded DNA template is denatured into two single strands, an annealing step where the primers bind to the complementary target DNA sequences, and an extension phase where DNA polymerase synthesizes a complementary copy of the target strand. The entire cycle is repeated for 25 to 35 times. Theoretically, the target DNA sequence is doubled in each cycle. During a routine testing process, target DNA sequences are amplified into millions of copies (2^n, n = 30-40). This ability to amplify small quantities of target DNA gives the technique its great sensitivity. PCR tests usually can detect less than 10 copies of target sequence. Cross contamination can therefor be a major concern. When the same diagnostic test is performed week after week the laboratory risks contamination with large amounts of amplified DNA (amplicons). The inadvertent introduction of small amounts of this material into a test sample before PCR amplification will result in a false positive test result. Laboratories must have sufficient safeguards established to avoid this risk of sample contamination. While other potential sources of sample cross-contamination exist, amplicon contamination is by far the most important. It has been reported that very few laboratories use specific methods to destroy amplicons and thus prevent sample cross-contamination.[2]

The final step in the testing process involves the confirmation of amplified (positive) test samples. By determining that the amplified product has the correct genetic code, the specificity of the test process is greatly enhanced. This is generally accomplished by hybridizing a nucleic acid probe, to the internal sequence of the amplified DNA. The DNA probe has enzymes or fluorochromes covalently attached to it, which are used to visualize or detect the amplified product. If the hybridization test is positive, it confirms that the amplified product represents a true positive sample.

Several diagnostic laboratories in the United States offer molecular-based veterinary testing. The number of laboratories and available tests should increase as applied technologies become more widespread. While several laboratories may offer molecular testing for a particular disease, the methods and practices utilized are not identical. This can lead to variability in test results among those labs. It has even been reported that a number of laboratories doing PCR testing lack sufficient safe guards (controls) to ensure that the results are accurate.[2] It is imperative that a practitioner keenly evaluate the available testing before selecting a laboratory to submit samples to. Validation of a laboratory's testing should be supported by scientific presentation and publication. Performance of these tests in the field of use should also be reviewed. Only then will a practitioner be able to select laboratories that are best prepared to give valid results.

Molecular Based Tests

Historically, the application of molecular based testing in veterinary medicine has been most extensively applied to diseases of avian, and in particular, psittacine species. Currently, validated tests exist for Psittacine Beak and Feather Disease virus, avian Polyomavirus, Pacheco's Disease virus, and Chlamydiophila (psittacosis) infections. These tests can be utilized to provide a confirmed diagnosis of infection in both ante-mortem and post-mortem samples. They also can detect sub clinical infections in "carrier" birds and can be used to assess the level of environmental contamination in physical facilities. Culturette swabs of cut tissue sections and environmental surfaces are used for post-mortem and environmental testing respectively. Preferred sample submission and test interpretation for individual diseases are listed below.

Psittacine Beak and Feather Disease (PBFD)

Whole, unclotted blood and post-mortem tissue swabs are the preferred samples to submit for testing birds for this infection. Currently, the majority of birds that test positive do not exhibit feather abnormalities or other outward signs of PBFD disease. Most of these are sub-clinically and transiently infected with the PBFD virus. Birds commonly come in contact with the virus through environmental contamination during shipment, in pet stores, at bird shows etc… Although infected with the virus, these birds do not show clinical disease. With a mature, functioning immune system, most birds are capable of mounting an effective and protective immune response, which results in elimination of the PBFD virus. Retesting of these individuals 90 days later is recommended, at which time most will show a negative test result.[3] Chronic, low-grade clinical infections are evident particularly in lovebirds and lorikeet species. These birds serve as a source of infection to other birds and contaminate environmental areas. The ability to compartmentalize this virus has been theorized during which infected birds may test blood negative. Current research is investigating this theory, which has yet to be substantiated. The inability to detect these individuals may however be due to inadequacies in test design.

The genome of the PBFD virus has been shown to vary, as much as 12% among isolates from different species.[4] Depending on design, DNA based tests may not be able to identify this infection in all species of psittacine birds. The problem has been most evident in detecting infected lory and lorikeet species. Conserved regions in the genome however do exist and a properly designed test should be able to detect infection in various psittacine species.[4] Interpretation of test results is listed in Table 1.

Avian Polyomavirus

Birds may be screened for infection with this virus using whole, unclotted blood or cloacal swabs. Avian Polyomavirus is thought to shed intermittently with shedding being most evident in clinically ill birds. During a neonate outbreak, the ability to detect infected birds is similar between whole blood and cloacal swabs. Birds that become ill and recover from the disease often become non-clinical carriers. They can harbor and shed the virus well into their adult life.[5] Blood testing is

much more reliable in detecting these infected individuals. Swabs of liver, spleen, and bursa can be used to confirm the diagnosis in post-mortem cases. Test sample interpretation is listed in Table 2.

Pacheco's Disease Virus

The cause of acute illness and death in adult psittacines, Pacheco's disease is incompletely understood.[6] Many strains of herpes virus belonging to at least five serologically distinct subtypes exist, but not all infections appear to cause disease in all birds. Conure and Amazon parrot species are frequently implicated as carriers of the virus however infections also appear to be far more common than previously thought. A DNA based assay has been developed to identify birds infected with this virus.[6] Cloacal swabs are the best sample to submit when screening birds, followed by fecal swabs, and whole, unclotted blood. One cloacal swab test will identify 75% of infected individuals; two tests, 30 days apart will detect 95% of those infected. Like herpes virus infections in other species, infected birds are considered to be infected for life.

Chlamydiophila

Avian chlamydiophila is an important infection that has historically affected aviculture. Impact ranges directly from overt clinical disease and mortality to the often non-diagnosed effects on growth, health, and reproduction. The organism exhibits a pronounced variability in host susceptibility, pathogenicity, course of disease, and diagnostic parameters. While numerous diagnostic methods have become available over the years, test results are often equivocal, making a confirmed diagnosis in a live bird elusive.

A molecular based test to identify birds infected with *Chlamydia psittaci* has been reported.[7] The test appears ideally suited to detecting states where infectivity is low or where a rapid assay is desired. It provides a confirmed diagnosis of chlamydial infection in the clinically inapparent and/or persistently infected state. It also offers the advantage of providing a sensitive method for chlamydia detection, which is not dependent upon a host immune response. Infectivity studies have shown that upon initial infection, oral/choanal swabs almost always immediately test positive. The organism becomes detectable in cloacal swabs around 10 days post infection. By 15 days post-infection, both swabs and blood test positive for infection.[7] It is recommended to submit a combined choanal and cloacal swab, and whole unclotted blood on each individual to be tested. Post-mortem liver and splenic swabs will also confirm infected individuals. It should be noted that doxycycline can interfere with the PCR test process. Samples should be acquired before treatment is initiated.

Conclusion

Molecular based testing offers a powerful means to diagnose and manage infectious disease in psittacine birds. It is important that practitioners understand the underlying technology and know how to properly submit samples for testing. Prudent sample collection and handling is essential to

insure accuracy of test results. They should also utilize only those laboratories offering tests with proven performance through scientific publication, presentation, and field use. These laboratories must have sufficient quality control and assurance procedures to report accurate results. When these criteria are met, practitioners will receive meaningful information to aid them in the control of these infectious diseases.

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Table 1. PBFD DNA test interpretation.

Age	Test result	PBFD Clinical signs	Interpretation
Neonate	Positive	None	PBFD viral nucleic acid was identified in the sample; Bird should be considered infected and may become clinical; Isolate and retest the bird in 30-90 days.
Juvenile to adult	Positive	None	PBFD viral nucleic acid was identified in the sample; Bird should be considered exposed and infected, but will most likely become immune and eliminate the virus; Isolate and retest in 30-90 days.
Neonate, juvenile to adult	Positive	Yes	PBFD viral nucleic acid was identified in the sample; Bird should be considered infected and clinical; Isolate.
Neonate, juvenile to adult	Negative	None	PBFD viral nucleic acid was not identified in the sample; bird should be considered negative for PBFD infection
Neonate, juvenile to adult	Negative	Positive	Consider other disease etiologies; run swab test from affected feather pulp for PBFD and APV.

Table 2. Avian polyomavirus blood test interpretation.

Age	Test result	APV Clinical signs	Interpretation
Neonate	Positive	None	APV viral nucleic acid was identified in the sample; Bird should be considered infected and a potential source of infection to other birds; bird may become clinical, generally within 2 wk; Isolate and retest the bird in 30-90 days.
Juvenile to adult	Positive	None	APV viral nucleic acid was identified in the sample; Bird should be considered exposed and infected, but will almost always become immune and eliminate the virus; Isolate and retest in 30-90 days.
Neonate	Positive	Yes	APV viral nucleic acid was identified in the sample; Bird should be considered infected and a source of APV infection to other birds; bird is clinical and may become a persistently infected "carrier" if it recovers. Isolate and retest at 30-90 days.
Juvenile to adult	Positive	Yes	APV viral nucleic acid was identified in the sample; bird may be exhibiting a rare case of Adult APV disease; An immuno-suppressed state is considered and the bird should be tested for PBFD.
Neonate, juvenile to adult	Negative	None	APV viral nucleic acid was not identified in the sample; bird should be considered negative for APV infection.
Juvenile to adult	90 day Positive Retest	None	APV viral nucleic acid was identified in the sample; bird is persistently infected and may serve to spread APV to other birds; Bird will most likely remain non-clinical and will probably become negative after a variable period of time.

THE USE OF OXYGLOBIN® IN BIRDS: PRELIMINARY PHARMACOKINETICS AND EFFECTS ON SELECTED BLOOD PARAMETERS AND TISSUES

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Abstract

Clearly, tissue oxygenation is the most important function of the cardiovascular and respiratory systems. Because of their highly specialized respiratory system, birds are more sensitive to the effects of hypoxia than mammals. As in other species, anemia has many causes including malnutrition, infection, immune-mediated diseases, parasites, or blood loss from hemorrhage. Traditional treatment for severe anemia includes either a whole blood or concentrated red blood cell transfusion. In some patients, multiple blood transfusion may be required, which is often problematic.[1-3]

For years synthetic blood substitutes have been investigated. Progress in purification techniques of bovine hemoglobin made Oxyglobin® (Biopure Corporation, Cambridge, MA 02141) available to veterinarians in 1998. This colloidal product contains 13g/dL of polymerized bovine hemoglobin and is labeled for use in dogs as supportive treatment for any type of anemia. With Oxyglobin® tissue oxygenation is improved by (1) increasing the oxygen content of blood, (2) expanding the vascular volume, (3) allowing circulating molecules of hemoglobin in plasma to reach small vascular spaces unavailable to red cells, and (4) providing a higher potency for oxygen release to the tissues than red cells.[4-8]

Hemoglobin-based oxygen carriers are compatible with any blood type; therefore the need for homologous donors and cross matching is eliminated. Theoretically, this solution could be used in any species. The objectives of this study were: (1) to determine the pharmacokinetics of a single dose of 15ml/kg of Oxyglobin® in healthy chicken, (2) to identify Oxyglobin®-associated acute changes in hematologic parameters (CBC) and plasma chemistries, (3) to evaluate selected acute tissue and organ changes (histopathology) at 1 and 7 days post infusion using histopathology, and (4) to determine acute morbidity/mortality that occurs acutely in experimental birds during the 7-day study.

Twenty-two healthy adult SPF White Leghorn chickens ranging in weight from 1.1 to 2.1 kg were utilized (16 experimental and six control). On the day of the study, each bird was manually restraint without sedation and a 24-ga Teflon catheter was placed intravenously (i.v.) in the basilary vein. The birds were randomly divided in two groups (A and B). Each bird randomly received either 15 ml/kg i.v. of Oxyglobin® (experimental) or Hetastarch® (Baxter Health Care, Deerfield, IL 60015) (control)

over 5 min. Blood was also collected for the pharmacokinetic analysis prior to the infusion in all birds, and at the following times: in group A, at 5, 15, 30, 60 min, 2, 4, 6, 12, 24 hr, 2, 3, 4, 5, 6, 7 days post infusion. In group B, blood was collected at 15 min, 6, 10, 14, 18, 24 hr post infusion. Blood was submitted for CBC and chemistry panel prior to the infusion, then at 1, 3 and 7 days post-infusion. The group A birds were euthanatized on day 7 and the group B birds were euthanatized on day 1 post-infusion. Complete necropsies were performed on each animal and the tissues submitted for histologic examination.

Results are summarized in Table 1. A 2-compartment pharmacokinetic model gave the best curve fit to the data (Fig. 1). The distribution phase (α) had a mean half-life of 15.71 and the elimination phase (β) had a mean half-life of 15.67 hr. Levels of hemoglobin reached baseline values by day 3. The elimination rate K_{elim} was 0.069. The value for the area under the curve (AUC) was 63.17; the transfer rate from the first compartment to the second compartment K_{12} was –0.0092 and from the second compartment to the first K_{21} was 0.028.

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Table 1. Mean and standard deviation (SD) of serum concentration of hemoglobin in g/dL after a single i.v. slow bolus of Oxyglobin® administered at 15 ml/kg of body weight.

			Time post-infusion													
	Base-line	Post Infusion	min		hr							days				
			15	60	4	10	12	14	18	24	48	3	4	5	6	7
mean g/dL	0.04	4.29	4.69	4.41	3.50	2.51	2.29	1.74	1.29	0.83	0.09	0.03	0.04	0.04	0.05	0.04
SD	0.02	0.80	0.98	1.06	1.10	1.09	1.00	1.03	0.90	0.71	0.07	0.02	0.02	0.02	0.04	0.03

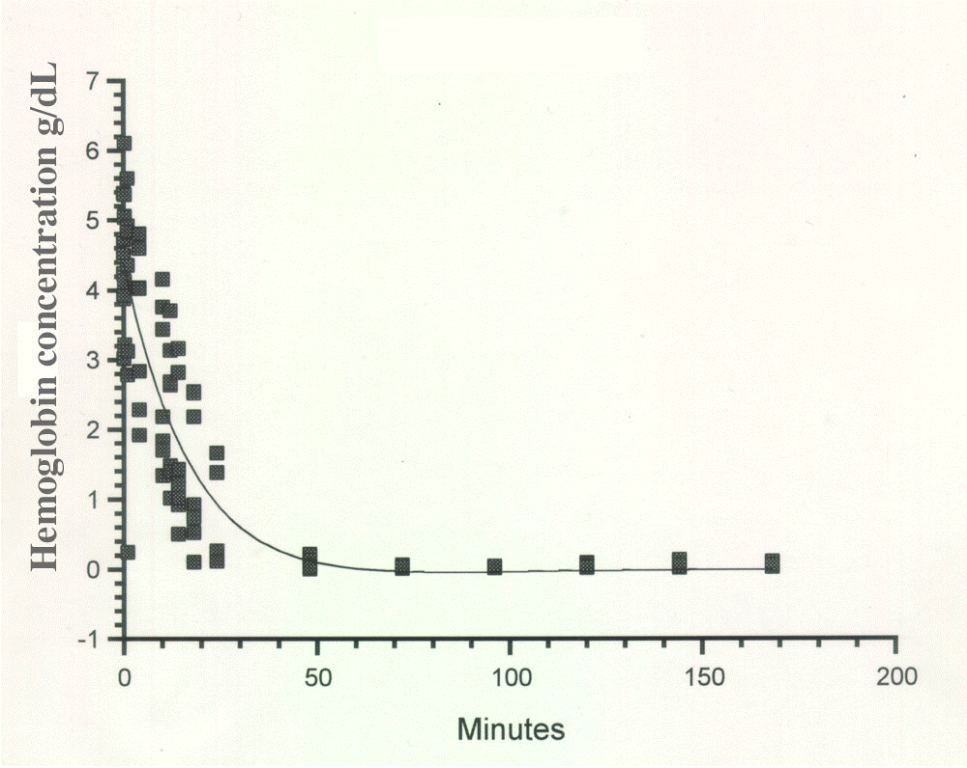

Figure 1. Serum concentrations of hemoglobin after a single i.v. slow bolus of Oxyglobin® administered at 15 ml/kg of body weight.

DIODE LASER SURGERY AND ITS APPLICATION IN BIRDS

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Abstract

Since Albert Einstein theorized the concept of lasers in 1916 considerable development has resulted in an array of equipment available to the human and veterinary surgeon. The term LASER stands for Light Amplification by the Stimulated Emission of Radiation and relies upon the production of electromagnetic radiation in response to photon emission by the lasing medium.[1] The characteristics of a laser are directly attributed to the lasing medium, be that CO_2, helium, argon, gold, ruby, or gallium aluminum arsenide (GaAIAs). The use of lasers in avian surgery, while not commonplace, is certainly gaining in popularity with various publications describing and supporting their use.[2]

A class IV GaAIAs diode laser creates a wavelength in the range of 790-830 nm which is transmitted from laser base unit to surgical site by a solid quartz-core, fiber-optic cable. A visible light beam is combined with the invisible laser beam to facilitate aiming. Laser fibers come in a variety of sizes (400-1000 mm) and shapes, including flat, conical and orb. Operator safety requires all personnel to wear protective glasses.

One of the advantages of the diode laser is the ability to use 400 mm fibers through rigid and flexible endoscopes, or in hand-pieces for open surgery. The Diomed Surgical Diode Lasers and fibers (Diomed Inc, 23 Main Street Suite 240, Andover, MA 01810 USA) appear to be compatible with the 2.7 mm telescope and protective 14F sheath with 5F instrument channel (64018BS, 67065CC; Karl Storz Veterinary Endoscopy America, 175 Cremona Drive, Goleta, CA 93117 USA).

Previous reports have described the use of diode lasers for ablating renal masses and gonads, and coagulating the infundibulum/oviduct to prevent reproductive activity.[3,4] In addition, lasers have been used to excise cutaneous masses and ablate small air sac granulomas. The endosurgical vaporization of bacterial and mycotic granulomas attached to the ventrolateral aspect of the lung or the air sacs was accomplished using a 400 mm fiber on a power setting of 3.5-6.5 watts. Where such granulomas are in close association with vital structures or large vessels, a pulsed power setting of 0.1 seconds and interpulse interval of 0.2 seconds helped reduce collateral heating and tissue damage.

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THERAPY FOR MYCOTOXICOSIS IN SANDHILL CRANES (*Grus canadensis*) IN FLORIDA

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Abstract

The sandhill crane is one of 14 crane species in the world. Two subspecies of sandhill crane reside in Florida; the Florida sandhill crane (*Grus canadensis pratensis*) and the greater sandhill crane (*Grus canadensis tabida*). The Florida subspecies is considered threatened due to its low reproductive potential, specialized habitat requirements and habitat destruction. The Florida sandhill crane is non-migratory and nests in Florida; whereas, the greater sandhill crane is a migratory species, spending winters in Florida and the remainder of the year in the Great Lakes states. The U.S. Fish and Wildlife Service and Canadian Wildlife Service have established a non-migratory population of whooping crane (*Grus americanis*), a critically endangered species, in Florida.

From 1981 to 1991, numerous injured and ill sandhill cranes were presented to the University of Florida Veterinary Medical Teaching Hospital (VMTH) by the Florida Freshwater Fish and Game Commission for a variety of problems.[2] Cholangiocarinomas, hepatic disease, and a "neck paresis syndrome" were seen with unusual frequency.[1,2] Mycotoxins are the most likely underlying etiology in two of these conditions, cholangiocarcinoma and the "neck paresis syndrome." This presentation will focus on the neck paresis syndrome. This syndrome was first noted in 1985 in sandhill cranes in Florida when wildlife officials began monitoring the birds in areas near peanut fields. The peanut harvest in Florida occurs in late October. The syndrome is triggered by the onset of cold weather, usually in late December or early January and continues for approximately 3 mo. Approximately, 5-10% of the flocks (up to 300 birds) in Alachua, Levy, and Marion counties are affected annually by this syndrome. An estimated 9,500 sandhill cranes died in Gaines County, Texas and Roosevelt County New Mexico between 1982 and 1987 from this syndrome. Trichothecene and other mycotoxins produced by *Fusarium* spp. were isolated from waste peanuts that the birds were feeding on and were considered to be the most likely cause of the clinical signs the cranes were showing.[2,4]

In the past in Florida, predators focused on roosts with affected birds or the birds would die of starvation or dehydration. Now birds are taken by wildlife officials before they die of the syndrome. Numerous cranes have been admitted to the VMTH with drooping necks and severe dependent

cervical edema. A thorough diagnostic evaluation was performed on all cases prior to instituting therapy. This included a physical examination, complete blood count, plasma biochemical profile, and plasma bile acid level. An equine encephalitis viral screen, fecal direct and flotation, whole body and cervical radiographs, electromyogram, muscle biopsy, and a liver biopsy were performed on selected cases. Birds with this condition that died received a complete postmortem examination. Consistent abnormalities included elevation of aspartate aminotransferase (AST), creatine phosphokinase (CPK), fibrinogen, and the white blood cell count. Similar changes have been noted in previous reports on this condition.[3] Most birds had histologic changes to the liver including chronic lymphocytic hepatitis, biliary hyperplasia, hemosiderosis, and hepatic necrosis. Cholangiocarcinoma was observed in one bird with this syndrome. No histologic changes were noted in cervical muscle biopsies performed on several birds. However, an electromyogram in one bird revealed changes consistent with a peripheral neuropathy. Pesticide screens on gizzard contents and selected tissues on three cranes were negative. Mycotoxin analysis on peanut stubble samples collected from November to February from peanut fields used by the cranes in Alachua county were inconclusive.

Upon arrival to the VMTH, most of these birds were moderately to severely dehydrated and in shock. Blood samples were obtained and then the birds were treated for shock and dehydration. The cranes were unable to eat or drink in this condition, thus force feeding and parenteral fluid therapy were instituted until the head could be held up in a normal position. For the first several cases, the crane's head would be manually held in a normal position for 5 to 10 min four times daily. With this technique, recovery times would be approximately 3 to 4 wk. Some of these birds died from secondary diseases such as disseminated visceral coccidiosis (DVC) and aspergillosis. In order to decrease recovery time and stress of handling, a pulley system was developed. A piece of roll gauze was placed through the external nares of the bird, then the roll gauze was extended over to the next enclosure and a 1-kg weight was attached to the other end. This elevated the head of the bird and allowed it to move back and forth in the enclosure. Recovery time with this technique was 24 to 48 hr. The most important part of the therapy appears to be elevating the head long enough for the cervical edema to subside and isolating the birds from further toxin exposure.

Eleven sandhill cranes were successfully rehabilitated and released using this technique from 1987 to 1991. Six of these were sited for 1 yr post-release and one for 3 yr post-release. This treatment regimen continues to be successful and could be utilized by other institutions encountering this problem in sandhill cranes and potentially other crane species.

Rendering moldy peanuts inaccessible to cranes by conventional tilage as soon after harvest as possible has resulted in reduced crane mortality in Florida and other areas where cranes feed on peanuts.[4] There are plans to establish a second migratory flock of whooping crane which will nest in Wisconsin and winter in Florida. If this projects proceeds, the resultant population will have the potential to be exposed to waste peanuts in the same areas and at the same rates as the sandhill cranes described above. This increases the importance of a quick diagnosis and initiating the treatment procedure.

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POPULATION: THE ULTIMATE CHALLENGE FOR ENVIRONMENTAL ACTIVISM

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Abstract

Global human population growth is placing stresses on wildlife species and habitats which challenge efforts to maintain Earth's biodiversity. It is the purpose of this analysis to summarize what is currently understood about human population growth and its impacts on wildlife populations, in order to better inform efforts to take direct action to prevent or mitigate those impacts. A review of recent findings about the population-environment nexus and the experience of the National Audubon Society in addressing population issues serves as the basis for this analysis.

During the twentieth century human population exhibited an unprecedented scale of growth, nearly quadrupling in just 1 century to over 6 billion people. At the current annual growth rate of approximately 1.4%, our population could double again by the middle of this century. Of particular concern to the fate of the rest of Earth's species is the fact that the human populations inhabiting most of the 25 recognized global biodiversity hotspots are both denser and growing faster than the world average.[1]

Human populations can cause the extinction of other species through a number of mechanisms, including hunting and deliberate extermination, habitat degradation or destruction, and introduction of exotic species, each of which tends to be aggravated by growth in human numbers.[2] Birds are particularly sensitive to these impacts, and recent trends in their numbers are indicative of the pressures created by human population growth. Fully half the species migrating between the United States and Latin America have experienced significant declines in recent years, while Latin America's human population more than doubled in only 30 yr. This extremely rapid rate of growth overwhelms the developing economies of the region, leading nations to exploit their natural wealth to try to solve their economic and social problems. The migratory birds are also impacted by population growth in the United States, the third most populous nation on Earth, where growth-driven habitat fragmentation contributes to their declining numbers.[3]

In response to a concerted international effort to address the causes of rapid human population growth, the global rate of growth has been declining in recent years. One dramatic example of this decline is Mexico, where the fertility rate has dropped to less than half of what it was in 1970. The experience of the last 30 yr demonstrates that five factors are generally present in countries which experience significant declines in fertility rates: improved status for women, improved employment opportunities for women, better education for girls and women, reduced infant mortality rates, and increased access to modern means of contraception.[3]

Because continuing growth of the human population can undermine progress made on every other front in the protection and restoration of populations of wild species, it is clear that any successful effort to improve the global environment must be accompanied by direct action to address our own species' population growth. Zoo veterinarians and other zoo professionals are in a uniquely influential position to address this issue. Their credibility as scientifically grounded conservators of wild species lends weight to their arguments when they educate the public or advocate to decision-makers and the media on behalf of policies that will address population growth while also improving the lives of people and the survival of wildlife species around the world.

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HUMAN POPULATION PRESSURES: FROM AWARENESS TO ACTIVISM

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Abstract

In the several minutes it takes to read this abstract, approximately 1000 people will be added to the planet. With rapid population growth and increased consumption in industrialized countries, one-third to one-half of Earth's land surface has been transformed, and 50% of the original forest cover has been cleared.[2] A six-fold increase in global freshwater consumption occurred between 1900 and 1995 alone, with studies estimating that by 2025, two out of every three of people on Earth will live in water-stressed conditions.[5,14]

Wildlife is feeling the human footprint on resources as humans have changed hydrologic cycles, contaminated air and water, converted land for agriculture and industry as well as urban sprawl, and altered climate patterns. Population pressures and over-use of resources are pushing diverse and important ecosystems over the brink. A study of 50 countries in Asia and Africa found that the loss of natural habitat was greatest in areas of high population density and least in low-density areas.[6] Extinction rates are currently estimated anywhere between 100 to 1,000 times greater than normal.[7,12] The loss of top predators, such as Florida panthers and cougars, and keystone species, such as prairie dogs and orangutans, due to human population pressures are causing imbalances in ecosystems that are yet to be fully understood.

While population growth is a driving force behind a number of the world's environmental problems, water quality and water quantity may be the most critical. Water is the most precious resource; only about 1% of the planet's water—contained in rivers, lakes, wetlands, and shallow aquifers—is easily accessible for human use.[10] Currently, humans use over half of all available freshwater. The use and misuse of water resources are resulting in less, free and clean water to support wildlife. If consumption per person remains steady, by 2025, humans could be using 70% of the total because of population growth alone. If per capita consumption reached the level of developed countries, humans could be using 90% of available water each year.[8]

As humans have diverted, dammed, and altered water systems, the loss of rich freshwater ecosystems and the wildlife that depend on them are increasing. Close to 69% of all freshwater dolphins are threatened with extinction, along with 70% of freshwater otters. In North America, 67% of all mussels, 51% of crayfish, 37% of fish, 40% of amphibians and 75% of all freshwater mollusks are either rare, imperiled or already gone. Of California's native fish, 63% are extinct, endangered, vulnerable or in decline. In Europe, 42% of all freshwater fish are threatened or going extinct, as are 33% of Australia's.[1,9]

In particular, cave or subterranean freshwater species, those known and many yet to be identified,

are being affected by human manipulation of the water tables; some of these species may have important ecologic functions that humans may not realize until it is too late.[13] Although a captive breeding program in 14 North American facilities may save it from the finality of extinction, the Chinese alligator will probably not survive in the wild beyond another decade.[2,11] Countless other species, both in the United States and abroad, may share similar fates.

Unfortunately, global data sets on biodiversity are lacking, and thus the planet may be losing species before they can even be identified. There is also incomplete information on the water needs of ecosystems. However, some have placed the global value of freshwater wetlands, including related riverine and lake systems, at close to $5 trillion per year. This is based on their value as flood regulators, waste treatment plants, and wildlife habitats, as well as for fisheries production and recreation.[4]

Although the ultimate value of identified and unidentified species may be unknown, there is growing recognition that rapid population growth, increased urbanization, and the consumption of resources combined are putting enormous pressure on the environment. One of the hardest parts in working toward a healthy future for all is making the link between what happens in our own backyards and the events in places very far from our own home. Zoo veterinarians and other zoo professionals are the ideal advocates for slowing population growth and reducing population pressures to help find a balance between people and wildlife. Despite the difficulty in valuing ecosystems, zoo officials appreciate the importance of protecting and maintaining biodiversity, wildlife habitat and the vast array of ecosystems that make up our planet.

Regardless of the rate at which human population grows in the coming decades, some level of population growth will occur. There is strong evidence that assistance for women's and girl's health, education, and reproductive services under international family planning programs helps lower fertility rates and slow population growth, thereby improving both human well being and environmental quality. The United States made a commitment at the 1994 International Conference on Population and Development (ICPD) in Cairo to provide funding for development assistance to help make family planning universally available by 2015. The United States has consistently fallen far short of fulfilling its obligations, ranking last among industrialized countries.

Success in ensuring a healthy future requires action at all levels—international, regional, national and local. On the international level, the Rio+10 Conference as well as the Cairo+10 Conference will be held in 2002 and 2004, respectively, offering humans the chance to evaluate trends and conditions and to debate future action agendas. Regionally, countries and organizations are discussing ecosystem management as a strategy for better addressing the needs of resources and wildlife. On a national level, discussions over changes to the Endangered Species Act may have immeasurable impacts on the survival of wildlife. Empowerment of communities on the local level to manage resources, particularly freshwater, is a potentially powerful tool that is not always explored. Zoo professionals can play significant roles on each of these levels by moving from awareness to activism on the issues.

A vital part of the long-term solution is recognition of the links between rapidly growing populations, escalating demand and shrinking supplies. Slowing population growth will mean that ecosystems that sustain life have the chance to adapt and replenish themselves to meet the needs of humans and wildlife for centuries to come. The work of the National Wildlife Federation's Population & Environment Program to help build the political will and behavioral changes necessary to ensure a sustainable future offers examples of ways in which zoo professionals can help work toward solutions to these complex issues. The Population & Environment Program's upcoming publications on *Population & Endangered Species* and *Population, Water & Wildlife: Finding the Balance* will provide for debate and dialogue on the interconnectedness of population and environment issues and how each and every person can make a difference in finding a balance between people and nature.

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CONSERVING ENERGY: ACTIVISM OR ACTION – MOTION OR MOVEMENT – REACTION OR RESULTS?

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Abstract

Activism means different things to different people in different positions. Yet the processes involved in taking action, and moving forward to result-driven solutions are generally the same.

Introduction

The word "activism" originates from the word "action," meaning "*to impel by causing change*" or "*expressing movement rather than a state of being.*" Webster's Dictionary defines "activism" as "*a theory or action based on militant action.*" Activism does mean different things to different people in different positions. To Congressional Staffers working on Capitol Hill and to professional staff working in Executive Branch agencies, activism often has negative connotations.

Purpose

This presentation provides ways in which the author believes, you, as veterinarians, animal care professionals, and scientists, can effect change, constructive change, on issues of interest to you, your organizations, Congressional staff and Members, and Executive Branch professionals at the local, regional and/or national levels. You can take the tips provided here (which are mine alone) and embrace them enthusiastically or erase them from your memory, or something in between, whichever you prefer!

Process

Impelling or influencing someone to effect the change you desire begins with communication. Following are 10 communication tips that I appreciate today, as a profession staffer (7 yr) with the U.S. Fish and Wildlife Service (Albuquerque, NM), and that I appreciated, as a scientific advisor (3 yr) for the U.S. House of Representatives (Merchant Marine and Fisheries Committee). These are also the tools I use in everyday situations.

Communication

The Basics
- Learn to listen...to listen very carefully
- Learn to speak only after really listening and thinking; use the "20/90 rule"

Information
- Know 360 degrees of the issue; do your homework
- Make scientific information/data clear, applicable and accessible

Attitude
- Attack the problem/solution, not the person
- Check your ego at the door

Creativity
- Find a clear window of time and space once or twice a day to do nothing by thinking
- "Go to the balcony" frequently

Decision-Making
- Invent and pursue multiple options and alternatives
- Fight for what's important to you; you cannot fight for everything; focus your attention

Make time for people. Have fun. Know the buzzwords of the day. Take risks. Know the purpose of your visits (for attempting to influence whomever), and communicate that purpose clearly and succinctly in one to two sentences.

Preparation

In preparing your presentation for influencing the person(s) with whom you plan to visit, answer three basic questions: (1) What is the issue? (2) So what? (3) Then what? (i.e., Explain what you are there to accomplish, what doing so could accomplish for the person to whom you are talking with, and what will be the results if this person chooses to base one or more decisions on your suggestions). Quiz yourself on any and all possible questions you might be asked and know the answers. Know your point of view; even more importantly, know what all the other points of view are, why they exist, and who holds those views. This is what builds your credibility and opportunity.

Ethics

Be open. Do not hide your agenda. Check your egos at the door (I cannot emphasize this point enough!). Do not play games. If you do not know the answer to a question, say so. Do not make us an answer. If you lie or fudge with just one person, remember that you will have to correctly remember that lie over and over again; it will catch up with you. Always speak the truth.

Science and Politics

Be completely aware that when dealing with most scientific issues nationwide, no matter at what level, that you will often lose. Don't get discourage, because where science and politics mix, "winning" for your cause 25% of the time is great success (e.g., when good science and good

politics coexist).

Influencing

When you begin a negotiation process, know that different people (including yourselves) are affected in different ways by different approaches to influencing. There are at least 10 different methods to use (Table 1).

In setting up a negotiation strategy, you must know where your endpoint is on the particular issue "continuum." Be realistic. If you have not pondered what your purpose is, when you reach your negotiating or influencing endpoint, you won't know you're there! Often with science, this is the biologic goal you wish to achieve and beyond which you cannot tolerate.

Attitude

Run toward action and challenge with facts. Know who you are speaking with. Make them like what they see. Keep your process clean.

Do not be confrontational, emotional, biased, cynical, or egotistical. Do champion your cause, support ongoing efforts, and pick your issues.

Conclusions

Before you begin your discussions, it is important to think about what it is you are trying to achieve, explore reference materials,[1-8] access different points of view, understand (if you can) the personality of the people you will be speaking with in order to choose some applicable negotiation styles, and then take action, which hopefully will move the issue forward to the decision-making mode and results!

> *Through the political and social changes that are normal in a democracy, I hope that you will always remember the reason that you first became Forest Service employees. Continue to advocate and teach the imperative of conservation and restoration. Enjoy yourselves and have fun. Get into the woods...or just enjoy the wild places with your family. Share with young people the love and respect for nature....Take in the splendor of an old growth forest, a prairie grassland, or jagged mountain. Follow your hearts and never allow your lives to be controlled by the desk bound....Above all, allow your commitment to your conservation ethic and the lands and waters that sustain us to take precedence over other political or organizational realities.* [Michael Dobeck, March 27, 2001]

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Table 1. Methods to use in a negotiation process.

1. Building alliances[a]	(Interpersonal, Building Support)
2. Appealing to friendship	(Interpersonal)
3. Appealing to others' values[b]	(Interpersonal, Inspirational)
4. Socializing	(Interpersonal)
5. Exchanging	(Intuiting, Determining Values & Questions)
6. Consulting[b]	(Interpersonal, Building Flexibility)
7. Persuading with logic[a]	(Managing Details, Problem-Solving)
8. Stating[a]	(Asserting with Facts)
9. Legitimizing	(Managing Details)
10. Modeling	(Persisting) (Bacon, 1994; Dalton, 1998)

[a]Methods I am most comfortable with.
[b]Methods I need to recognize and practice more frequently.

ENVIRONMENTAL ACTIVISM: ONE ZOO VETERINARIAN'S EXPERIENCE

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Abstract

Over the past 30 yr, first as a professional wildlife biologist and then as a zoo veterinarian, I have viewed and researched the degradation of the environment and loss of wildlife habitat in numerous environs of the world. In recent years my efforts for environmental protection have included both scientific studies and environmental activism. My personal activism has included lobbying of politicians through office visits and letter-writing, grass-roots organizing, public speaking, media interviews, and teaching. I view these efforts as important as my scientific contributions.

During the past few years I have focused on my activism efforts on human population and environment issues. To me the population/environment connection is all-encompassing and the most important issue facing us as inhabitants of planet "Earth." As a zoo veterinarian I have had numerous opportunities for activism in the zoo community. The public views zoo veterinarians in high regard, loves to hear our stories, and intently listens to our messages on environmental conservation. Organizations including National Audubon Society, National Wildlife Federation, Sierra Club, Zero Population Growth, and local environmental groups have provided numerous educational experiences and material resources to assist me in my activism efforts.

I am driven in my activism efforts for many reasons but primarily because I want my 16-yr-old daughter and her children to experience the beauty and wonders of nature as I have.

SURGICAL MANAGEMENT OF CLOACAL AND URINARY BLADDER PROLAPSE IN AN AFRICAN BULLFROG (*Pyxicephalus adspersus*)

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Abstract

Introduction

Cloacal and urinary bladder prolapses are relatively common in captive anurans. Potential causes include parasitic infestations (e.g., nematodes, protozoa), ingestion of foreign bodies (i.e., substrate), neoplasia and the feeding of excessively bulky food items, especially in larger species. The anatomy of the anuran cloaca is such that cloacal prolapses can easily involve the bladder. The urinary bladder in frogs can be variable in its structure ranging from globoid to almost bilobed in some species. It arises as an outpouching off the ventral surface of the urodeum, and is not directly connected to the mesonephric duct system. The urinary bladder serves as a storage reservoir for fluids and is capable of absorbing water in times of dehydration. These anatomic features allow the bladder to prolapse readily, but also allow for more options regarding therapeutic intervention.[1-6]

Case Study

A 1.0-kg 11-yr-old male African bullfrog (*Pyxicephalus adspersus*) was presented to the University of Georgia College of Veterinary Medicine Teaching Hospital for evaluation of a persistent tissue exteriorization from the cloaca. The frog was maintained alone in a 55-gallon aquarium with pea gravel substrate at room temperature. It was usually fed 1-2 adult mice or small rats per week. The frog was housed in a classroom during the school year. The exteriorized tissue from the vent had been obvious to the owner for 1 mo prior to presentation. During that period the client had been treating the frog by not feeding it, because food restriction resolved a similar problems 6 mo previously.

On physical examination the frog was noted to be in adequate body condition with no observable abnormalities beyond the exteriorized tissue, which was visibly diverse. It consisted of a blind ended light pink sack with a dense, rubbery, consistency ventrally that had a fringe of hyperemic and shredded friable tissue dorsally. Gentle attempts to replace the exteriorized tissue into the cloaca were unsuccessful.

Impression slides were made of the prolapsed tissue and stained with Dif-Quick and Gram's stain. Low numbers of gram positive and gram negative bacteria were noted. No parasitic organisms or ova were detected.

Whole body radiographs were taken and showed the presence of multiple irregular radiodense objects (subjectively considered to be gravel) within the gastrointestinal tract. The radiodense foreign bodies were considered small enough to pass naturally.

An exploratory laparotomy was performed on the frog in order to identify the exteriorized tissue and correct the problem. Enrofloxacin (5 mg/kg) was administered intramuscularly. The frog was premedicated with an intramuscular dose of butorphanol (0.4 mg/kg), then anesthesia was induced with a tricaine methanosulfonate bath (1 g/L buffered to a pH of approximately 7 with sodium bicarbonate). Following induction, the frog was placed on clean moist towels, intubated and maintained on isoflurane with intermittent positive pressure ventilation.

The coelom was approached via a ventral paramedian incision in order to avoid the ventral abdominal vein. The coelom was thoroughly explored. The hyperemic friable tissue was determined to be urinary bladder and was gently retracted into the coelom. The bladder was extremely traumatized and necrotic and the decision was made to surgically remove it as close to the urodeum as possible. The necrotic tissue was removed and the resulting defect was closed with 5-0 PDS in a simple continuous pattern. The identity or source of the other tissue protruding from the vent was not readily apparent. A colotomy helped determine that the tissue originated from the caudoventral aspect of the urodeum. The prolapsed tissue was reduced by a non-sterile assistant with the intent of removing it as close to the cloacal wall as possible via the colotomy incision. However, when reduced, the tissue mass completely occluded the mesonephric ducts and both ducts became extremely dilated within 5-10 min. Centesis was used to remove the urine (0.5 ml of urine was removed from both ducts) to prevent rupture of the ducts. The tissue was then pushed back through the vent and the colon was closed with 5-0 PDS in a simple interrupted pattern. The coelom was flushed with warm saline. The coelom was closed with 4-0 PDS in a simple continuous pattern in the body wall, and everting horizontal mattress sutures in the skin. The prolapsed tissue was then approached from the vent and retracted as far as possible from the cloaca. It was then transfixed with 2-0 PDS and removed. The remaining tissue retracted into the cloaca.

Recovery from anesthesia was rapid and complete. The frog was placed in a container of aged water in an incubator maintained at 80 F. The frog listed to the right side and was unable to completely inflate it's right lung for the first 2 days following surgery. Presumably the length of the frog's trachea was overestimated and the endotracheal tube was placed into the left mainstem bronchus. The frog did recover completely prior to its discharge from the hospital.

The frog was discharged with enrofloxacin (5 mg/kg i.m. q 24 hr for 10 days) and instructions to modify the husbandry. The gravel was removed from the bottom of the aquarium and the floor was left bare. The diet was reduced to 1-2 smaller 1/2 skinned mice once per week, and was expanded to include insects and fish.

Histologically, the excised tissue was consistent with cloaca with hemorrhage and fibroplasia suggestive of a long standing lesion. No organisms or neoplastic cells were observed.

Eight months post-operatively, the frog is reported to be apparently healthy with no recurrence of the prolapse.

Discussion

In this particular case it was thought that the prolapse may have occurred secondary to chronic ingestion of the gravel substrate and overfeeding of bulky prey items. Both of these factors could presumably cause protracted straining resulting in the deformation and eventual fibrosis of the cloacal tissue. It is theorized that once the deformed fibrotic tissue reached a sufficient size it was expelled from the cloaca dragging with it the urinary bladder.

This is a case in point of the importance of determining the origin/identity of prolapsed tissue. If the exteriorized tissue had simply been reduced into the cloaca and retained with stay sutures, this frog would have most certainly ruptured both mesonephric ducts and died.

Most cases of cloacal and bladder prolapse in anurans are not as complicated or chronic as the one in this report. The vast majority are diagnosed acutely and can be effectively treated without exploratory surgery. If the bladder is intact, it can be gently reduced after cleaning and lubrication. Stay sutures can be place across the vent or the bladder can be pexied via percutaneous placement of sutures facilitated with a lubricated cotton tipped applicator. If the bladder is severely traumatized and disrupted it is acceptable to simply ligated the tissue and remove the necrotic portion of the urinary bladder. In captivity under controlled conditions, the importance of the urinary bladder for survival is minimal and the animals appear to thrive without it.

An effort should be made to determine the cause of the prolapse in order to administer definitive therapy and avoid recurrence. Impression smears and cytology of all prolapsed tissues should be performed as well as direct mounts plus fecal flotation if a sample is available. It may also be useful to biopsy prolapsed tissue if possible. Radiographs (± contrast) and cloacal and/or coelomic endoscopy may also be performed in pursuit of a diagnosis.

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TOXICOLOGY STUDIES IN STELLER SEA LIONS (*Eumetopias jubatus*)

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Abstract

Scats (feces) from Steller sea lions (*Eumetopias jubatus*) were collected on rookeries as a medium to compare relative levels of selected organochlorine (OC) contaminants between the thriving eastern stock in Southeast Alaska (SEA) and the depleted western stock [Gulf of Alaska (GOA) and eastern Aleutian Islands (EAI)]. Matched sets of feces, blood, and blubber samples were collected from wild pup and juvenile Steller sea lions captured during Alaska Department of Fish and Game capture operations in SEA and Prince William Sound (GOA) as well as from captive adults at the Alaska SeaLife Center. These sets were used to evaluate relationships of contaminant levels and composition in the three media. Samples were analyzed for selected congeners of polychlorinated biphenyls (PCBs) and metabolites of dichloro-diphenyl-trichloroethane (DDT) by high performance liquid chromatography. Hair was also collected from wild caught individuals from SEA and GOA and subsequently analyzed for total mercury (Hg). In addition, we examined the utility of porphyrin profiles in feces as a biomarker of environmental contaminant exposure in both individual sea lion feces and from rookery scats. Blood levels of individual PCBs congeners and DDT metabolites were highly correlated with blubber levels. In contrast, feces were not well correlated with blubber or blood congener profiles. This result was expected since fecal OC levels reflect excretion of PCBs congeners not metabolized or retained in the body in addition to recent dietary intake. Therefore, use of fecal OCs were intended as a rough indicator of exposure levels, not as a reflection of the individual congeners in body depot stores. We determined that OC contamination in Steller sea lions from portions of the western stock (EAI) have significantly higher OC levels excreted in feces compared to the GOA and SEA. Additionally, the mean ratio of porphyrins in scats from rookeries were correlated with OC levels. Finally, total mercury analyses indicated significantly higher levels in hair in GOA pups compared to SEA. Therefore, adverse effects of both inorganic and organic environmental contaminants need to be considered as contributing factors in the continuing decline of the western stock of SSL. This research leads us to recommend that additional, extensive, effects-based contaminant research on Steller sea lions is warranted.

Introduction

Steller sea lion (SSL) numbers in western Alaska (west of 144°W) have declined by about 80% over

the past 20 – 30 yr and that population is now federally classified as endangered. In Southeast Alaska, sea lion numbers have nearly doubled over the same time period. While nutritional stress is the favored hypothesis for explaining the decline, other factors cannot be discounted. Organochlorine contaminants are present in the marine ecosystem of the North Pacific Ocean and relatively high concentrations have been found in the tissues of dead adult SSLs in Alaska. Several lines of evidence indicate that contaminants should be evaluated as an alternative hypothesis for explaining, in part, the decline in the western population of SSLs.

1. Levels of OCs in tissues of small samples of SSLs from Alaska have been "surprisingly" high.[6,8,9,15] In addition, sea otters (*Enhydra lutris*) sampled from the Aleutian Islands had much higher levels of both PCBs and DDTs than otters sampled from SEA.[13]
2. The OC levels reported for Alaskan SSLs are high enough to be of concern, relative to levels reported to be immunosuppressive in harbor seals (*Phoca vitulina*).[1,13]
3. Several observations have been made of animals from the western population that are suggestive of immunosuppression. Dermal, fungal patches have been observed more frequently on animals from the western population than in the thriving eastern population in SEA (unpublished data). Zenteno-Savin et al.[16] reported higher levels of the acute phase protein haptoglobin from SSLs in the western population than for animals in the SEA population.
4. The ability to metabolize OC compounds appears to be less well developed in a similar species, the southern sea lion (*Otaria flavescens*), than in other mammals.[14]
5. SSLs have extended lactation and nurse their young for up to several years,[10] providing an efficient mechanism for transfer of lipophilic, biomagnified contaminants vulnerable juveniles.
6. Reduced reproductive success has been reported as an adverse effect of high body burdens of OC contaminants.[2,5,12] SSLs from the depressed, western Alaska population were found to have high rates of reproductive failure; about 40% of pregnancies failed, primarily the result of middle and late term abortions.[10,11]

To determine if SSLs from the two stocks are exposed to significantly different levels of OCs, we measured OC concentrations in SSL scats collected on rookeries from portions of the ranges of the western and eastern stocks. We looked for correlations between contaminant levels and composition and population trends at individual rookeries and between the Southeast Alaska and western Alaska populations. Additionally, we examined the suitability of porphyrin profiles (intermediates in the biosynthesis of heme) in feces as a biomarker of OC exposure. We compared the profiles of PCB congeners and DDT metabolites in the blood, blubber and feces of individuals to examine the relationships between the difference matrices and the suitability of using less invasive sampling techniques, as well as comparing mean levels of contaminants between regions.

Methods

Samples of fresh feces were obtained per rectum or from observed defecation from SSL juveniles during the course of capture operations in SEA and the GOA during 1998-2000. Samples were collected with acetone-rinsed spatulas or forceps and transferred to acetone-rinsed foil or Teflon and sealed in a whirl-pac or into methylene chloride rinsed vials and frozen. From these same

individuals, blubber biopsies and blood samples were also obtained and frozen to –20°C until analysis. Feces as individual, recognizable defecations, were collected off 18 rookeries during 1998 and 2000 (Fig. 1) and handled similarly. No samples from the central or western Aleutian Islands were available. Rookery scats are primarily from adult females, as territorial bulls are fasting and pups are suckling at the season of collection. Ten to 12 scats per rookery were combined by homogenization and became a composite analyzed as a single sample from that site on that day. The same homogenate was used for both OC and porphyrin analyses.

Fecal, blood, and blubber samples were extracted and analyzed for selected OC contaminants using high-performance liquid chromatography (HPLC) with photodiode array detection.[7] Lipid concentrations of all samples were determined by thin layer chromatography with flame ionization detection so comparisons of OC contaminants could be made based on lipid weight (l.w.).

Porphyrins were extracted from dried fecal samples using a hydrochloric acid extraction protocol outlined in Taylor et al.[14] Characterization of the porphyrin profiles was accomplished using high performance liquid chromatography. Hair clipped in preparation of the blubber biopsy site was collected and prepared for total mercury determination by hot acid digestion (70% NNO_3/30% H_2SO_4) and analyzed by $SnCl_2$ reduction, dual gold amalgamation, and CVAFS detection (EPA method 1631 modified).

Results

Blood levels of individual congeners of PCBs and DDT metabolites were highly correlated with blubber levels (Table 1) and therefore blood sampling is a suitable alternative to the more invasive blubber biopsy for monitoring of OC contaminants. Congener profiles in feces were not well correlated with blubber or blood congener profiles. We determined that SSLs from portions of the western stock (EAI) have significantly higher OC levels excreted in feces compared to SSL in the GOA and SEA (Fig. 2). Additionally, the ratio of coproporphyrin III to uroporphyrin mean levels is higher and the mean ratio of protoporphyrin IX to coproporhyrin III is lower in the EAI and GOA rookeries than in the reference SEA rookeries. These results indicate that the animals from the western stock have a disruption at either or both the coproporphyinogen or protoporphyrinogen oxidase steps. The porphyrin ratios were correlated to OC levels but OC and mercury levels were inversely correlated. Therefore, increased corpoIII:uro may be an appropriate biomarker of OC exposure in SSL but not mercury exposure (at least not in suckling pups). Finally, total mercury analyses indicated significant higher levels in post-molt GOA (Prince William Sound) pups compared to SEA pups (mean ± SEM, 1986±342 ng/g GOA, $n = 15$; 876±116 SEA, $n = 7$, $P = 0.43$).

Discussion

These findings indicate that PCBs are present in the food web exploited by SSL in Alaska, at least as far west as the EAI. In SEA where SPCB levels were intermediate, the population has increased in recent years. The relatively high levels for the EAI, in comparison of the nearby GOA, are of interest as they suggest either a local source or a strong influence from the Bering Sea. The animals

inhabiting these areas appear to be at increased risk of adverse effects of environmental contaminant exposure.

The lack of correlation between fecal PCB congener profiles and blubber or blood was not an unexpected result. The number of animals with all three media analyzed was small. Also, fecal OC levels reflect excretion of PCB congeners not metabolized or retained in the body in addition to recent dietary intake. Thus, use of fecal OCs was intended as a rough indicator of the magnitude of exposure, not as a reflection of the individual congener concentrations in body depot stores.

Analyses of fecal composites for porphyrin metabolites, a suggestive biomarker of non-specific contaminant exposure, indicate a "backup" of the OC metabolic pathway in the GOA and EAI samples compared to SEA samples. In light of the results of these preliminary studies, the further use of biomarkers and other studies examining adverse health effects as a result of environmental contaminant exposure are currently being undertaken by the authors with additional collaborators.

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Table 1. Pearson correlation coefficients between selected PCB congener and DDT concentrations in blubber vs. blood of 10 Steller sea lions, 1998-2000 (ng/g l.w.). CBs-118, -128, -180 not significant, CBs-110, -170 had too few above detection limits to evaluate.

	CB-101	CB-105	CB-138	CB-153	pp'-DDE	ΣPCB	ΣDDT
r	0.792	0.951	0.880	0.864	0.932	0.884	0.934
P	0.00637	0.00357	0.000778	0.00267	0.00026	0.000696	0.00008

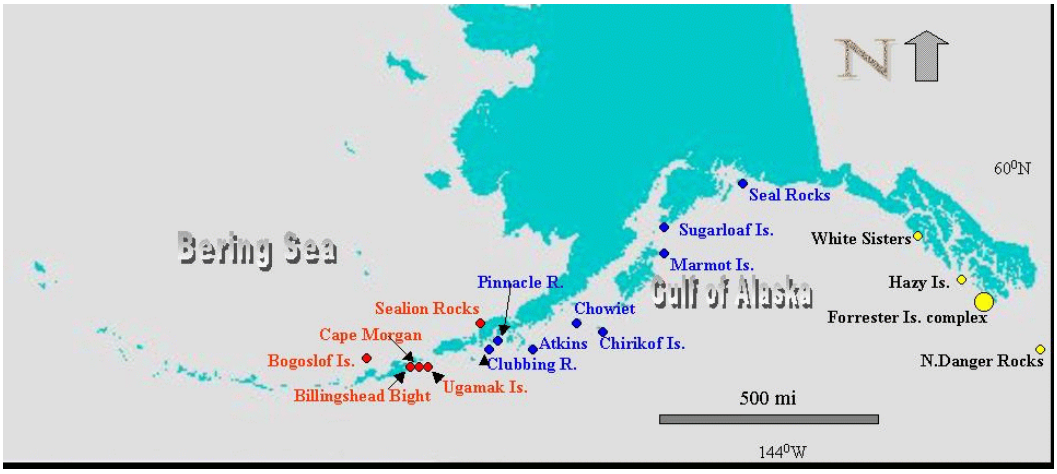

Figure 1. Steller sea lion (SSL) rookery and haulout scat sampling sites (1998 and 2000).

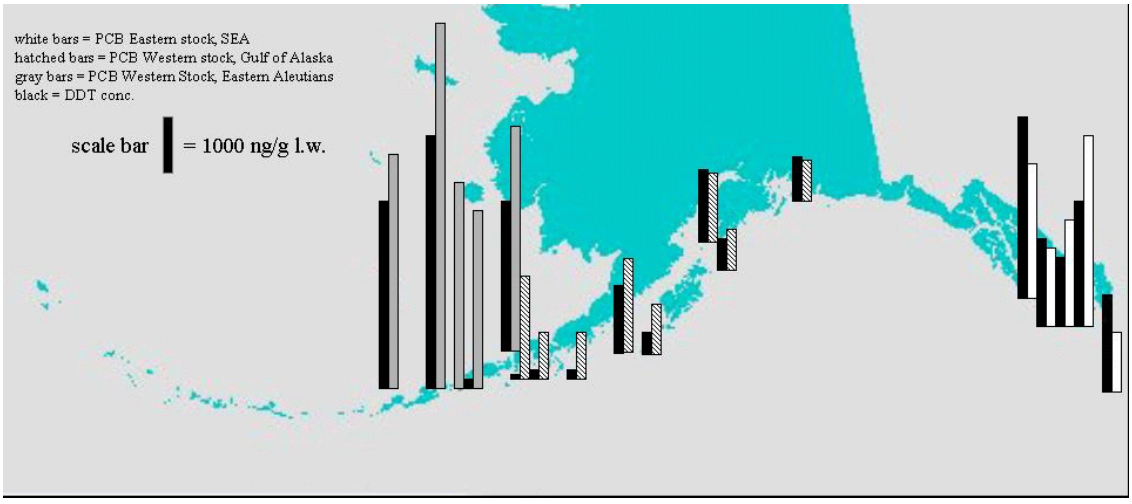

Figure 2. PCBs (shaded) and DDTs (black) in SSL scats collected at rookeries or haulouts (1998 and 2000).

CONGENITAL DIFFUSE HYPERPLASTIC GOITER ASSOCIATED WITH PERINATAL MORTALITY IN SEVEN CAPTIVE-BORN BOTTLENOSE DOLPHINS (*Tursiops truncatus*)

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Abstract

Seven perinatal dolphin mortalities associated with a histologic diagnosis of diffuse hyperplastic goiter were obtained from three separate zoologic or aquarium facilities (Table 1). Dolphins 1-2 were from the Edmonton Mall aquarium, Alberta, Canada. Dolphin 3 was from the Minnesota zoo, and dolphins 4-7 were from the Indianapolis zoo, United States. Five dolphins were males and two were females. Dolphin 2 was 6-8 wk premature, dolphins 1, 3 - 5 lived less than 1 day, and dolphins 6 and 7 were stillborn. All dams were wild caught. Dolphins 1 and 2 had the same dam, and Dolphins 4 and 6 had the same dam. Dams were different for dolphins 3, 5 and 7. Duration in captivity for the dams at the time calf births averaged 11.5 yr, with range of 6-23 yr. Thyroid morphology was compared with the histologically normal thyroids of two stranded wild bottlenose dolphins, a neonate and a 2-mo-old calf, that died of unrelated causes. Compared to the stranded dolphin calves and the published literature[3], histologic changes consistent with diffuse follicular goiter were seen in the thyroid glands of all captive born dolphins. Changes included reduced follicular luminal diameter, markedly reduced or absent luminal colloid, and hypertrophy of follicular epithelium. Additional histologic findings included lymphoid depletion in dolphin 2, sepsis in dolphin 3, and atrophy of fat in dolphin 7. Cause of death could not be determined for these animals, but was presumed to be due to metabolic derangements associated with the thyroid lesion, drowning, or dystocia.

Congenital goiters can be acquired or heritable, and are almost always diffuse follicular goiters, rather than nodular or colloid goiters.[1] Acquired diffuse follicular goiter is due to high or low levels of maternal dietary iodine, the latter sometimes exacerbated by concurrent goitrogenic dietary substances.[1,4,6] Diffuse acquired follicular goiter is well documented in humans, horses, and domestic ruminants.[1,2,4,6] Heritable diffuse follicular goiter is documented in humans and several breeds of cattle.[1] Because the dams to the dolphins of our study were wild caught, and there is a wide demographic spread in incidence of this condition, it seems unlikely that this represents a heritable disorder. The water used in the dolphin exhibits is treated through filters that can potentially remove microminerals. Ozone treatment of artificial seawater may alter the relative concentrations of iodine species in a closed tank system.[5] Additionally, a number of potentially goiterogenic compounds may

be present in the aquatic environment, including chlorine, chlorine compounds, ammonia, nitrates, nitrites, and urea.[5] We believe that the congenital goiter in these dolphin calves is an acquired disorder associated with derangements in thyroid and/or iodine metabolism; maternal and fetal thyroid and iodine values may be affected by inappropriate levels of iodine in the diet or in the aquatic environment. It is interesting to note that the dam to dolphin 2 subsequently died and had hyperplastic changes in the thyroid gland.

Additional studies at facilities with this problem should include: 1) evaluation of iodine levels in exhibit water and comparison with known values for natural seawater, 2) evaluation of thyroid values for affected neonatal and adult dolphins and comparison with known values for normal captive and wild dolphins; and 3) evaluation of thyroid to body weight ratios for affected juvenile and adult dolphins, and comparisons with known values for normal dolphins.

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Table 1. Neonatal dolphin mortalities associated with congenital goiter.

Dolphin	Location	System[a,b,c]	Sex	Age	calf wt (kg)	Histopathology	Dam
1 (V96-8144)	West Edmonton Mall	a	male	10 min	18.2	hyperplastic goiter	11-yr captivity
2 (99-12355)	West Edmonton Mall	a	male	6-8 wk premature	13.2	hyperplastic goiter lymphoid depletion	13-yr captivity
3 (9259/G97-1729)	Minnesota Zooligical Gardens	b	female	< 1 day	11.3	hyperplastic goiter sepsis	23-yr captivity
4 (G98-0595)	Indianapolis Zoo	c	male	6 min	14.1	hyperplastic goiter	10-yr captivity
5 (1562-94/ G00-0368)	Indianapolis Zoo	c	male	15 min	16.8	hyperplastic goiter	6-yr captivity
6 (10606-94/ G00-0376)	Indianapolis Zoo	c	female	stillborn	12.7	hyperplastic goiter	6-yr captivity
7 960138/G0 0-0757	Indianapolis Zoo	c	male	stillborn	15.4	hyperplastic goiter atrophy of fat	8-yr captivity

[a]Closed system, salt water synthesized from road salt obtained from salt bed. Water filtered through carbon and diatomaceous earth mechanical filters, chlorinated.
[b]Closed system, salt water synthesized by addition of salt, eight sand and gravel filters, ozonated.
[c]Closed system, salt water synthesized by addition of salt, 12 sand and gravel filters, ozonated and chlorinated.

ASSISTED MECHANICAL VENTILATION AND ITS AFFECT ON END-TIDAL CARBON DIOXIDE LEVELS IN ANESTHETIZED CALIFORNIA SEA LIONS

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Abstract

Anesthesia in many marine mammal species may be complicated by anatomic and physiologic adaptations to a marine existence. A highly developed diving reflex that includes bradycardia and prolonged apnea may be responsible for anesthetic complications reported in the past. Of special interest is a an apparent tolerance for increasing carbon dioxide levels without stimulation of respiratory centers that may lead to a potentially dangerous acidosis. Continuous monitoring of end-tidal carbon dioxide ($EtCO_2$) levels may bring new insight into the causes of marine mammal anesthetic complications. In addition, the physiologic effects and potential benefits of mechanically assisted ventilation have not been well described in marine mammals.

Thirty-five stranded California sea lions (*Zalophus californianus*) were anesthetized for a variety of clinical procedures at a rehabilitation center. The animals were given 0.02 mg/kg of atropine i.m. and, approximately 10 min later, 70 µg/kg of medetomidine with 1 mg/kg of a 1:1 solution of zolazepam and tiletamine i.m. All animals were intubated, given 100% oxygen, and maintained with isoflurane . During the procedure, isoflurane level, anesthetic plane, heart rate, respiratory rate, esophageal temperature, oxygen saturation, and $EtCO_2$ levels were monitored and recorded approximately every 5 min. Blood gas parameters were also recorded in some individuals.

Eighteen sea lions were anesthetized without providing assisted ventilation. Twelve animals were provided with mechanically-assisted ventilation at a rate of 6-8 breaths per minute (bpm) and a volume of approximately 20 ml/kg/breath. Mechanically-assisted ventilation was given to an additional five sea lions at the same volume but at a rate of 10-12 bpm. The ventilation volume of 20 ml/kg/breath was found to produce an inspiratory pressure of less than 20 cmH_2O in most animals. If the resulting inspiratory pressure was greater than 25 cmH_2O, the ventilation volume was decreased.

The $EtCO_2$ levels for animals with no assisted ventilation ranged from 26 to 80 mmHg with a mean ± SD of 51 ± 10 mmHg. The $EtCO_2$ levels in animals assisted at a rate of 6-8 bpm ranged from 20 to 65 mmHg with a mean ± SD of 47 ± 10 mmHg. In the sea lions provided with assisted ventilation at a rate of 10-12 bpm, $EtCO_2$ levels ranged from 32 to 53 mmHg and had a mean ± SD of 42 ± 6. The mean $EtCO_2$ levels that were recorded from animals receiving assisted ventilation at a rate of 10-12 bpm were significantly lower than those recorded from the other two groups of sea lions. Blood pH levels of greater than 7.5 were recorded in two animals that were ventilated at the higher rate and was likely indicative of alkalosis.

Mechanically assisted ventilation resulted in lower mean $EtCO_2$ levels in anesthetized California sea lions, and appeared to decrease the occurrence of very high levels (greater than 60 mmHg). It is likely that mechanically-assisted ventilation is not required for all anesthetized sea lions but should be considered for procedures of a relatively long duration, for animals that must be a maintained in a position that may interfere with normal thoracic expansion, or for those animals in which rising $EtCO_2$ levels are observed. For most sea lions a volume of 20 ml/kg/breath at a rate of 10-12 bpm is recommended initially, but should be adjusted for each individual. Measurement of blood gas parameters is also recommended due to the possibility of developing an alkalosis in sea lion as a result of mechanical ventilation.

ANESTHESIA AND POSTOPERATIVE CARE FOR A GASTROTOMY IN A HARBOR SEAL *(Phoca vitulina*)

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Abstract

A 29-yr-old female harbor seal *(Phoca vitulina)* was evaluated because of a 3-wk decline in total food consumption. Despite the decline in food quantity consumed, she had a willingness to eat, and appeared to be bright and alert. She was sedated with butorphanol tartrate (Torbugesic, Fort Dodge Animal Health, Fort Dodge, IA 50501 USA) 3 mg i.m. to assist in the physical exam. The seal weighed 59.8 kg and her body condition was thin. The physical exam was otherwise unremarkable. Abdominal radiographs revealed approximately 75 to 100 rocks of 1 to 4 cm, four to five coins and one AA alkaline battery within the stomach. The complete blood count and serum chemistries were within normal limits. Initial fluid therapy consisted of 2.5% dextrose and 0.45% sodium chloride (Baxter Healthcare Corp. Deerfield, IL 60015 USA) 3 L given s.c., enrofloxacin (Baytril 100, Bayer Corp. Shawnee Mission, KS 66201 USA) 325 mg and vitamin B complex (Butler Company, Columbus, OH 43228 USA) 5 ml were both given by i.m. injection. Ongoing treatment with enrofloxacin (325 mg i.m., s.i.d.) and 2.5% dextrose and 0.45% sodium chloride 3 L given s.c., s.i.d.) was continued while further treatment plans were organized.

Two days after the initial evaluation, she was immobilized for gastric foreign body removal. Prior to chemical immobilization, a pre-anesthetic 1.2 mg dose of atropine sulfate (Atropine SA, Butler Company, Columbus, OH 43228 USA) injection was given i.m. Approximately 15 min post atropine injection, tiletamine hydrochloride and zolazepam (Telazol, Fort Dodge Animal Health, Fort Dodge, IA 50501 USA) 50 mg by i.v. injection into the epidural vein at the level of the lumbar spine was given. After induction, she was then intubated with an 11-mm diameter cuffed silicone endotracheal tube (Jorgensen Laboratories, Inc., Loveland, CO 80538 USA) and placed on isoflurane (Isovet, Schering-Plough Animal Health Corp, Union, NJ 07083 USA) through a precision vaporizer. Mechanically assisted ventilation (MDS Matrix, Orchard Park, NY 14127 USA) was provided at a rate of six breaths per min and a volume of between 700 – 1200 ml of oxygen per breath in order to produce an inspiratory pressure of no greater than 25 cm of water. An 18-ga × 5.1-cm catheter (Quick-Cath, Trovenol Laboratories, Inc., Deerfield, IL 60015 USA) was placed into the epidural vein at the mid-lumbar area and lactated ringer's solution (Lactated Ringer's Injection USP, Baxter Healthcare Corporation, Deerfield, IL 60015 USA) given at a rate of 5-10 ml/kg/hr. Additional atropine 0.6 mg by intramuscular injection was given 91 min after the initial injection.

Isoflurane level (1 ± 0.5%), mechanical ventilation volume (800 ± 100 ml O_2), heart rate (86 ± 13

beats/min), respiratory rate (7 ± 2 breaths/min), temperature (34.1 ± 2°C), oxygen saturation (SpO_2) (96 ± 4%), and endtidal carbon dioxide ($EtCO_2$) (41 ± 5 mm Hg) were monitored and recorded every 5 min for the duration of the procedure. The total time on isoflurane and the ventilator was approximately 2.5 hr. Recovery was uneventful with the animal starting to spontaneously breathe approximately 11 min after the isoflurane was turned off. Butorphanol tartrate 3 mg by intramuscular injection was given approximately 9 min after the animal was extubated to provide post operative analgesia as well as mild sedation to limit movement to prevent additional trauma to the surgical site.

Endoscopic evaluation of the stomach confirmed the presence of the foreign bodies and revealed an inflamed gastric mucosa without evidence of ulceration or necrosis. Endoscopic assisted retrieval was not attempted due to the quantity of rocks present. Water gastric lavage retrieval using a standard equine stomach tube (Jorgenson Labs, Loveland, CO 80538 USA) 1.2 cm ID attached to a flexible vinyl tube 3.7 cm ID used for outflow was attempted but also unsuccessful. Removal via gastrotomy was elected.

A standard ventral midline approach to the abdomen was used via an incision approximately 18 cm length and the stomach exteriorized. The stomach serosa was unremarkable and a gastrotomy was performed in a relatively avascular region of the stomach mid-way between the between the lesser and greater curvatures. The foreign bodies were easily removed manually and with a sterile tablespoon. The gastric mucosa and submucosa were closed with an apposing simple continuous suture pattern using 2-0 monofilament polyglyconate (Maxon, Sherwood Medical, St. Louis MO 63103 USA). The seromuscular layer was closed with an inverting interrupted lembert suture pattern using 2-0 monofilament polyglyconate. The abdominal wall was closed with simple continuous suture pattern using 0 monofilament polyglyconate. Additional simple interrupted 0 monofilament polyglyconate sutures were placed in the abdominal wall at 2-cm intervals. The subcutaneous fat layer was closed in two layers with 2-0 monofilament polyglyconate in a simple continuous suture pattern. Finally, the subcutis was closed using 2-0 monofilament polyglyconate in a simple continuous suture pattern and the skin was closed using 35-mm surgical staples (Kendall, Mansfield, MA 02048 USA). The incision was sealed with formulated cyanoacrylate (Nexaband, Veterinary Products Laboratories, Phoenix, AZ 85013 USA).

The postoperative and convalescent care was uneventful. Prophylactic enrofloxacin 300 mg given i.m. and ceftriaxone sodium (Rocephin, Roche Laboratories Inc., Nutley, NJ 07110 USA) 1.4 g i.m. were started preoperatively and continued every 24 hr while the animal was NPO. Food and water were withheld for the first 36 hr. A regimen of small multiple meals was then started and a normal level of food consumption was gradually achieved within 7 days. Once the seal began eating, the injectable antibiotics were discontinued and oral antibiotics, ciprofloxacin (Cipro, Bayer Corporation, West Haven, CT 06516 USA) 375 mg b.i.d. administered for 7 days and Amoxicillin trihydrate (Amoxi-Tabs, SmithKline Beecham Pharmaceuticals, Philadelphia, PA 19101 USA) 1.2 g b.i.d., were administered for 21 days. In addition, cimetidine hydrochloride (Cimetidine, Geneva Pharmaceuticals, Inc., Broomfield, CO 80038 USA) 400 mg b.i.d., p.o. was administered for 7 days.

She was "dry docked" and confined to a 3.5 × 8 m outdoor run with smooth rubber matting (Rail Trax Mats, Lab Safety Supply, Janesville, WI 53546 USA) and the incision was monitored daily. She was hosed with water 15 min three times daily to assist in thermal regulation. Four days post surgery; she was transferred to a small holding pool of approximately 2,750 L (726 gallons) to which she was allowed access during the day. She was "dry docked" at night and the incision was checked in the morning prior to allowing her access to the pool. Water changes were made when the pool became contaminated with feces.

Seven days post surgery; there was a 5-cm dehiscence of the most cranial aspect of the surgical incision. At this site, the surgical staples were missing and the subcutaneous fat layer was exposed. Close monitoring revealed no further dehiscence and the wound was left to heal by secondary intention. The "pool protocol" was revised and the seal was allowed access to the pool once it was filled with water and 2.5 kg of sodium chloride salt (Solar Salt, Cargill, Inc., Minneapolis, MN 55440 USA). The pool water continued to be changed when fecal contamination was apparent and salt was added to each fill. The seal continued to be "dry docked" each night and the incision was checked in the morning prior to allowing her access to the pool.

Twenty-one days post surgery, the cranial area of dehiscence was forming a healthy granulation bed and the rest of the incision appeared fully healed. She was sedated with butorphanol tartrate 4 mg i.m. injection and the remaining skin staples were removed. Naloxone (Narcan, Endo Pharmaceuticals, Inc., Chadds Ford, PA 19317 USA) 1 mg i.m. effectively antagonized the effects of the butorphanol tartrate. The next day, she was transferred back to her regular exhibit.

I feel that there were two important factors that were instrumental in the successful outcome of this case. First, the use of a ventilator during the procedure insured good alveolar gas exchange. In our personal experience, unassisted ventilation has produced low SpO_2 (< 80%) and high $EtCO_2$ (> 60 mm HG) particularly in procedures requiring a surgical plane of anesthesia of long duration. This situation during anesthesia is complicated in phocids by initiation of a dive response that includes bradycardia and apnea potentially leading to hypoxia and anesthetic complications. In this particular case, mechanically assisted ventilation appeared to have helped to prevent hypoxia and contributed to a successful anesthesia.

Secondly, a closely controlled postoperative environment was important to promote good wound healing and maintain proper thermoregulation. There were concerns that constant pressure on the surgical incision and the significant ventral subcutaneous fat layer relatively void of blood supply would be problematic if the animal remained dry docked for the entire post operative period. On the other hand, though preferred to maintain the animal's physiologic needs and keep weight off of the surgical incision, the potentially contaminated aquatic environment had the potential to compromise wound healing as well. A balance was struck between keeping the animal out of the water to allow good wound sealing and in the water to take weight off of the incision and allow proper thermoregulation. In order to give the surgical incision the best chance for uneventful healing, the optimum post operative environment must include: 1) a smooth surface for "dry docking," 2) controlled pool access, 3) the ability to monitor the incision site daily, and 4) the ability

to maintain the pool water as clean as possible.

ACKNOWLEDGMENTS

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AN OUTBREAK OF HEPATOPANCREATITIS IN TIGER-TAIL SEAHORSES (*Hippocampus comes*)

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Abstract

The tiger-tail seahorse, *Hippocampus comes,* takes its name from its tail color of black or gray with cream or yellow bands. This striking appearance makes it a very popular species for home tanks and larger exhibits at commercial aquariums. In nature, this seahorse species is distributed mainly in the western central pacific area of Malaysia, Singapore, Vietnam and the Philippines. Although not endangered, the tiger tail seahorse may play an important role in the understanding of disease processes in this and threatened species of seahorses.

Between 6/11/00 and 8/12/00, 13 tiger tail seahorses in quarantine at the National Aquarium in Baltimore, Maryland developed a fatal disease characterized clinically by fluid-filled vesicles around the abdominal pouch, cervical region and beneath the mandible. The affected seahorses were lethargic and anorectic. Despite treatments with trimethoprim sulfamethoxazole baths (960 mg/10 gallons 6-8 hr s.i.d.), metronidazole baths (250 mg/10 gallon s.i.d.) or injectable ceftazidime (22 mg/kg i.m. q 72 hr) the 13 seahorses died over a 6-wk period. Necropsy findings on five animals showed soft, pale pouch skin containing grayish raised foci. The hepatopancreas had randomly distributed yellowish-white foci. Histopathologic examination of these tissues showed necrosis of the skin with ulceration, and necrosis with inflammation of both the hepatic and pancreatic components. Large basophilic intracytoplasmic inclusion bodies were evident within degenerated cells in the hepatopancreatic lesions. In several animals, similar necrotizing lesions were also found in the nasal passage, kidney, extrahepatic pancreatic tissue and coelomic fat. Pure cultures of *Vibrio alginolyticus* were isolated from the skin and liver lesions of one animal. In samples from the same animal a cytopathic agent was detected in three fish cell lines in an attempt to isolate a virus; however, subcultivation produced no further cytopathic effect and identification of the agent was unsuccessful.

The nature of the lesions and the presence of large basophilic inclusion bodies suggest that a viral agent could be involved. The necrotic lesions and inclusion bodies resemble those in infectious pancreatic necrosis virus (IPNV), a birnavirus of fish. *Vibrio alginolyticus* is considered an opportunistic invader of already damaged tissues and a weak pathogen in stressed fish. Further studies including transmission electron microscopy of the inclusion bodies in the fixed tissues and development of seahorse cell lines are currently underway.

DISSEMINATED MYCOSIS CAUSED BY *Exophiala*-LIKE FUNGI IN CAPTIVE COLD-WATER MARINE FISH

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Abstract

Dematiaceous fungi in the genus *Exophiala* are commonly isolated from plant materials, decaying wood, sewage sludge, and wood pulp samples.[11] The genus contains several species acting as opportunistic pathogens, causing cutaneous, subcutaneous and systemic clinical diseases in man.[5] In fish, naturally occurring disease with high mortality has been reported predominantly in intensively farmed salmonids including Atlantic salmon (*Salmo salar* L.),[6,8-10] cutthroat trout (*Salmo clarki)*,[2] and lake trout (*Salvelinus namaycush).*[2] Reports in non-salmonid fish are limited to a single epizootic in farmed channel catfish (*Ictalurus punctatus* Rafinesque)[3,7] and reports in individual captive scup (*Stenotomus versicolor*),[1] Atlantic cod (*Gadus morhua*),[1] common seahorse (*Hippocampus hudsonius*),[1] Sargassum triggerfish (*Xanthichthys ringens*),[1] brown clownfish (*Amphiprion sebae*),[1] and smooth dogfish (*Mustelus canis* Mitchill).[4] This is a report of a cluster of deaths, occurring over a period of 4 yr at the Shedd Aquarium in three species of captive cold water marine fish, attributed to cutaneous and disseminated infections with *Exophiala*-like fungi.

Affected fish included four lumpfish (*Cyclopterus lumpus*) an Atlantic wolffish (*Anarhichas lupas*) and a thorny back ray (*Platyrhinoidis triserata*). All fish were wild caught and maintained in the same water system, with biologic filtration and UV sterilization of water between tanks. The lumpfish were housed together in the same tank as juveniles, but different species never occupied the same tank simultaneously. Enclosure substrate was minimal consisting of concrete and crushed coral.

Fish were in captivity for at least 1 yr prior to observation of clinical signs. Signs were nonspecific and included anorexia and muscle wasting of between 1 mo and 1.5 yr duration. All four lumpfish (designated A,B,C,D) developed single to numerous crateriform skin ulcerations predominantly on the head. The ray had a single cutaneous ulceration. No cutaneous lesions were noted in the wolffish. Clinical decline resulted in death in lumpfish A and B, the ray, and wolffish, and euthanasia in lumpfish C and D. Necropsies were performed by Zoo Pathology staff (lumpfish B, and D) and Shedd Aquarium staff (lumpfish A, C, wolffish and ray) with submission of formalin fixed tissues.

Gross lesions recorded in the lumpfish were: multifocal 1-4 mm diameter areas of branchial necrosis and multifocal to coalescing slightly raised black foci in heart, spleen, kidney, liver, mesentery, skeletal muscle, gastrointestinal tract, peritoneum, and eye (B); discrete granuloma-like lesions in heart, spleen, and kidney (A). Wet mounts of cutaneous, branchial, and internal lesions in lumpfish B, revealed large numbers of 2-3 μm diameter, right angle branching fungal hyphae. The wolffish had numerous 2-3 mm diameter white foci in the epicardium and a single 3-mm diameter aggregate of soft, friable, yellow material in the spleen. No internal lesions were noted in the ray.

The distribution of histologic lesions is presented in Table 1. Cutaneous lesions in lumpfish B,C, and D, consisted of severe necrotizing, granulomatous and ulcerative dermatitis with large numbers of intralesional fungal hyphae. Often immediately subjacent skeletal muscle was similarly affected. In lumpfish B and C vascular invasion was evident, with fungal hyphae visible in vascular lumens often incorporated within thrombi composed of fibrin, erythrocytes, and eosinophilic granular cells. In H&E stained sections hyphae were light brown, parallel walled, 2.5 μm diameter, septate, and exhibited occasional to frequent right angle branching. All visceral lesions listed in Table 1 were similar, characterized by necrosis with admixed and/or surrounding small to moderate numbers of macrophages and lymphocytes, rare eosinophilic granular cells and moderate to large numbers of intralesional similar fungal hyphae. Lumpfish C had multifocal areas of hepatic necrosis with intralesional fungal hyphae and no evidence of an inflammatory response.

Fungal cultures of lumpfish B kidney, and wolffish spleen, each yielded *Exophiala*-like fungi, which were not identical to each other or to any of the established species of *Exophiala*. Further morphologic and DNA analyses of the isolates are currently underway in order to achieve more definitive identification.

The similar histologic appearance of fungal hyphae and lesions in all fish in this series, whilst not definitive, made it highly likely all were infected by an *Exophiala*-like fungus. Hence exophialosis may be a more common cause of death in captive cold water marine fish than previously suspected, and should be considered a differential diagnosis in fish with granulomatous disease, both with or without cutaneous lesions.

The fish all occupied the same water system and were in captivity for a prolonged period of time prior to clinical decline, suggesting persistence of the fungus somewhere in the aquarium environment. Given the pattern of cutaneous ulceration in multiple lumpfish, colonization of skin or opportunistic infection of clinically inapparent cutaneous abrasions, followed by vascular dissemination to visceral organs seemed the most probable pathogenesis. The predominance of lumpfish in this case series suggested a species susceptibility. Possibly their bottom dwelling life style resulted in protracted contact with substrate containing the organism, or predisposed them to cutaneous abrasions and subsequent fungal colonization.

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Table 1. Distribution of histologic lesions.

	Lumpfish A	Lumpfish B	Lumpfish C	Lumpfish D	Atlantic Wolffish	Thorny back ray
Skin	N/A[a]	+	+	+		N/A
Gill	+	+			+	
Heart	+	+			+	
Spleen	N/A	+			+	
Kidney	+	+				+
Liver	+	+	FHN[b]			
Mesentery		+				
Skeletal muscle		+				
Gastrointestinal tract		+				
Peritoneum		+				
Vertebral bone					+	
Spinal cord					+	
Eye		+				

[a]N/A lesion not submitted for histology.
[b]Fungal hyphae and necrosis present but no evidence of an inflammatory response.

MULTICENTRIC LYMPHOMA IN A CLOUDED LEOPARD

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Abstract

A 13-yr-old female, clouded leopard (*Neofelis nebulosa*) housed at the Rosamond Gifford Zoo was evaluated for a two-day history of lethargy and inappetence. Upon visual exam, the animal was unwilling to move and showed signs of abdominal discomfort. She was immobilized with a medetomidine/ketamine combination and maintained on isoflurane for evaluation. Physical exam findings included thin body condition, a moderate degree of muscle atrophy, 10% dehydration, alopecia of the distal tail, a rigid spine, and a firm, irregular mass in mid-abdomen. Blood was collected for a complete blood cell count and serum biochemistry. Abdominal radiographs confirmed the presence of a mass in the mid-abdominal region, which appeared to be related to the spleen. Both kidneys were smaller than normal and the left kidney was irregular in shape. Ultrasound examination of the abdomen confirmed the presence of the mass and allowed a higher degree of suspicion that the mass was splenic in nature. A fine needle aspirate of the mass was performed and yielded a cytologic diagnosis of lymphoma based on the presence of a prominently dominant population of lymphoblasts admixed with a reactive population of inflammatory cells.

Results of the complete blood cell count were not available and the biochemistry analysis was largely normal except a marked azotemia. Three treatment options were explored: 1) euthanasia, 2) chemotherapy with adriamycin, cytotoxin and prednisone which is currently the most successful treatment utilized in domestic cats for lymphoma and 3) treatment with prednisone alone. The following day, the animal was started on prednisone therapy at 2 mg/kg and cephalexin at 10 mg/kg as a prophylactic measure. During the following month, the animal's activity increased and her appetite was normal; however, she exhibited a progressing degree of muscle wasting. One month after the initiation of prednisone, the cat's appetite became irregular. Oral therapy with sucralfate at I gram twice daily was initiated. The cephalexin was discontinued at this time, as this drug is reported to cause nausea and anorexia in domestic cats. Over the next month, the cat's level of activity decreased significantly and weight loss progressed. Serial urinalyses performed on samples obtained opportunistically showed a marked bilirubinuria. Two months after the initial diagnosis, the cat was immobilized for further evaluation of its hepatic/renal function and to reevaluate the progression of the splenic lymphoma. Due to the animal's poor body condition and poor response to therapy, euthanasia was elected.

Significant gross necropsy findings included generalized lymphadenopathy, a multifocally discolored spleen with poorly demarcated, coalescing, 1-3 mm, white areas, and a liver with poorly

demarcated, irregular, multifocal 1-3 mm white areas and welldemarcated, spherical, white to pink multifocal nodules. The medullary cavity of the femur was filled with a dark red, firm material, with multifocal, poorly circumscribed 1-3 mm white areas. The kidneys contained circular, cortical, fluid-filled cavities. The subcutaneous tissue was bright yellow.

Histopathologic diagnosis indicated that the architecture of the lymph nodes had been replaced by a sheet of small and medium sized lymphocytes in a coalescing follicular arrangement. The spleen was multifocally infiltrated with small and medium sized lymphocytes, similar to those found in the lymph nodes, as was the liver. The renal cysts were thought to be an incidental finding. The majority of the bone marrow cells were of the same neoplastic population as seen in the liver, spleen and lymph nodes. A final diagnosis of multicentric lymphoma was concluded based on histopathologic findings.

PHARMACOKINETICS OF ACYCLOVIR IN TRAGOPANS *(Tragopan* sp.*)*

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Abstract

Introduction

Acyclovir is a synthetic purine nucleoside analogue (guanosine) with in vivo and in vitro antiviral activity against varicella zoster and herpes simplex types 1 and 2 in humans.[4,7] Inhibitory activity is due to incorporation, inhibition and inactivation of herpes viral DNA.[4,7] Acyclovir is marketed as an oral preparation and appears to have the least potential for adverse effects when compared to other antiviral drugs.[4] Previous studies of acyclovir in quaker parakeets reported efficacy and few adverse effects at 40 mg/kg i.m. and 80 mg/kg p.o., t.i.d. as initial trial dosages.[5,6]

Acyclovir was used in tragopans (*Tragopan caboti* + *T. temminckii*) to determine absorption, distribution, and linearity of the drug after a single dose. This study was designed in response to multiple peracute deaths in multiple pheasant species at the Bronx Zoo. Affected birds showed minimal or no clinical signs prior to death. The diagnosis of herpesviral infections on postmortem histopathologic evaluation encouraged the need for investigation of a potential treatment for future cases. There is no known published information on the efficacy or safety of acyclovir in tragopans or on herpesvirus infections in pheasant species.

Methods and Materials

Five healthy adult tragopans, three males and two females weighing 0.96 to 1.8 kg, at the Wildlife Conservation Society (Bronx, NY) were in the study. Birds were fasted 12 hr prior to drug administration, and no treatments were given for at least 1 mo prior to study initiation. Each bird was given doses of 40, 80 and 120 mg/kg oral suspension (Alphapharma USPD Inc., MD) by gavage. Blood samples of approximately 1 ml per sample were obtained from the jugular, deep ulnar, brachial or metatarsal veins at 1, 2, 6, 12 and 24 hr post drug administration. Trials were repeated at 1- to 2-wk intervals to allow for drug elimination from the subjects.

Results were obtained with reverse phase HPLC with a C-8 column and UV detection.[1,2,10] Validation was done with banked, untreated plasma from the same birds used in the study.

Results

All animals were clinically healthy throughout all treatments as determined by physical examination, hematology and plasma biochemistries. Complete blood counts preformed prior to and during the study revealed no evidence of abnormalities that would indicate clinical illness.

The plasma acyclovir concentration vs. time curve is shown in Fig. 1. The AUC_{tlast} significantly increased following the 120 mg/kg dose when compared to both of the lower doses, but not from 40 to 80 mg/kg. This increase was not linear. The values for C_{max} also showed similar results, but the increase was only significant between the 40 and 120 mg/kg treatments. There were no detectable levels of acyclovir in plasma at 24 hr with 80 mg/kg dosage.

Discussion

Acyclovir is probably the most readily available, effective compound to administer for treatment of herpes virus in captive avian species, at a reasonable cost. Potential for side effects also appears to be less with acyclovir (in comparison to the basic antiviral chemical compounds famciclovir, valciclovir and interferon).[4,7-9] Cost and decreased potential for side effects make acyclovir appealing to the clinician with potential herpes viral infection in their avian collection. These facts also make acyclovir attractive for consideration for future viral studies in birds.

The nonlinearity of acyclovir pharmacokinetics in tragopans makes dose extrapolation difficult, if not dangerous. When pharmacokinetic parameters do not respond in a linear fashion (i.e., as dose doubles, plasma C_{max} and AUC double), then one cannot reliably predict the pharmacodynamic effects or efficacy of a given drug. However, the results from this study suggest that 120 mg/kg p.o. b.i.d. may be the best dosage in this species to achieve effective plasma concentrations for treatment and prevention of herpesvirus infections. Precaution should be taken with prolonged therapy, as long-term chronic toxicity has not been evaluated in this species.

A detectable plasma level of acyclovir was noted at 24 hr with 40 mg/kg dosage only in one bird, and may be attributed to individual variation in metabolism or absorption of the drug.

A moderate elevation in bile acids was noted on several birds during the study. Birds were not fasted prior to phlebotomy for biochemistry samples. Increased bile acids in these birds could be due to postprandial sampling or liver compromise. Lack of adequate bile acid reference values for this species prohibits definitive interpretation of these results.

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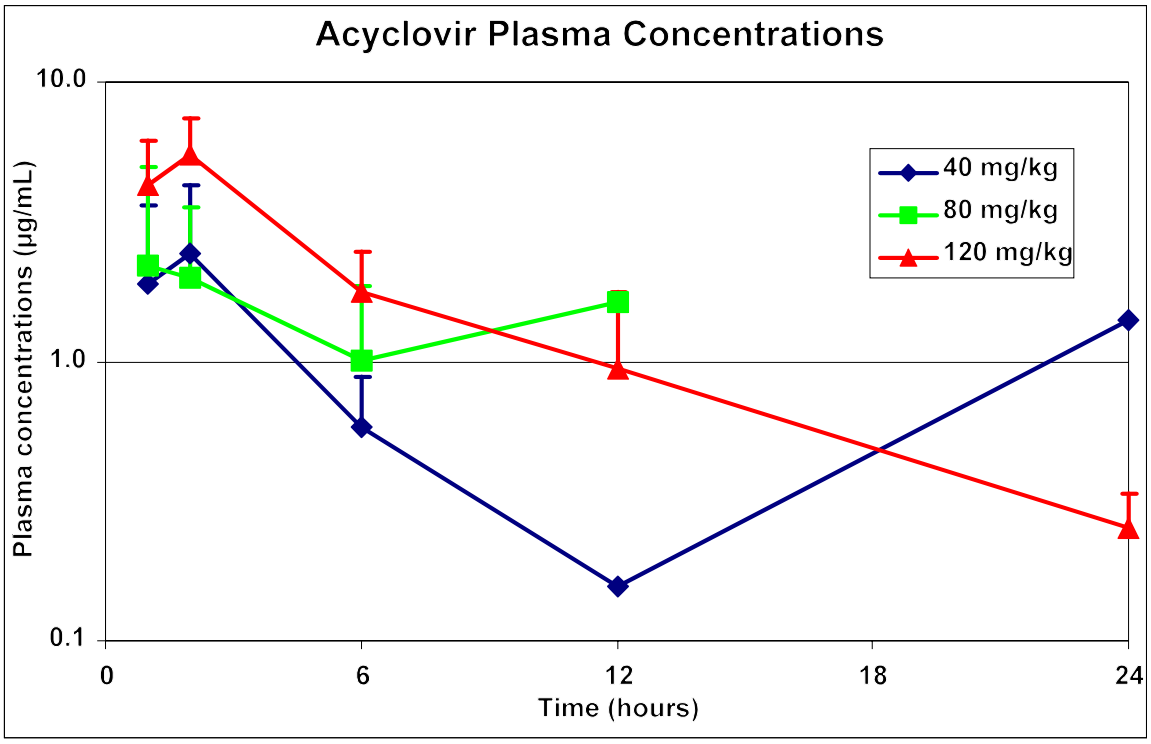

Figure 1. Plasma concentration of acyclovir versus time.

TOXICOSIS ASSOCIATED WITH TOPICAL ADMINISTRATION OF IVERMECTIN IN DOG-FACED FRUIT BATS (*Cyanopterus brachyotis)*

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Abstract

Eleven of 40 dog-faced fruit bats administered one drop of 1% ivermectin topically developed generalized paresis. Six of the bats recovered within 24 to 48 hr. The remaining five died or were euthanatized within 3-5 days of the development of clinical signs. Histologic evaluation revealed renal tubular necrosis in three of the five bats.

Introduction

Ivermectin, an endo- and ectoparasiticide, potentiates the action of gamma amino butyric acid (GABA) by increasing its release and binding at post-synaptic receptors.[3,12] Since GABA is an inhibitory neurotransmitter its potentiation by ivermectin further decreases nerve conduction. In mammals, GABA receptors are restricted to the central nervous system, which is not easily penetrated by the large avermectin compounds. In contrast, in many invertebrates these receptors regulate peripheral muscle activity,[3,12,13] making ivermectin an effective and safe parasiticide for use in mammals.

In dogs, safe and effective ivermectin dosages range from 0.006 mg/kg orally for heartworm prevention to 0.2 mg/kg by subcutaneous or intramuscular administration for the treatment of mites and nematodes.[15] An exception is in collie breeds, which may show toxic effects at dosages as low as 0.1 mg/kg.[15] This susceptibility appears to be associated with a more permeable blood-brain barrier.[14,15] In cattle, horses, sheep, and birds the recommended dosage is 0.2 mg/kg orally, by subcutaneous or intramuscular administration for susceptible parasites.[15]

Ivermectin toxicosis is well recognized but its pathogenesis is not well understood. High dosages (e.g., > 2 mg/kg orally) in dogs cause mydriasis, ataxia, tremors, depression, emesis and stupor.[12,16] In some dogs paralysis, weakness, coma and death may occur.[12] Clinical signs of toxicosis have been reported in cattle, horses and swine at oral dosages of 4, 2 and 30 mg/kg respectively.[3,16] The oral LD_{50} in mice, rats, dogs and rhesus monkeys is 25, 50, 80 and 24 mg/kg respectively.[2,3] Topical LD_{50} for rats and rabbits is > 400 mg/kg.[3] Therapeutic doses of ivermectin are well below these LD_{50} values. The following case report describes toxicosis in a captive collection of dog-faced fruit bats associated with topical administration of ivermectin.

Case Report

Five adult male dog-faced fruit bats (*Cyanopterus brachyotis)*, weighing 30-40 g, were evaluated at the Veterinary Medical Teaching Hospital (VMTH), University of Florida, because of sudden onset of generalized paralysis. These animals were housed indoors with other fruit bats in free-flight enclosures at a private facility and maintained on a diet of fresh fruit, vitamins and protein supplements.

The day before admission these and 35 other bats were administered a 1% ivermectin solution (Ivercide® 10 mg/ml, Phoenix Pharmaceutical, Inc., St. Joseph, MO) as part of an annual physical examination and preventive care program. Each bat was administered one drop from a 0.5ml insulin syringe (Terumo Medical Corporation, Elkton, MD) on the skin of the chest or the patagium (membranous wing). The following morning, 11 of the 40 treated bats had fallen from their perches and were lying either on the ground or in their food bowl, unable to move. Two days later another animal was found in a food bowl.

Six bats recovered within 24-48 hr. The first four bats admitted will be identified as bats A, B, C, D and the final one, E. Physical examination of bats A-D revealed spontaneous respiration but they could not eat or perch and had minimal movements in all parts of the body. They were placed in an incubator at 25°C and tube fed every 2 hr with 0.4 ml of a carbohydrate liquid supplement (Carbo-Fuel®, Twin Laboratories, Inc., Ronkonkoma, NY). One bat could lap up fluid on its own. Bat A was given 0.02 mg of flumazenil (Romazicon®, Hoffman-LaRoche, Basel, Switzerland) by subcutaneous administration. The following day three bats died spontaneously and one was euthanatized. Bat E, evaluated 5 days after the ivermectin treatment because of weakness, died spontaneously 2 days after admission. All bats were submitted for necropsy evaluation.

Histopathologic examination of representative tissues from bats A, B, and C revealed mild to moderate acute tubular necrosis with evidence of tubular epithelial regeneration. A proliferative glomerulonephritis, with marked cortical tubular distension and tubular proteinosis was present in bat C. In addition, bat C also had a severe hemorrhagic gastroenteritis with intralesional fungal organisms. A suppurative bronchopneumonia was present within bat E.

Discussion

The paralysis/paresis observed in these bats was most likely due to ivermectin. This diagnosis is based on the clinical signs, and the recent history of ivermectin administration. Other differential diagnoses for acute paralysis include spinal cord injury, polyneuropathy, polymyopathy, metabolic abnormalities and other toxicants.[11] These were ruled out because of the acute onset of the clinical signs in combination with multiple animal involvement and the history of recent topical ivermectin therapy.

Ivermectin is used percutaneously in canaries, finches and budgerigars.[18] Reported topical dosages for psittacines and pigeons are 200 µg/kg of 1% ivermectin and one drop of 0.8% ivermectin

respectively.[18] One drop of the bovine formulation of ivermectin on the skin of small birds is common practice.[4]

The volume of one drop of 1% ivermectin from an insulin syringe was determined to be approximately 0.0054 ml. Based on this volume, the dose of ivermectin applied topically to these bats was calculated to be 1.8 - 2.1 mg/kg. Topical application of ivermectin and abamectin (a compound that differs from ivermectin by a single double bond in chemical structure[3] in rats, rabbits and monkeys has shown poor dermal absorption of these compounds because of their large size. Absorption is thought to be less than 1% of the dermally applied dose.[3] In goats the peak plasma level of ivermectin after topical application was considerably lower compared to oral administration but, was detectable in the plasma longer (192 days topical dosing versus 144 days in oral dosing).[19] If 0.5% of the drug was absorbed[3] the calculated absorbed dosage for each bat was approximately 0.009 - 0.012 mg/kg. This is comparable to safe therapeutic dosages in other mammal and bat species.[8]

Given the estimated dose appears within safe limits for use in other mammals, there are few possible reasons why these animals showed clinical signs of toxicity: 1) more than one drop was administered per bat, 2) increased drug absorption through the patagium, 3) oral ingestion of the drug through auto- and allogrooming, and 4) this species is more sensitive to ivermectin.

Some of the animals received the topical dose on the skin of the ventral thorax whereas others received it on the patagium. The patagium is a thin membrane-like wing containing a generous blood supply[10] and it is possible that absorption is increased because of the thin barrier between the drug and underlying vasculature. Unfortunately it was not possible to relate topical application site to toxicosis because the site of administration was not recorded.

The use of flumazenil on one bat was to reverse benzodiazepine mediated CNS depression. It is reported that avermectins potentiate benzodiazepine receptor binding.[3] No change was seen in the animal after administration.

The acute tubular necrosis observed in three of the five bats is consistent with a toxic or ischemic insult.[9] Possible causes for ischemic insult include hypotension (associated with hemorrhage, dehydration, or vasodilation), vasoconstriction or thromboembolism. Known nephrotoxic substances include aminoglycosides, some heavy metals, ethylene glycol and many plants.[9] Although it was difficult to assess hypotension at presentation, there was no evidence of hemorrhage and hydration was assumed to be maintained with the fluid diet. However, gastroenteritis of bat C may have led to dehydration in this animal. There was no history of either drug therapy or access to toxic plants or chemicals in this group of animals.

Renal damage attributable to ivermectin toxicity has not previously been reported. The exception is in humans with onchocerciasis in which glomerular and tubular kidney dysfunction was noted following ivermectin treatment and likely related to microfilarial load.[1,7] In teleost fish ivermectin is readily taken up by the tubular epithelial cells and transported in the kidney proximal tubules by

a p-glycoprotein transporter.[6] Teleost tubules are functionally identical to mammalian renal proximal tubules.[6] Additionally in a study in which pig kidney cells were bathed in ivermectin solutions of various concentrations, cell death occurred within 24 hr at 40 µg/ml.[17]

It is not completely clear why these bats showed ivermectin toxicity and why three of the animals suffered renal tubular necrosis. It is possible that this species represents a group particularly sensitive to the ivermectin compound and that the renal damage may have been related to administration of ivermectin. It is, therefore, recommended that 1% ivermectin solution not be topically administered to dog-faced fruit bats.

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CUTANEOUS POX VIRUS INFECTION IN TWO GIANT ANTEATERS (*Myrmecophaga tridactyla*) WITH CLINICAL AND PATHOLOGIC FEATURES SIMILAR TO HUMAN MOLLUSCUM CONTAGIOSUM

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Introduction

Pox viruses cause disease in a wide range of mammals, birds and reptiles. The six genera of pox viruses known to infect mammals include: *Orthopoxvirus* (mouse pox, rabbit pox, horse pox, cow pox, elephant pox, many others); *Parapoxvirus* (contagious ecthyma, seal pox, red squirrel pox, others); *Capripoxvirus* (goat pox, others); *Leporipoxvirus* (hare fibroma, myxomatosis, squirrel fibroma, others); *Suipoxvirus* (pigs), *Yatapoxvirus* (primate tanapox); and *Molluscipoxvirus* (human molluscum contagiousum).[6,8] A seventh genus contains several unassigned viruses including cetacean pox virus, gray kangaroo pox, molluscum like virus of horses, mule deer pox and others.[6,8] Marennikova et al. (1976) described fatal pox virus infection in two giant anteaters with severe rash, vesicular dermatitis and pulmonary infection.[4] The virus isolated from these anteaters was similar to cowpox virus (genus: *Orthopoxvirus*).[4]

Molluscum contagiosum is a disease of humans characterized by proliferative cutaneous lesions in children and adults. Children are infected by close contact or fomite inoculation. In adults, molluscum contagiosum is a sexually transmitted disease that is seen with increased incidence in immunosuppressed individuals (i.e., HIV infection or chemotherapy patients). Skin lesions due to molluscum contagiosum are clinically recognized as small slightly raised umbilicated lesions on the face, limbs or trunk. Histologically, the skin lesions are characterized by endophytic nodular hyperplasia of the epidermis. Large eosinophilic cytoplasmic inclusions (molluscum bodies) compress the nucleus to the periphery of infected keratinocytes. In this report, we describe cutaneous pox virus infection in two giant anteaters (*Myrmecophaga tridactyla)* with clinical and histopathologic lesions resembling those seen in molluscum contagiosum of humans.

Case Report

Anteater #1

An adult female giant anteater was treated over an 8-mo period for cutaneous *Mycobacterium* sp. infection in the inguinal region. During one of the surgical procedures performed to treat the Mycobacterial infection, an area of roughened and irregular skin was noted on the lateral cervical region cranial to the right shoulder. Two 4-mm punch biopsies from this region were submitted in

10% neutral buffered formalin for histopathologic examination. The anteater was euthanatized several weeks later due to uncontrolled spread of the Mycobacterial infection. At necropsy, close examination of the previously biopsied site on the lateral cervical region revealed numerous small (2-4 mm diameter) slightly raised nodular lesions. In many areas the lesions were confluent with each other giving the skin the roughened texture that was described clinically. Examination of the lesions under a 10x magnifying lamp revealed many of the lesions had a small umbilicated central region. Several lesions were placed in formalin for histopathologic examination.

Anteater #2

Following the diagnosis, treatment and euthanasia of the female anteater described above, an adult male anteater was immobilized for complete physical examination. Several raised cutaneous lesions were discovered on the caudal palmer surface of both right and left front feet. These lesions were pale tan in color and measured 2-3 mm in diameter. The lesions occurred individually or in small groups of 2-3 nodules. Punch biopsies of two lesions were submitted in formalin for histopathology. Examination of the submitted lesions under a 10x magnifying lamp revealed one of the two lesions had a small umbilicated central region. At the time of preparation of this abstract, numerous cutaneous lesions remain on the palmar surface of both front feet. Rare lesions are also present on the hind feet.

Microscopic Examination

The histopathologic characteristics in the skin lesions submitted at all time periods from both anteaters were similar. The epidermis was diffusely irregularly hyperplastic and was covered by a thick dense layer of orthokeratotic keratin. In multifocal areas there was marked endophytic hyperplasia of the epidermis forming a multilobular intradermal mass. The lobules comprising the mass were separated by thin bands of dermal collagen. The lobules of hyperplastic epidermis were characterized by a central core of enlarged (20-30 micron diameter) round to oval eosinophilic keratinocytes. This core of enlarged keratinocytes was surrounded by a rim of normal appearing keratinocytes resembling those of the stratum basale. Scattered hyperplastic lobules had a central core of keratin and degenerate keratinocytes. Keratinocytes comprising the center of hyperplastic lobules contained large (10-20 micron diameter) hypereosinophilic intracytoplasmic inclusions that occupied the majority of the cytoplasm and displaced the nucleus to the periphery of the cell. Similar inclusions were also present within degenerate keratinocytes intermixed with keratin at the center of hyperplastic lobules and on the epidermal surface. Dermal inflammation was present in any of the lesions examined.

Electron Microscopy

Transmission electron microscopy revealed large numbers of brick shaped pox virus particles within the cytoplasm of keratinocytes that contained eosinophilic intracytoplasmic inclusions on histopathology. These pox virus particles measured approximately 200 nm in length and contained the characteristic “hour glass” central core. The nucleus of infected cells was displaced to the

periphery of the cell. Cellular changes associated with pox virus infection included mild vacuolization of the cytoplasm and swelling of the nuclear membrane.

Discussion

Benign cutaneous pox virus infection has not been previously reported in giant anteaters. The clinical presentation and pathologic lesions seen in these two giant anteaters differ greatly from a previous report of fatal systemic cowpox infection in two giant anteaters from the Moscow Zoo.[4] In that report, cutaneous lesions of pox virus infection were described as a severe rash and a hemorrhagic vesicular dermatitis that was intensely pruritic. Cutaneous lesions in the two anteaters reported here consisted of small benign umbilicated nodules that were discovered as incidental findings during treatment for a separate disease process or during a routine physical examination. These nodules do not seem to cause any discomfort or pruritis in the currently infected anteater and there was no evidence of systemic spread of the pox virus infection in the anteater that was necropsied following euthanasia for cutaneous mycobacterial infection. Efforts are underway to isolate and grow the pox virus responsible for the cutaneous lesions reported here. We believe the pox virus responsible for the lesions in these two anteaters is a different virus than the cowpox virus believed to be responsible for the deaths at the Moscow Zoo.

A unique finding in the lesions examined from these two anteaters was the consistent presence of endophytic hyperplasia of the epidermis associated with pox virus infection. In authors' experience and in most reported cases, lesions associated with pox virus infection are nodular exophytic masses. The endophytic nature of the cutaneous pox lesions in these cases is similar to the lesions described in the human disease, molluscum contagiousum. Molluscum contagiosum has never been experimentally transmitted to animals and only a few cases of "molluscum contagiosum-like disease" have been reported.[7,9] Reports of molluscum contagiosum-like disease include those from horses, chimpanzees and a red kangaroo.[1,3,10] Cases of molluscum contagiosum-like disease were diagnosed in those species based on clinical signs and histopathologic lesions similar to the human disease. The pox virus responsible for molluscum contagiosum-like disease in horses has been demonstrated by in situ hybridization to be closely related to the human molluscum contagiosum virus and the possibility of cross-species transmission of molluscum contagiosum virus has been discussed.[9]

Molluscum contagiosum of adult humans and the molluscum contagiosum-like disease of adult horses are diseases that are often seen in association with immunosuppression.[2,5,9] One of the two pox virus infected anteaters (case #1) was concurrently infected with *Mycobacterium fortuitum*. It is suspected that the severe granulomatous inflammatory process present associated with the Mycobacterial infection may have led to immunosuppression that predisposed this anteater to pox virus infection. An obvious predisposing condition has not been discovered for the infected male anteater (case #2), although the stress of captivity could play a minor immunosuppressive role.

In this report, we have described two cases of molluscum contagiosum-like disease in giant anteaters. Efforts to further characterize the virus by molecular methods are underway at this

writing. Those molecular results will help us classify this disease as another report of molluscum contagiosum-like disease or as another example of possible cross species transmission of the molluscum contagiosum virus.

The prevalence of pox virus infection / disease in captive giant anteaters is unknown. Detailed examination of the skin may be required for diagnosis of this subtle disease. Definitive diagnosis is easily made by skin biopsy and histopathology. Treatment has not been attempted with the currently infected anteater.

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MYOCARDIAL FIBROSIS IN AN ORANGUTAN

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Abstract

Myocardial fibrosis has increasingly been documented as an important cause of mortality in zoo primates. The changes have been associated with hypertensive heart disease, atherosclerosis, obesitas, and hypovitaminosis E.[1-7] Viral infections have been discussed, but the pathogenesis is more probably a non-infectious disease. Histologically the lesions are similar to chronic ischemic heart disease in elderly humans, except that in the great apes atherosclerotic lesions may be absent, and deaths occur in middle-aged animals.[5]

A 16-yr-old male Sumatran orangutan (*Pongo pygmaeus*), born in an English zoo, lived in a Swedish zoo for 12 yr together with a clinically healthy female without producing any offspring. During a 22-mo period recurrent diffuse respiratory symptoms were treated with a variety of antibiotics, bromhexine, and prednisolone. The male was continuously lethargic and at times uncoordinated, and only prednisolone appeared to alleviate some of the symptoms.

The ape was found dead and necropsy was performed the same day. The orangutan weighed 192 kg and was obese.The major gross finding was severe effusions, with hydropericard (> 0.8 L), hydrothorax in the left pleural cavity (> 0.7 L) and hemothorax (> 3.0 L) on the right side. The lungs were congested, compressed and edematous. Approximately 1 L of clear watery fluid was found in the air sacs. The liver was severely enlarged with a reticulated appearance. Large amounts of deeply infiltrating subcutaneous edema was present in the torso, and the eyelids were swollen and edematous. The heart APPEARED normal in shape, the myocardium and the valves were grossly unremarkable.

Histologic examination showed a severe diffuse fibrosis of the myocardium without any inflammatory cells present. The myocytes were multifocally hypertrophied and alternatively atrophic, with fibrous connective tissue in between muscle cells. Signs of atherosclerosis was not seen. Chronic pulmonary congestion with severe perivascular and interstitial fibrosis as well as chronic liver congestion was noted. Moderate focal lymphocyte airsacculitis was seen. Vitamin E and selenium levels in muscle and liver tissue were found to be within normal range.

The diagnosis was myocardial fibrosis. As no inflammatory reaction was seen in the heart, a viral infection was considered as less probable to have caused the long-term symptoms and extensive changes in the heart. The respiratory symptoms could have been due to both repeated infections and to heart disease, but the chronic changes in lungs and liver tissues point to a heart problem. A hypertensive heart disease together with obesity had probably over time produced the extensive fluid

effusions and finally ended with a ruptured pulmonary vessel leading to a fatal hemorrhage to the pleural cavity. As this condition has been increasingly documented in captive great apes, further investigations is needed in the possible pathogenesis. Considering the comparative histology of the human condition of chronic ischemic heart disease, an overview of diets and management of the captive primates may be called for.

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MEASUREMENT OF GLOMERULAR FILTRATION RATE, RENAL PLASMA FLOW AND ENDOGENOUS CREATININE CLEARANCE IN CHEETAHS (*Acinonyx jubatus jubatus*)

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Abstract

Glomerular filtration rate (GFR), renal plasma flow (RPF) and the creatinine clearance rate (CCR) were determined in 12 captive cheetahs (seven females and five males ranging in age from 1.5 to 7.5 yr; mean 5 ± 2.5 yr) during general anesthesia with Telazol® and isoflurane by measuring the urinary clearances of inulin, para-aminohipppuric acid (PAH), and endogenous creatinine (CCr), respectively. The GFR and the RPF were stable during the procedure, with mean values of 1.59 ± 0.17 ml/min/kg body wt for GFR and 5.12 ± 1.15 ml/min/kg body wt for RPF. While the mean value for CCr of 1.47 ± 0.20 ml/min/kg body wt was significantly ($P<0.05$) less than the corresponding value for GFR, the mean difference between GFR and CCr was only 0.11 ± 0.02 ml/min/kg and the CCr was highly correlated with GFR ($R^2 = 0.928$; $P<0.0001$). The measurement of CCr in cheetahs should provide a reliable estimate of GFR, facilitating the early detection of renal disease in this species.

Introduction

Renal disease is a leading cause of mortality and morbidity in captive cheetahs.[5] The genetic homogeneity of these animals may predispose them to diseases such as renal amyloidosis and glomerulosclerosis that ultimately cause their death.[1,5,6,8] Unfortunately, efforts to characterize and control these diseases are hampered by the fact that they are often only confirmed on postmortem examination.[1,8] Early detection of renal disease in captive cheetahs may allow the development of a program to screen these animals, characterize the natural progression of the disease(s), study interventions, and introduce appropriate management techniques. This study evaluated methods to determine glomerular filtration rate (GFR), renal plasma flow (RPF) and endogenous creatinine clearance (CCr) in captive cheetahs, and to determine the relationship between GFR and CCr in this species.

Methods

Urinary clearances were measured in 12 captive cheetahs from White Oak Conservation Center, seven females and five males ranging in age from 1.5 to 7.5 yr (mean 5 ± 2.5 yr). All cheetahs had been previously diagnosed with lymphoplasmacytic gastritis by histologic examination of gastric

biopsies. *Helicobacter* spp. were present in gastric biopsies of five of the cheetahs. Animals were immobilized with tiletamine and zolazepam (Telazol®, Fort Dodge, Fort Dodge, IA 50501 USA; 3.4 ± 0.5 mg/kg i.m.), intubated, and maintained with isoflurane (Abbott laboratories, North Chicago, IL 60064 USA) in oxygen**.** Subcutaneous administration of inulin (Sigma Chemical Co., St. Louis, MO 63178 USA; 220 mg/kg) and PAH (Sigma Chemical Co., St. Louis, MO 63178 USA; 8.25 mg/kg) occurred at t = 0, and was repeated at t = 25 min. A urinary catheter was aseptically placed for initial urine collection at t = 0, with 2-3 additional 30 min collection periods starting after t = 50. Blood was collected for serum at t = 0 and t = 50 min. Inulin and PAH concentrations in urine and plasma were determined by anthrone reaction methodology.[2,3] Creatinine concentration in urine and serum were determined by a semi-automated method (Spectrum CCX, Abbott Diagnostics, Irving, TX 75038 USA).

The glomerular filtration rate (GFR), renal plasma flow (RPF), CCr were expressed as urinary clearance of inulin, PAH and endogenous creatinine, respectively. Clearances were calculated as ml/min/kg body wt by using the standard clearance formula: (urine concentration of substance × urine volume)/(plasma concentration of substance × duration of period × body wt in kg). For each animal, the mean of the clearance determinations for the two or three periods was taken to represent the value for clearance for that animal. Linear regression analysis was used to evaluate the nature of the relationship between GFR and CCr (Statview 4.5, Abacus, Berkeley, CA 94710 USA).

Results

Urinary clearance values and associated parameters are listed in Table 1. The GFR and RPF were stable during the procedure, with mean values (ml/min/kg body wt) of 1.59 ± 0.17 for GFR and 5.12 ± 1.15 for RPF. The mean value for GFR was significantly greater than the corresponding value for CCr ($P < 0.05$), with a mean difference of 0.11 ± 0.02 ml/min/kg body wt and the ratio CCr/GFR was significantly ($P < 0.05$) less than 1.00. GFR and CCr were highly correlated (Fig. 1), with an R^2 value of 0.928 ($P < 0.0001$).

Discussion

Early detection of renal insufficiency is necessary to characterize and control renal disease in captive cheetahs. In this study, urinary clearance of inulin and PAH were assessed as measures of GFR and RPF, and the reliability of urinary clearance of endogenous creatinine was evaluated as an estimate of GFR in this species. GFR and endogenous creatinine clearance were well correlated in this study. In domestic cats, there has been controversy surrounding the correlation of inulin and endogenous creatinine clearance,[4,7,9,10] primarily because of the presence of noncreatinine chromogens which confounds the procedure technically. In captive cheetahs, the strong relation and small difference between CCr and GFR support the use of endogenous creatinine clearance as a measure of renal function, possibly because noncreatinine chromogens comprise a relatively small fraction of the total measured SCr in cheetahs. The use of CCr to estimate GFR simplifies the procedure, as endogenous creatinine clearance is less technically challenging than GFR determination using inulin. Endogenous creatinine does not require injections or specialized laboratory assays and might prove

to be an appropriate addition to routine health screening in captive animals. The correlation between CCr and GFR provides a rationale for testing additional animals to further evaluate CCr as an early indicator of renal disease in captive cheetahs.

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Table 1. Urinary clearance values obtained in 12 captive cheetahs.

Parameter	Mean ± standard deviation
GFR (ml/min/kg body wt)	1.59 ± 0.17
RPF (ml/min/kg body wt)	5.12 ± 1.15
CCr (ml/min/kg body wt)	1.47 ± 0.20
CCr/GFR	0.93 ± 0.05

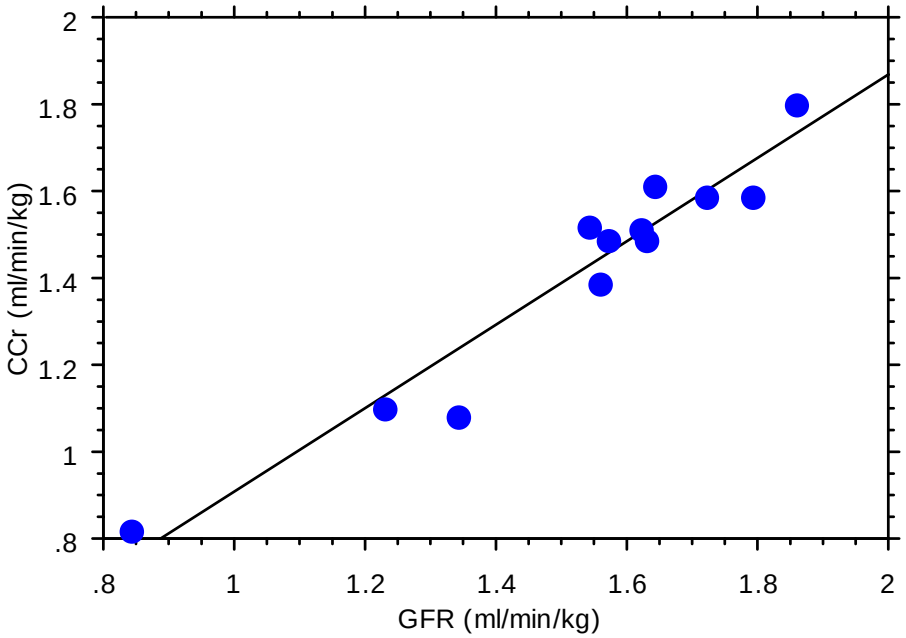

Figure 1. Relation between urinary clearance of inulin (GFR) and endogenous creatinine (CCr) in 12 captive cheetahs. Although the mean value for GFR (1.59 ± 0.17 ml/min/kg body wt) was significantly greater than the corresponding value for CCr (1.47 ± 0.20 ml/min/kg body wt), the mean difference of 0.11 ± 0.02 ml/min/kg was small and the two clearance determinations were highly correlated with an R^2 value of 0.928 ($P < 0.0001$).

ETODOLAC AS AN ADJUNCT TO MANAGING OSTEOARTHRITIS IN CAPTIVE BENGAL TIGERS (*Panthera tigris bengalis)*

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Abstract

Introduction

Osteoarthritis (OA) is a serious malady of numerous species kept in zoologic collections. Clinical and radiographic signs can be seen in middle-aged animals and certainly in geriatric patients. This condition is especially noted in primates and cats from the genus *Panthera*.[6] Medical management of domestic species includes rest, analgesics, and even surgical management. Glycosaminoglycans and glucosamines have recently found a place in zoologic medicine and have their value.[7] Analgesics include opioids and non-steroidal anti-inflammatories (NSAIDs). Opioids have limitations and may even be contraindicated in felids. NSAIDs have limitations with metabolism as well in these animals but with careful monitoring may be effective.

While animals in zoologic collections serve several purposes their educational value may be the most significant. As such it is well to think of them as companion animals and mange them as such to maximize their health and welfare. One such aspect of this care is behavioral modification/enrichment including the training of animals to facilitate medical management. The combination of this type of management has led to the use of a novel therapeutic approach in osteoarthritis in large cats to a satisfactory conclusion.

Case Report 1

A female Bengal tiger (Accession No. 50386) was first diagnosed with osteoarthritis of both coxofemeral joints on physical exam in may 1998 at 10 yr of age. Pertinent medical history included a chronic struvite cystitis that was eventually controlled with ammonium chloride and antibiotics as needed. In spring and early summer of 1999 the tiger was noted by the care taking staff to have a decrease in activity especially swimming, moving off and onto a bench, running, and stretching up trees on the display. By July conditioning to allow voluntary blood draws from the lateral tail vein was a steady husbandry feature. Because this was in place the decision was made to try etodolac as an analgesic for the hip osteoarthritis and suspected spinal arthritis seen in large felids. Etodolac was started at 5 mg/kg p.o. q 48 hr for five doses, 600 mg at an estimated weight of 120 kg based on the latest physical exam. At the end of this course the response was judged to be excellent by the staff. The tiger was then tapered off to 600 mg q 72 hr again with an overall improvement in activity and comfort level. When the dose was extended beyond this interval there was some notation that she would be stiff in the mornings and subjectively did not appear to be as comfortable as before. The 72-hr interval was reinstated and has been in place ever since. Serum

chemistries from blood samples taken from the tail vein while awake prior to etodolac therapy (*n* = 3) revealed the following means: BUN 34 mg/dl, Cr 2.3 mg/dl, albumin 3.7 g/dl, AST 21 IU/L. Samples taken after the start of therapy (*n* = 5) revealed the following means: BUN 39.4 mg/dl, Cr 2.34, albumin 3.4 g/dl, AST 20.2 IU/L. No statistic comparisons were attempted because of the small sample sizes but it appears that the etodolac has not affected these parameters. Subjectively this tiger has responded extremely well by running onto the display, climbing trees, and especially by swimming.

Case Report 2

A second female Bengal tiger (Accession No. 50385), was diagnosed radiographically with coxofemeral osteoarthritis in 1998. Several courses of chondrotin sulfates and glycosaminoglycans were initiated but appeared to be ineffective at improving the tiger's condition. In spring of 1999 she developed a left forelimb lameness and a slight right rear leg lameness. A course of etodolac was instituted on this animal as above at the same dose and interval. Blood was collected in the same manner as well. The clinical response was not as dramatic as in the first tiger so a physical exam was performed. Severe proliferative osteoarthritis of the left elbow was seen radiograhically. Several large osteophytes were surgically removed in an attempt to alleviate the discomfort and to return to normal use. Because the proliferation was so vast, this was minimally effective. Etodolac was re-instituted after the surgery. This tiger is not as reliable for voluntary blood sampling as the previous case, so awake sampling is limited but a single samples taken prior to the etodolac revealed a BUN 24 mg/dl, Cr 2.5 mg/dl, albumin 3.4 g/dl, AST 21 IU/L. Post therapy values are essentially identical with the means (*n* = 2) of BUN 27.5 mg/dl, Cr 2.65 mg/dl, albumin 3.6 g/dl, AST 30.5 IU/L. Subjectively this animal does move better than before the etodolac and the hind limb lameness is essentially resolved but the forelimb lameness persist. This tiger has however become the most dominant one of the group of three females and will run and jump upon her bench readily.

Discussion

Osteoarthritis is a disease of captivity in large felids. In a study of museum specimens OA could not be documented in wild felids of the genus *Panthera*.[6] Degenerative spinal disease is another musculoskeletal condition that affects this genus in captivity that may warrant analgesic use.[4] Analgesics for management of OA in this genus ranges from aspirin to glycosaminoglycans and glucosamines.[7] Etodolac has not been studied in cats and is not recommended for this species because of the toxic effects of other NSAIDs.[2] In one review NSAIDs are not even listed as a therapeutic option for domestic cats with OA.[3] Etodolac at approximately 5 mg/kg q 48 hr and then tapering to effect has resulted in good clinical resolution in one tiger (Case Report 1) and a combination of surgery and etodolac has resulted in satisfactory results in the other (Case Report 2). Hepatotoxicity associated with NSAID use are typically hepatocellular in nature and dogs on short course of etodolac have seen transient hypoalbuminemia.[1] Monitoring of serum chemistries demonstrated no change in liver or renal function over a protracted course of therapy and no clinical signs of side effects were observed in either cat. Neither cat had a gastroscopic exam to rule out gastric ulceration but no gastrointestinal signs have been observed. Objective evaluation such as

gait analysis is not practical in this situation. Subjective evaluation from clinical staff and caretakers are in consistent agreement that both cats are improved since the etodolac therapy has been instituted. This evaluation may be the best overall assessment of pain control.[1] While both cats have varying degrees of coxofemoral OA, the tiger in Case Report 2 also had severe changes in both elbows with one requiring surgical removal of free osteophytes. While her clinical improvement has improved her overall quality of life she has not responded as well as the tiger in Case Report 1. Etodolac has been reported to not only arrest the progression of osteoarthritis but also if instituted early enough actually reverse its clinical effects leaving only residual pathology (i.e., osteophytic changes).[5] Early diagnosis through a progressive preventive medicine program along with operant conditioning will allow early interventions and appropriate follow-ups to ensure the highest standard of care for captive wildlife.

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TICK TOXICITY IN ZOO ANIMALS IN EASTERN AUSTRALIA

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Abstract

Tick toxicity caused by the Australian paralysis tick, *Ixodes holocyclus*, which occurs along a coastal band of eastern Australia is reported in several exotic and native species held at Taronga Zoo, Sydney, Australia. The toxin causes a progressive paralysis which may be fatal and in domestic dogs is usually accompanied by cardiopulmonary dysfunction and esophageal weakness or paralysis. In the affected zoo animals, neuromuscular weakness has been the predominant feature of toxicity, but despite this cardiopulmonary function should be assessed in every case. Affected animals are treated with an antiserum containing concentrated gamma globulins prepared by hyperimmunizing dogs. Live ticks should be removed and antiserum should be administered as early as possible in the course of the disease to reduce the risk of fatality and minimize the recovery time. Antisera reactions occur in domestic animals, so animals should be premedicated with a combination of corticosteroids, atropine and/or adrenaline to reduce the risk of such reactions. The availability of preventive acaricidal preparations for zoo animals effective against *I. holocyclus* are limited.

Introduction

Tick toxicity is well documented in Australia with the vast majority of cases caused by *Ixodes holocyclus*. The tick occurs in a narrow coastal band from northern Australia to Lakes Entrance in Victoria. There are several genera of ticks worldwide that have been associated with paralysis, but the Australian paralysis tick (*I. holocyclus*) causes a progressive paralysis which may be fatal if untreated. Although native fauna, particularly the Peramelidae (bandicoots) and Phalangeroidea (possums) are the natural hosts of the tick, all warm-blooded animals are susceptible, including birds and humans. Monotremes and marsupials appear to be less susceptible to toxicity than eutherian mammals. Large numbers of domestic pets and livestock are affected annually but fatality is most common in dogs and cats.

The toxin causes an ascending flaccid lower motor neurone paralysis due to the blocked transmission of acetylcholine at the neuromuscular junctions.[4] In dogs this is often accompanied or preceded by dysphonia and/or vomiting and/or regurgitation and/or gagging and retching, associated with esophageal weakness or paralysis.[6] Pulmonary congestion and oedema are a common feature of toxicity in domestic animals.[2] Respiratory dysfunction in tick toxicity cases is complex and has been poorly understood although, recent studies have shown that the toxin is cardiotoxic.[3] The speed of onset of signs and rate of progression is extremely variable being influenced by tick, host and environmental factors.

Case Reports

Case 1: Tropical dingo, *Canis familiaris dingo*
A 3-yr-old female dingo presented with rapid respiration, moist lung sounds, excessive salivation and profound neuromuscular weakness causing an inability to stand. Acetylpromazine (ACP 2, Delvet, Asquith, N.S.W., Australia) was used for sedation and an *Ixodes* tick was removed from her left shoulder. The animal was given dexamethasone (Dexapent, Troy Laboratories, Smithfield, N.S.W., Australia) i.v. and then treated with tick antiserum (AVSL *Ixodes holocyclus* antivenom, Australian Veterinary Serum Laboratories, Lismore, N.S.W., Australia) 0.56 ml/kg i.v., frusemide (Frusemide injection, Troy Laboratories, Smithfield, N.S.W., Australia) s.c. and given supportive care. The pulmonary oedema worsened over the next 24 hr and a second dose of antiserum 0.3 ml/kg i.v. was administered, along with more frusemide and continued supportive care. Improvement of the pulmonary oedema began within 2 days. She was lifting her head with some voluntary movement within 3 days but was not able to walk until 8 days post-treatment. She recovered successfully.

Case 2: Binturong, *Arctictis binturong*
An 11-yr-old male binturong presented with an ataxic hindlimb gait and was retching frothy fluid. He was anesthetized and an *Ixodes* tick was removed from the head. Dexamethasone and atropine (Atrosite injection, Troy Laboratories, Smithfield, N.S.W., Australia) were given i.v., followed by the administration of tick antiserum 0.5 ml/kg i.v., topical application of fipronil spray (Frontline Spray, Merial Australia, Parramatta, N.S.W., Australia) and supportive care provided. Improvement was seen the following day and recovery was complete in 4 days.

Another episode of tick toxicity occurred in this animal 16 mo later. On this occasion the binturong presented with a crouching gait. Anaesthesia was induced and an *Ixodes* tick was removed from the right axilla. The animal was premedicated with dexamethasone i.v., acetylpromazine s.c. and atropine s.c. prior to the administration of tick antiserum 1.4 ml/kg i.v. and 0.1 ml s.c. injected at the tick attachment site. He was sprayed with fipronil and provided with supportive care until complete recovery 5 days later.

Case 3: Ring-tailed lemur, *Lemur catta*
A 7-yr-old male lemur presented with ataxia and miosis and an *Ixodes* tick was removed from the sternal area. The lemur was premedicated with dexamethasone i.v. and atropine i.v. prior to the administration of antiserum 0.6 ml/kg i.v. and 0.5 ml s.c. injected at the tick attachment site. The animal was much improved the following day and fully recovered in 3 days.

Case 4: Malayan sun bear, *Helarctos malayanus malayanus*
A 3-yr-old female sun bear presented with lethargy and inappetence of 48 hr duration. The animal was anesthetized, examined and hospitalized with no diagnosis reached. The following day it was noted that the animal appeared to exhibit mild paresis and was reluctant to walk. The animal was anesthetized again and a tick search performed, but none were found. Despite this, tick toxicity was suspected. She was premedicated with dexamethasone i.v., tick antiserum 0.6 ml/kg i.v. was

administered, fipronil was applied topically (Frontline Top Spot, Merial Australia, Parramatta, N.S.W., Australia) and supportive care was given. There was no evidence of pulmonary oedema. Improvement was noted the following day and she was more ambulatory. Recovery was complete 3 days post-treatment.

Ten months later, the sun bear suffered a second episode of tick toxicity, presenting with lethargy, mild hindlimb ataxia and coughing. She was anesthetized and an *Ixodes* tick was removed from the ventral thorax. Dexamethasone i.v. and acetylpromazine s.c. were given and then tick antiserum 0.65 ml/kg i.v. and 1 ml s.c. at the tick attachment site were administered. Topical fipronil was applied. The animal was fully recovered in 2 days.

A third episode of tick toxicity in this animal occurred 1 mo later. The sun bear was found crawling on her ventrum around the enclosure. She was anesthetized and an *Ixodes* tick was removed from the right axilla. Antiserum 0.5 ml/kg i.v. and 0.5 ml s.c. at the tick attachment site were administered after premedication with dexamethasone i.v., atropine s.c. and acetylpromazine s.c.. It was noted that the heart rate increased from 50 bpm to 150 bpm when the antiserum was administered i.v., respiration became more erratic and vomiting occurred. Oxygen saturation of hemoglobin decreased from 98% to 60% 10 min later. Intermittent positive pressure ventilation with oxygen was commenced and continued for 10 min until the respiratory rate had settled and oxygen saturation improved. The animal was awake 7 min later. Despite premedication with dexamethasone and atropine prior to the administration of tick antiserum to reduce the risk of an antiserum reaction, it was suspected that an antiserum reaction had occurred. Respiratory arrest occurred 2 hr after recovery from the general anaesthetic and resuscitation was unsuccessful.

At necropsy it was revealed that there was massive pulmonary congestion and alveolar hemorrhage. This was combined with subendocardial hemorrhage which appeared to be due to increased permeability of the microvasculature, as there was no obvious vasculitis or hemorrhage surrounding large blood vessels.

Case 5: Red Panda, *Ailurus fulgens fulgens*
A 7-yr-old male red panda presented with moderate ataxia, predominantly in the hind quarters. Under general anaesthesia, an *Ixodes* tick was removed from the face. The animal was premedicated with dexamethasone i.m. prior to the administration of tick antiserum 1 ml/kg i.v.. Fipronil was applied topically and supportive care given. Gradual improvement of the ataxia occurred over the next few days with apparent neuromuscular recovery in 4 days. However, the appetite and demeanor of the animal remained poor. Two weeks later the animal was hospitalized again due to inappetence and lethargy. Over a 5-wk period there was gradual clinical deterioration and extensive diagnostic tests were performed. There was evidence of decompensated renal failure, potentially exacerbated by the episode of tick toxicity. It was suspected that the animal was in compensated renal failure at the time of tick attachment. A renal biopsy revealed chronic interstitial nephropathy and moderate to severe membranous glomerulopathy. The animal was euthanatized.

Case 6: Squirrel monkey, *Saimiri boliviensis*
A 14-yr-old male squirrel monkey was found weak and unable to stand with an *Ixodes* tick visible on the neck. The animal was anesthetized, the tick removed and the monkey was premedicated with dexamethasone i.v. and atropine i.v. prior to being treated with tick antiserum 2.5 ml/kg i.v.. Fipronil was applied topically and supportive care given. No pulmonary edema was detected on clinical examination or radiographs. The animal recovered fully in 2 days.

Case 7: Fennec fox, *Fennecus zerda*
A 4-yr-old male fennec fox presented in recumbency, was unable to stand and no withdrawal reflexes were exhibited in all four limbs. The animal was still able to lift his head. He was anesthetized and an *Ixodes* tick was removed from the left thorax. Dexamethasone i.v. and atropine i.v. were given prior to the administration of antiserum 0.7 ml/kg i.v. and fipronil spray was applied topically. No pulmonary oedema was evident on clinical examination or radiographs. The neuromuscular weakness progressed and a few hours later the animal was not able to lift his head. A second dose of antiserum 0.7 ml/kg i.v. was given 3 hr after the initial dose. The following day the animal was sitting up on his sternum with some hindlimb movement. It took 8 days for this animal to be able to stand and 10 days until the animal exhibited a normal gait. Considerable supportive care was necessary during this recovery phase, including intravenous fluids and expressing the bladder.

Case 8: Red collared lorikeet, *Trichoglossus haematodus rubritorquis*
An 11-yr-old male lorikeet presented with flaccid paralysis and hyperpnoea. Two *Ixodes* ticks were removed from the head of the bird. Dexamethasone i.v. was given prior to treatment with tick antiserum 0.5 ml/kg i.v. and 0.02 ml s.c. at the tick attachment site. Five hours later, the bird received a further 0.5 ml/kg antiserum i.v. The lorikeet improved considerably within 24 hr and was fully recovered in 3 days.

Discussion

In the last few years, tick toxicity has become a significant concern at Taronga Zoo, Sydney, Australia. In addition to the reported cases, there have been suspected cases in a fishing cat, another squirrel monkey and other birds, of which none received treatment. The majority of cases have presented with varying degrees of neuromuscular weakness. In dogs, pulmonary congestion and oedema commonly occur due to the cardiotoxic effects of the toxin causing reversible myocardial depression.[3] For the majority of dogs that die, death is due to progressive pulmonary dysfunction rather than progression of paralysis.[3,5] Cardiopulmonary effects have only been noted in the tropical dingo. In the fatal episode in the Malayan sun bear, severe pulmonary congestion and alveolar hemorrhage were evident at necropsy however, it is unknown if this was a direct result of the tick toxicity or an antiserum reaction. Although pulmonary congestion and oedema have not been commonly detected in the zoo animal cases, cardiopulmonary function should be assessed in every case. The use of diuretics and arteriodilating drugs would be beneficial in cases of pulmonary oedema and in severe cases ventilatory support may be required.

Esophageal muscle weakness or paralysis occurs in most cases of tick toxicity in dogs.[6] A poor gag reflex, saliva pooling, megaesophagus and aspiration pneumonia are often noted in dogs.[6] Again, this has not been specifically diagnosed in any of the zoo cases. The sun bear presented with coughing during it's second episode of toxicity and the binturong presented with retching of frothy fluid during it's first episode. It is possible that these clinical signs were related to some degree of oesophageal weakness.

Treatment protocols are currently based on those for domestic dogs and cats, as there have been no studies in other species. Few documented experiments based on therapy exist. Following further research and a National Tick Paralysis Survey in dogs completed in 1998, there have recently been new recommendations for the management of tick toxicity.[5] Animals are treated with an antiserum which contains concentrated antitoxic gamma globulins prepared by hyperimmunizing dogs against the potent toxin produced by *I. holocyclus*. Little is known about ideal dose rates for the antiserum and its pharmacokinetic properties. However, the antiserum should be given as early as possible in the disease, as there is a delay of 8-12 hr before signs of reversal of paralysis are seen, during which time the animal tends to clinically deteriorate.[6] The suggested intravenous dose rate of antiserum in dogs is 1 ml/kg, but a range of 0.1-8 ml/kg has been used, adjusted according to the severity of clinical signs.[5] It is important to give a sufficient dose initially as supplementary doses seem far less effective with prolonged recovery times. Antiserum has most commonly been administered at a dose rate of 0.5 - 1.0 ml/kg in the cases reported in this paper. Most of the affected zoo animals have survived, but perhaps their recovery time may be reduced by using slightly higher doses.

Most zoo animals will require anaesthesia for assessment, diagnosis and treatment. However, all forms of stress or anxiety should be avoided prior to treatment and during the recovery phase. Anxiolytic drugs such as phenothiazines, benzodiazepines and opioids can be used during the recovery phase to minimize stress.

Antisera reactions are a concern following administration. Most reactions in dogs are associated with the Bezold-Jarisch reflex which results in bradycardia and hypotension, rather than anaphylactic reactions. Atropine administered prior to the antiserum will prevent or reduce the chance of this reflex being activated.[1] Atropine also has the added benefit of reducing saliva pooling, but is contraindicated if an animal is hypertensive. Anaphylactic reactions are more likely to occur in zoo animals, as they do in domestic cats given that the antiserum is derived from dogs. It is not clear whether repeated treatment with the antiserum increases the risk of anaphylaxis. Dexamethasone has been routinely used in an attempt to prevent or minimize anaphylactic reactions to the antiserum however, adrenaline is the drug of choice for preventing and treating anaphylactic reactions. Thus, anaphylactic reactions may be better prevented by the prior administration of subcutaneous adrenaline.[6] Corticosteroids will not prevent Type 1 anaphylactic reactions, but may moderate some of the consequences.

Live ticks should be removed from an affected animal. In the past it was considered important to kill the tick before removing it to prevent further toxin release during removal. The National Tick Paralysis Survey in dogs in 1998 indicated that recovery times were significantly lower in cases

where live ticks were removed at the time of treatment, however, mortality rates were similar whether the tick was removed alive or killed in situ before removal.[5] It has also been a routine measure to inject a small volume of antiserum subcutaneously at the tick attachment site in an attempt to bind the toxin before it circulates. The same survey indicated that this procedure has no bearing on the clinical outcome.[5] The antiserum administered intravenously will "mop up" existing unbound toxin and will neutralize any new toxin released from the tick attachment site.

As vegetation and natural substrates are provided in most exhibits and the zoo is surrounded by bushland, it is difficult to control ticks in the environment. Unfortunately, preventive acaricidal preparations that are effective against *I. holocyclus* which can be readily applied to zoo animals are limited. The most suitable preparations available in Australia for use in zoo animals are fipronil and cythioate (Proban tablets or liquid, Boehringer Ingelheim, North Ryde, N.S.W., Australia). The fipronil requires topical application every 2 wk and the cythioate is given orally every second day. At Taronga Zoo the sun bears, red pandas and binturongs are trained for fipronil application, the fennec foxes receive cythioate and the dingos are searched daily for ticks during the tick season (September-May).

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CHRONIC SEVERE ULCERATIVE MYCOTIC VENTRICULITIS CAUSED BY *Candida krusei* IN A YELLOW-FRONTED AMAZON PARROT (*Amazona ochrocephala*)

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Abstract

The carcass of a 0.301 kg male yellow-fronted Amazon (*Amazona ochrocephala*)was submitted for necropsy because the bird had died under "suspicious" circumstances. On gross postmortem examination, there was profound emaciation with marked pectoral muscle wasting. The prominent findings were in the coelomic cavity. There was displacement of the viscera to the right side from abnormal nodular to myxoid thickening over the serosal surface of the ventriculus. The liver was pale and mottled with necrotic margins, and merged with a caseous mass that was adherent to the ventriculus. Histopathology revealed marked inflammation in the ventriculus, extending transmurally from the submucosa through the layers of the muscularis, as well as focal extensive ulceration of the mucosa. This ulcer was coated with a necrosuppurative exudate in which were numerous and conspicuous yeast and bacterial colonies. Fungal elements were also identified in the liver and were associated with various degree of necrosis and inflammation. Histopathologically, fungal elements were consistent morphologically with *Candida* sp., presumed to be *C. albicans*. However, culture from tissue and from caseum yielded *Candida krusei*, which has not previously been reported as causing candidiasis in birds. *Candida krusei* was identified based on morphologic and biochemical features.[2,11] Bacteriologic culture additionally revealed a heavy growth of *E. coli*, and *Enterococcus* sp., presumably secondary to septic coelomitis from the perforating ulcer.

Candida krusei is a pathogen of increasing concern in humans[7,8] in which it causes invasive candidiasis.[3,4,7,8]Controversy exists in medicine over the use of fluconazole in immunocompromised individuals with fungal disease, as it may predispose them to infection with the more aggressive, fluconazole-resistant *C. krusei*.[3,4,7,8] There is strong evidence that biochemical properties of *C. krusei* result in poor to limited efficacy of fluconazole and some other azoles.[1,5,9,12,13]

Candida krusei has been documented sporadically in other animals.[6,10] Infections with *C. krusei* in birds may be more common than this single case report suggests. Candidiasis in birds is often diagnosed through cytologic examination of specimens such as crop wash or fecal smears, and gastric candidiasis may also only be disclosed through histopathologic examination of formalin-fixed tissues. In either case, the yeast is usually assumed to be *Candida albicans* and rarely is cultured from specimens or tissues. This report suggests that speciation of the causative yeast may have substantial clinical significance, especially if systemic disease is suspected and fluconazole is being considered as a therapeutic option. In addition, *Candida krusei* infections in birds may constitute a more serious zoonotic threat to immunocompromised individuals than *C. albicans*.

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OCULAR FINDINGS IN A COLONY OF MOUSE LEMURS (*Microcebus murinus*) FROM THE PARIS ZOO

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Abstract

Introduction

Grey mouse lemurs are found throughout the dry deciduous forests and spiny deserts of the south and west cost of Madagascar. This is a primarily insectivorous species**,** which also consumes fruits, flowers, leaves and small vertebrates. Grey mouse lemurs are nocturnal, solitary foragers who congregate at daytime sleeping sites. Their lifetime expectancy is thought to be around 6 yr in the wild, 10 yr in captivity.

Because of the general concern about the space of habitat destruction in Madagascar, all species of *Microcebus* are listed as endangered by USDI and are on CITES appendix I. The mouse lemurs are still relatively common and may be the only Madagascan lemurs not undergoing a general decline.

Recently, scientists' interest for mouse lemurs grew because *Microcebus* have cerebral lesions with ageing which are similar to lesions found in Alzheimer patients in humans. First mouse lemurs arrived in Paris zoo in 1990. Our collection is reaching now 71 individuals, most of them are off public. Some of the older specimens are part of a behavioral study to evaluate their cerebral ageing. Among the tests they undergo, is a visual discrimination test.[1] In 1998, more and more specimens could not be tested because of visual deficiency, and an increasing number of cases of cataracts was observed even on relatively young animals. Thus, we decided to perform an ocular examination on all the animals in order to determine the incidence of cataract in the colony, describe the type of cataract, its progression and attempt to identify its possible cause.

Methods

Over a 1-yr period, 75 mouse lemurs (45 males, 30 females) between 7 mo and more than 10 yr of age, underwent an ocular examination. Seven and 36 of them, respectively, were re-examined 5 mo and 13 mo after the initial examination. Ocular examinations were performed under subdued illumination. Pupillary light reflexes (direct and indirect) were assessed with a Finoff transilluminator. Slit-lamp biomicroscopy (Kowa SL-2) examination was performed before and after pupil dilation using one drop of 1 % tropicamide (Mydriaticum 1 %, MSD laboratory). Fundoscopic examination was performed with an indirect ophthalmoscope (Heine EN20) and a 28 D lens.

Observations for each animal were recorded on a separate form. Results of ocular histopathologic findings were available for five animals that died during that period.

Results

Ocular lesions were observed in 45 lemurs. These lesions included cataracts (36/45), keratitis (4/45), corneal dystrophy (2/45), corectopia (1/45), chorioretinitis scars (1/45) and phthysis bulbi (1/45). Blindness due to cataract or to total opacification of the cornea was seen in 14 cases.

Cataracts were predominantly bilateral (34/36) and all stages of progression were observed (21/36 incipient, 7/36 immature, 4/36 mature and 4/36 hypermature). A majority of incipient cataracts (19/21) were posterior subcapsular opacities. Cataract-associated lesions were observed in 10 animals and included hyphema (one case), posterior synechiae (five cases), pupil seclusion (three cases), keratitis or corneal edema (three cases). Cataracts affected both males (n = 18) and females (n = 18) and were observed in animals as young as 3 yr and 10 mo old. Cataract incidence increased with time and more than 50 % of lemurs over 6 yr of age were diagnosed with cataract.

Discussion

Cataracts in this colony of mouse lemurs appeared to be acquired and slowly progressive. In 11 cases, no cataracts were observed at initial examination but were diagnosed subsequently. Initial opacities were predominantly localized, both clinically and histologically, to the posterior subcapsular area. Later stages included anterior and posterior subcapsular opacities progressing to nucleo-cortical cataracts.

No cases of uveitis in the absence of cataract were diagnosed, suggesting that, when present, the uveitis was a lens-induced inflammation.

Early age of onset observed in some lemurs, as well as lens morphology, tends to exclude that these may be senile cataracts. Possible causes of these cataracts include nutritional, hereditary and infectious disorders.

Conclusion

We report an important number of cases of acquired slowly progressive bilateral cataracts in a colony of mouse lemurs (*Microcebus murinus*). Further studies need to be addressed to determine the aetiology of this blindness causing disease.

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DIAPHRAGMATIC HERNIAS IN CAPTIVE CHEETAHS (*Acinonyx jubatus*)

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Abstract

A 7-yr-old, male castrated cheetah (*Acinonyx jubatus*) at White Oak Conservation Center, Yulee, FL USA and a 9-yr-old, male intact cheetah at the Central Florida Zoo, Sanford, FL USA were diagnosed with hiatal hernias. Both cats exhibited clinical signs of intermittent inappetence, vomiting, and regurgitation. On thoracic survey radiographs of both cheetahs, a soft tissue density in the caudal thorax was seen at the location of the esophageal hiatus. Endoscopic examination of both cheetah showed pooling of gastric fluid in the distal esophagus, esophagitis, and bulging of the gastric mucosa through the esophageal hiatus. In one cheetah, medical therapy with omeprazole was unsuccessful in reducing the incidence of regurgitation. Consequently the cheetah was surgically treated by closure of the esophageal hiatus, esophagopexy, and gastropexy. Following surgery, the regurgitation ceased except for a brief period (5 days; 7 wk after surgery). The cheetah died suddenly 12 wk after the surgery. Necropsy revealed that the gastropexy had stretched and allowed the stomach, the proximal duodenum, the spleen, and a large amount of omentum to become entrapped in the thorax. The stomach filled with gas causing compression of the lungs and heart. The second cheetah was treated medically with success for 7 mo using omeprazole. The cheetah was euthanatized at that time following development of clinical signs consistent with leukoencephalomalacia. Necropsy of this cheetah showed a portion of the liver was damaged from being entrapped in the hernia, but the distal esophagitis had resolved. The two cheetahs in this report did not share a common ancestor within the previous five generations, according to the international Cheetah SSP studbook. Forty-two of 68 institutions with captive cheetahs responded to an e-mail survey and the results revealed a prevalence of 0.81% (2/247 cheetahs) for this abnormality. Both cheetahs had gastritis associated with *Helicobacter*, but it is unknown what influence this had on the formation of their hiatal hernias.

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COCCIDIOIDOMYCOSIS IN PRZEWALSKI'S HORSES (*Equus caballus przewalskii)*

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Abstract

Infection with the saprophytic fungus *Coccidioides immitis* typically results in subclinical or self-limiting infections with granulomas in the lungs and local lymph nodes.[3] The Zoological Society of San Diego maintains a herd of Przewalski's horses at the Wild Animal Park that are part of the global captive breeding population. Because of an apparent high prevalence of coccidioidomycosis in this herd,[2] necropsy records and slides from Przewalski's horses (n = 30, 15 males, 15 females) greater than 1 mo of age that died between 1984 and 2000 were reviewed. Coccidioidomycosis was the single leading cause of death in this population resulting in the death of 33% of these horses. Affected horses tended to be younger (average age 2.5 yr) than unaffected animals (average age 13.1 yr) at the time of death and males were over- represented (eight males, two females). Affected animals were housed in multiple enclosures. Although the founder base for the Przewalski's horse is limited, multiple lineages appear to be affected and in most cases, the dams, sires, and half siblings of affected animals were not affected. Lesions in affected horses included chronic granulomas in the lungs and tracheobronchial lymph nodes of all animals with variable involvement of the brain, spinal cord, liver, kidney, skeletal muscle, spleen and other regional lymph nodes.

To determine if this high prevalence was the result of species predisposition or environmental factors, necropsy records from other species of equids housed at the Wild Animal Park that had died during this same time period were also reviewed. During this same time period, only two individual animals from populations of Somali wild ass (*Equus africanus somalicus*) (n = 5), Grevy's zebra (*Equus grevyi*) (n = 19), Transcaspian kulan (*Equus hemionus kulan*) (n = 15), Persian onager (*Equus hemionus onager*) (n = 31), and Eastern kiang (*Equus kiang holdereri*) (n = 6) had pulmonary granulomas due to infection with *C. immitis*. No equids other than the Przewalski's horses were diagnosed with systemic coccidioidomycosis.

Because systemic coccidioidomycosis rarely occurs in domestic animals with adequate cell-mediated immune function,[1,3,4] studies are ongoing to evaluate the immune function of Przewalski's horses. The number and distribution of $CD21^+$ B cells and $CD3^+$ T cells including CD4 (helper) and CD8 (cytotoxic) T cell subsets were quantified in circulating blood of Przewalski's horses (n = 4) using flow cytometry. Preliminary analysis suggests that lymphocyte profiles in Przewalski's horses are similar to those in domestic horses. Studies are ongoing to evaluate immune function and specific responses to *C. immitis* antigens of Przewalski's horses in comparison to domestic horses.

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FATAL *Nocardia* sp. MENINGITIS AND SPINAL ABSCESS IN A FOUR-YEAR-OLD LOWLAND GORILLA (*Gorilla gorilla*)

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Abstract

In late January of 2000 a 4-yr-old Lowland gorilla from the Calgary Zoo's troop exhibited lethargy, mild fever, one episode of vomiting and mild pain when swallowing. These signs responded well to non-steroidal anti-inflammatory drugs (NSAID), and were consistent with "flu-like" signs observed in the rest of the troop intermittently throughout the winter. In early March, the animal was again somewhat lethargic with a decreased appetite and, head-holding behavior, as before the signs were responsive to NSAID. On March 9th, the animal was separated from the troop and anesthetized with medetomidine and ketamine; physical exam and radiographs of skull and chest were normal. Blood cultures (aerobic and anaerobic) were negative, rectal culture revealed normal flora, and a palpebral TB test was non reactive. Results of the CBC and serum chemistries indicated a mild anemia and mildly elevated C reactive protein (indicator of inflammation). Mild signs persisted for several days including an occasional hunched posture and stiff neck. Urinalysis and a test for fecal occult blood were carried out. Both were normal. On March 15th, a developing strabismus of the left eye was noted and during a second immobilization a full body computer tomography scan including a contrast study of the CNS, chest radiographs, blood chemistry screen and counts and a repeat blood culture were carried out. C reactive protein remained mildly elevated, all other results were within normal limits and no changes were seen on the CT scan. A human viral panel for respiratory viruses showed no significant exposure. Tests for rheumatoid factor, anti-nuclear antibody and evidence of streptococcal disease (ASOT) were negative. The gorilla was placed on azithromycin therapy orally for 2 wk; while he did seem to improve over the next week, occasional head holding, strabismus and a tendency to hold his neck stiffly continued.

The gorilla's condition was stable for about 2 wk following antibiotic therapy; during this time he was observed to be irritable and somewhat lethargic. On April 16th the animal was markedly depressed, lying in a curled position and wincing his face at regular intervals. At this time he was anesthetized for chest radiographs, thoracic and abdominal ultrasound examination, blood culture and lumbar CSF collection. The CSF obtained was yellow tinged and very mildly clouded. Protein was elevated, as was the white cell count (60% lymphocytes, 22% neutrophils), glucose was lower than normal. Aerobic and anaerobic bacterial cultures, viral culture, mycobacterial culture, fungal culture and fungal and acid fast staining of CSF were all negative; volume collected was insufficient for *Herpes* virus PCR. Serology for *Toxoplasma*, and several non-respiratory human viruses was negative for exposure. Viral culture of feces was also negative. Therapy with ceftriaxone, a third generation cephalosporin was instituted. Clinical improvement was fairly rapid and injections continued for a 21-day treatment. After 9 days of treatment (on April 25th) a second CT scan

revealed no focal lesions in the CNS, although there was slight increases in ventricle size noted by comparison to the March 16th scan. At the end of treatment (on May 8th), a magnetic resonance imaging scan (MRI) was performed of head and spine to rule out focal lesions such as a paraspinal abscess. This procedure revealed an increased signal at the ventral surface of the brainstem, and a mild increase in the size of the ventricles, compatible with a resolving meningitis and an associated mild bout of communicating hydrocephalus. CBC and serum chemistry values, as well as C reactive protein were all within normal limits.

For the next 5 wk, the gorilla's behavior and appetite were normal. He gained weight and resumed his position within the troop as the dominant young animal. Some subtle changes in attitude were noted in early June, as well as a stiff neck and mild muscle wasting of the cervical spine. He had a mild facial sweat and slept more often over the next week. On June 13th, the animal was immobilized. Physical exam was normal, CSF collected was clear with a mild elevation in protein and normal cell counts. CBC was normal however there was still an elevated C reactive protein. Bacterial and fungal culture of CSF, as well as acid fast staining and *Herpes* virus PCR were all negative. Rectal culture revealed normal flora, a repeat viral culture of stool was also negative. Urinalysis was normal, a second palpebral intradermal tuberculosis test was non reactive. Ceftriaxone was administered for 4 days until all negative results were obtained. During the following week, the young gorilla exhibited normal behavior some days and was somewhat quiet and sleepy on other days. Decreased hand-eye coordination and a developing weakness in the right arm prompted a second MRI exam on June 29th. This procedure showed the same increased signal in the ventral brainstem, no change in ventricle size, and two new small cystic lesions in the cervical spine. There was little increased signal associated with these signals, and the combined opinions of pediatric neurologists, a pediatric neurosurgeon and radiologists indicated these were most likely syrinxes formed in response to the chronic low grade communicating hydrocephalus. Other ruleouts included transverse myelopathy, infectious myelitis and spinal abscesses. Serology for *Brucella* sp., *Borrelia* sp. and *Cryptococcus* was negative. CSF had normal color and cell counts. There were no organisms on gram stain, no fungal elements or yeast were seen, and there was no cryptococcal antigen detected. Stereotactic biopsy of the spinal lesions was considered, but not pursued due to the difficulty in accessing this expertise and in providing post-surgical care. In humans, spinal abscesses are rare and on this basis conservative medical treatment was chosen: ceftriaxone i.m., high dose dexamethasone p.o. and ranitidine p.o. were instituted, with immediate response. All neurologic signs disappeared within 48 hr, and the animal had very good attitude and appetite.

On July 12th, the antibiotic was changed to oral chloramphenicol, and the steroid dose was being tapered. On July 19th, MR imaging indicated shrinkage of the cystic lesions in the spine. Clinically, the animal was doing quite well, though occasional tremors and facial sweats persisted. On August 9th, near the end of planned dexamethasone therapy, he took a turn for the worse with a return of right arm weakness, lethargy and a horizontal nystagmus. He responded positively for several hours after an increased dose of dexamethasone, and an MRI exam was set-up for the following day. On August 10th, he was responsive at 7 a.m. but deteriorated to a comatose state by 10 a.m. Dr. Mark Hamilton, a pediatric neurosurgeon, placed an emergency ventriculoperitoneal shunt surgically. CSF seen at surgery was clear and initially at high pressure (though not measured). An MRI that

afternoon indicated multiple focal brain lesions. Despite intensive care over the next 36 hr, the gorilla's condition continued to deteriorate, and when spontaneous breathing began to fail, euthanasia was elected on August, 12th 2000.

Necropsy revealed some chronic pneumonic lesions as well as subacute multifocal pulmonary necrosis caused by *Aspergillus* sp. The acute multifocal necrotizing meningoencephalitis was also caused by *Aspergillus* sp. The chronic spinal lesions were abscesses and contained organisms identified tentatively in histologic preparations as *Nocardia asteroides*. Immunohistochemistry is pending for final identification. Retrospective fungal serology on samples from March, April and May were negative for *Aspergillus* (several species) and a number of other fungal agents. *Nocardia* is the assumed pathogen for the protracted illness in this animal.

While there are many cases of *Nocardia* infection in the human literature, it is considered a rare pathogen, responsible for less than 2% of cerebral abscesses. Nocardial spinal abscesses are considered extremely rare with only four cases reported.[2] Early biopsy and aggressive surgical reduction are considered appropriate when neurologic nocardial lesions are identified. Delays in obtaining a biopsy have been related to the accumulation of non-specific or unhelpful results.[1] Mortality in human cases of cerebral nocardiosis can be as high as 80%, often related to difficulties in identifying an organism and associated with late or inappropriate therapy.[3] Human cases of nocardiosis are often, though not exclusively associated with immunosuppression or immunoincompetence. Preliminary investigations reveal no exposure of this animal to HIV or simian foamy virus.

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USE OF ORAL TILETAMINE-ZOLAZEPAM FOR SEDATION AND TRANSLOCATION OF CAPTIVE YELLOWTAIL JACKS (*Seriola lalandi*)

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Abstract

A population of sub-adult yellowtail jacks, acquired from the wild in early 1998, was housed in a three-story, 539,600 L (142,000 gallon) natural seawater exhibit at the Aquarium of the Pacific, along with other temperate teleost and cartilaginous fishes. Diet consisted of pieces of capelin, herring, squid hand-fed twice daily by volunteer divers. By June 2000, several jacks had developed superficial abrasions of the rostrum and flanks and/or mild kyphosis or scoliosis. Lesions were attributed to limited tank width (approximately 25 m), and plans were made to translocate 15 adult fish into a holding tank for ultimate transfer to another aquarium's significantly larger habitat.

To minimize stress and skin abrasions during transport, sedation or anesthesia of fish is recommended.[1,4-6] Attempts to remove the jacks (approximately 6-9 kg each) from the exhibit using ketamine i.m. (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA 50501 USA), delivered via modified Hawaiian sling, had limited success. The jacks proved difficult for staff divers to isolate and dart safely underwater, and a recommended dosage range of 66 - 88 mg/kg ketamine i.m.[2] was likely never reached due to volume limitations per dart.

Oral sedation was considered preferable in terms of safety and ability to deliver doses to the jacks individually. Published reports of successful oral sedation of large teleost fish are scarce.[4] Our initial trials using a variety of sedatives and anesthetics injected into food pieces and hand-fed to the jacks were ineffective; the fish apparently detected an abnormal taste or texture and immediately regurgitated medicated food.

Tiletamine-zolazepam (Telazol, Fort Dodge Animal Health, Fort Dodge, IA 50501 USA) has proven efficacy in multiple species and a rapid onset of action, particularly at low doses.[3] To our knowledge, there are no published reports of sedation of teleost fish using tiletamine-zolazepam. We used Telazol in its dry form for oral sedation of 14 yellowtail jacks. First, total powdered drug in each 500 mg vial of tiletamine-zolazepam was weighed and mg of active drug per mg of powder calculated (usually 0.5 mg active drug/mg powdered drug). Powder equivalent to 35 mg of active drug was measured and placed in a gelatin capsule. A dosage range of 8-9 mg/kg p.o., or an average of 70 mg per yellowtail jack, yielded the best results overall.

Capsules were placed behind the gills of a thawed capelin and immediately fed to individual jacks by staff divers within the exhibit. Attempts were made to identify individual fish swallowing medicated food and to note time of ingestion. Restricting intake of food to only the yellowtail jacks was challenging for divers; therefore, it took six procedures to remove 14 fish. One yellowtail jack

never took medicated feed and thus remained on exhibit.

Within 6-8 hr of ingestion, fish receiving an intended 70-mg dose began to show signs of sedation consisting of dark coloration, altered swim patterns, and loss of righting ability. Divers approached affected fish, trapped them in a hand-held net, and moved them to the surface. Although obviously sedated, fish remained reactive to touch, especially around the peduncular region. During acclimation to the holding tank, jacks were restrained within a canvas stretcher. Blood samples for complete blood counts, serum chemistries, and serum banking were collected from the vertebral vein using a lateral approach. Due to net-induced skin abrasions, all jacks received broad-spectrum antibiotics i.m. before transfer to a 30,400 L (8,000 gal) round holding tank.

Maximum sedative effect of oral tiletamine-zolazepam was 8-12 hr. Recovery from sedation, defined as return of normal swimming patterns and equilibrium, ranged from 12-48 hr. All fish refused to eat while in holding for 15 days. Skin lesions due to netting and contact with the tank sides were treated with nitrofurazone immersion (2 mg/L, renewed every 72 hr, for a duration of 7 days).

Four (28.6%) of the yellowtail jacks died during oral sedation procedures. Husbandry staff observed or suspected these four fish of taking additional doses of medicated food for estimated total doses of 140 or 160 mg (an average 15 - 20 mg/kg p.o. per fish). One of the four fish (25%) had a seizure-like episode and was found dead on the bottom of the tank shortly after a medicated feed session, and the other three fish (75%) failed to regain normal equilibrium post-procedure, sustained traumatic skin abrasions, and died from septicemia secondary to severe integumentary compromise. Liver, spleen, kidney, and serum samples from all four fish were banked for future analysis.

Sedation and translocation was successful in 10 out of 14 (71.4%) yellowtail jacks at our facility using oral tiletamine-zolazepam at a dosage of 8-9 mg/kg.

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PLASMA BUPIVACAINE LEVELS IN MALLARD DUCKS (*Anas platyrhynchos*) FOLLOWING A SINGLE SUBCUTANEOUS DOSE

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Abstract

Birds may be more sensitive to the toxic effects of local anesthetics compared to mammals but the pharmacokinetics of bupivacaine are unknown in birds. The purpose of this investigation was to determine the fate of bupivacaine following a single subcutaneous injection. Bupivacaine (2 mg/kg of a 0.5% solution) was given subcutaneously to eight adult female mallard ducks. Blood samples of 1.0 ml were drawn from the jugular vein prior to bupivacaine administration (0 hr) and at 0.25, 0.5, 1, 2, 3, 4, 6, and 12 hr after administration. Samples were assayed by high performance liquid chromatography and pharmacokinetic parameters were calculated from data plotted to the best-fit curve for samples from 15 to 180 min. The absorption time ($T_{1/2abs}$ = 13.9 min) appeared to be shorter than the elimination time ($T_{1/2elim}$ = 28.1 min) which may, in part, explain why birds are susceptible to toxic effects of local anesthetics. Maximal plasma concentration (C_{max}) of 0.08 ± 0.06 μg/ml was observed (T_{max}) at 0.52 ± 0.01 hr, but high plasma levels were evident at 6 and 12 hr (0.061 and 0.074 μg/ml, respectively). The appearance of subsequent high levels of plasma bupivacaine is unique to this study and may also contribute to the apparent avian sensitivity to toxic effects of local anesthetics.

Introduction

Local anesthetics, such as lidocaine and bupivacaine, function by blocking ion channels thereby preventing impulse conduction of pain.[6] In avian species, local anesthetics are used for operative and/or post-operative pain relief.[14] In domestic chickens, bupivacaine has produced effective analgesia in two pain models.[9,11] Birds may be more sensitive to the toxic effects of local anesthetics compared to mammals, as higher doses (3.5 - 4.5 mg/kg)[15] produce toxic effects in dogs compared with birds (2.7 - 3.3 mg/kg).[11] However, pharmocokinetics of local anesthetics in avian species are unknown. The purpose of this investigation was to determine the fate of bupivacaine following a single subcutaneous injection.

Materials and methods

Eight adult female mallard ducks, F2 progeny of wild strain adults, weighing 1060 ± 10 g, were used for this study. Ducks were given bupivacaine (2 mg/kg, 0.4 ml/kg of a 0.5% solution, Marcaine, Sanofi Winthrop, Chatham, Ontario, Canada) subcutaneously in the ventral abdomen, between 0900 and 1100 hr. Blood samples of 1.0 ml were drawn into heparinized syringes before drug administration (0 hr) from the jugular vein and at 0.25, 0.5, 1, 2, 3, 4, 6, and 12 hr after

administration. Samples were centrifuged and frozen at -20 °C until analyses were preformed. Concentrations of bupivacaine in plasma were determined by high-performance liquid chromatography.[10]

Pharmacokinetic modelling was performed using the computer program WIN-NONLIN, version 1.1 (Statistical Consultant Inc. Lexington KY, USA). Standard pharmacokinetic parameters were calculated from data plotted to the best fit curve for samples from 15 to 180 min according to a one compartment first order, first order elimination model. Area under the curve (AUC), 0 to 3 hr was computed by trapezoidal rule. All pharmacokinetic parameters were calculated for each animal and data are reported as mean ± standard deviation.

Results

One duck was excluded from the analysis as results were below detection limit of the assay from 15 to 180 min. The remaining data only fitted the model for up to 180 min. For this period the harmonic means for absorption half-life ($T_{1/2abs}$) was 13.9 min and elimination half-life ($T_{1/2elim}$) was 28.1 min. Subcutaneous administration gave an $AUC_{(0\,to\,180\,min)}$ of 0.14 ± 0.08 µg/h/L with a maximal plasma concentration (C_{max}) of 0.08 ± 0.06 µg/ml which was observed (T_{max}) at 31.6 ± 6.5 min. High levels were also evident in five of eight ducks at 360 min (0.061 ± 0.05 µg/ml) and in six of eight ducks at 720 min (and 0.074 ± 0.04 µg/ml) The plasma concentration vs. time distribution is shown in Fig 1.

Discussion

Metabolism of local anesthetics is very important because their toxicity depends largely on the balance between their rate of absorption and elimination.[5] In this study the absorption rate was slower than the elimination rate, which may, in part, explain why birds are more sensitive to toxic effects of local anesthetics compared with mammals. Chickens receiving high doses of intra-articular bupivacaine (2.7 - 3.3 mg/kg) had evidence of toxicity (recumbency, drowsiness, and signs of distress immediately after injection).[11] In dogs, plasma levels of 2.8 µg/ml are associated with increased QRS duration and conduction time,[8] but plasma levels as low as 1.5-2.3 µg/ml, in humans can cause dizziness and drowsiness.[7] Plasma levels required for clinical signs of toxicity in birds are unknown, but in order to avoid any serious side effects 2 mg/kg was chosen for this study based on a previous study in chickens.[11] Plasma levels attained in the current study were well below levels required for toxicity in other species.

It is difficult to estimate the activity of local anesthetics from blood concentration as the amount of drug present at the site determines local anesthetic efficacy. Bupivacaine is considered to have long duration of action in comparison to other local anesthetics. When used as infiltration anesthesia, it provides approximately 2.5 to 6 hr of analgesia in dogs.[15] Although it is difficult to compare studies with differing methodologies, a 15 min i.v. infusion of 3.4 mg/kg bupivacaine in dogs had an elimination half-life of 39.1 ± 13.3 min.[2] The length of action of bupivacaine in mallard ducks is possibly shorter as $T_{1/2elim}$ (28 min) is rapid; however, further studies are needed to confirm this.

In mammals, local anesthetics are distributed throughout all body tissues but the relative concentration in different tissues varies. Although skeletal muscle does not show any particular affinity for local anesthetics, the highest percentage of an injected dose is found in skeletal muscle because it is the largest reservoir.[1] Local anesthetics are rapidly extracted by lung tissue, as extravascular pH of the lung is low relative to plasma pH and results in ion-trapping of local anesthetics.[12] This first-pass lung uptake represents drug distribution not drug clearance.[12] It is unknown if the avian lung is capable of similar extraction. The pH of arterial blood in chickens is 7.42 - 7.52[13] indicating that birds are possibly more susceptible to ion trapping in the lung than mammals. However, uptake by the lung may be counteracted by greater plasma binding that occurs at higher blood pH values.[1]

In this study, C_{max} of 0.08 ± 0.06 was observed at 0.52 ± 0.01 h but high levels were also evident at 6 and 12 hr. The appearance of these high levels of plasma bupivacaine 6 and 12 hr after administration has not been recorded in other species. Pharmacokinetics of bupivacaine can depend on time of administration and, as it is highly protein bound, it may be influenced by circadian variation in protein binding.[3] In birds, the lung and pectoral and gastrocnemius muscles have relatively low perfusion rates at rest but during exercise, blood flow to these tissues can increase by three to five times.[4,16] It is possible that periods of activity resulting in increased blood flow through the muscle and lung, with concurrent pH changes, may produce intermittent redistribution of bupivacaine. This may also contribute to avian sensitivity to local anesthetics and suggest that delayed toxicity may be possible.

This study suggests that bupivacaine may be shorter acting in ducks than it is in mammals. A shorter absorption time, as compared to elimination time, may in part, explain avian sensitivity to local anesthetics, however, more information on drug distribution is required to draw concrete conclusions. In addition, sequestration and redistribution of bupivacaine may result in a delayed toxicity but mechanisms are unknown. Pharmacokinetics may contribute to the sensitivity of avian species to local anesthetics but other possible mechanisms could be involved. The avian blood brain barrier is not as complex as that of mammals[17] and this may allow for higher concentrations of local anesthetic in the brain. More studies are necessary to elucidate the mechanism of the toxicity of local anesthetics in avian species.

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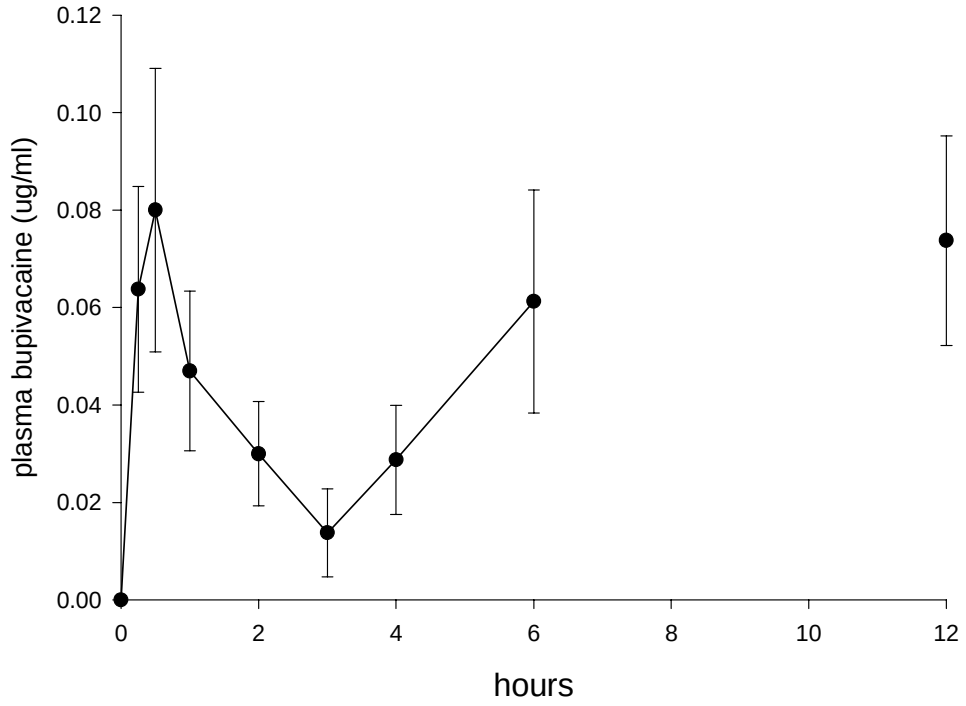

Figure 1. Plasma concentration of bupivacaine (µg/ml) following subcutaneous injection of 2 mg/kg of a 0.5% solution in female mallard ducks. Six and 12 hr samples represent high levels in plasma bupivacaine concentration.

MORTALITY OF MALE SPECTACLED EIDERS (*Somateria fischeri*) AND KING EIDERS (*Somateria spectabilis*) GIVEN PROPOFOL, BUPIVACAINE, AND KETOPROFEN

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Abstract

Twenty free-ranging spectacled eiders (*Somateria fischeri*) and 11 free-ranging king eiders (*Somateria spectabilis*) were anesthetized with propofol for the surgical implantation of satellite transmitters. For analgesia, bupivacaine at 2-10 mg/kg was infused into the incision site and ketoprofen at 2-5 mg/kg was given intramuscularly at the time of surgery. Four of 10 male spectacled eiders and five of six male king eiders died 1-4 days after surgery. None of 10 female spectacled eiders and only one of five female king eiders died during the same post-operative period. None of 20 female common eiders (*Somateria mollissima*) anesthetized with propofol, bupivacaine, and propofol and none of 20 female common eiders anesthetized with isoflurane and given ketoprofen (2-5 mg/kg) died within 4 days of release. Significant histopathologic findings from two dead male king eiders were severe renal tubular necrosis, acute rhabdomyolysis, and mild visceral gout. Gross necropsy findings in three additional dead male king eiders were consistent with visceral gout. Propofol has been used as an anesthetic in other avian species without problem, and it is unlikely that it is unsafe to use in male eiders. We strongly suspect that the perioperative use of ketoprofen caused lethal renal damage, but bupivacaine toxicity or cumulative toxicity of ketoprofen and bupivacaine may also have occurred. Male eiders may have been more susceptible to renal damage than females because of behavioral differences they exhibit during their short stay on land. The combination of propofol, bupivacaine, and ketoprofen should not be used to anesthetize free-ranging male eiders. Propofol alone or isoflurane should be used. The perioperative use of non-steroidal anti-inflammatory drugs should be avoided in any bird that may be predisposed to renal insufficiency.

COMPARISON OF THE ANESTHETIC AND CARDIORESPIRATORY EFFECTS OF MEDETOMIDINE-KETAMINE, MEDETOMIDINE-BUTORPHANOL-KETAMINE, AND MEDETOMIDINE-BUTORPHANOL-MIDAZOLAM IN RING-TAILED LEMURS (*Lemur catta*)

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Abstract

Ring-tailed lemurs, *Lemur catta*, are prosimian primates endemic only to the island of Madagascar. Due to an accelerating rate of natural habitat destruction, they are currently listed as vulnerable to extinction by the World Conservation Union (IUCN).[8] Approximately 1,000 captive ring-tailed lemurs worldwide are currently registered with the International Species Identification System (ISIS).[8,11] Scientific studies and health management practices aimed at conservation of this threatened species often necessitate the use of anesthetic agents for restraint. Unfortunately, information on the use of anesthetics in lemurs is scarce and mostly limited to brief mention of agents used for field capture, without reference to effectiveness or physiologic response.[2,5,6] Very high doses of ketamine (30 –-35 mg/kg) or tiletamine:zolazepam (20 mg/kg) appear to be necessary for rapid immobilization of lemurs in the wild.[5] At these concentrations 3-5-hr recovery times are to be expected (K.E. Glander, personal communication).

The purpose of our study was to evaluate three anesthetic drug combinations as potential replacements for ketamine and tiletamine:zolazepam use in captive and free-ranging lemurs. All three combinations include the potent alpha$_2$-adrenergic receptor agonist, medetomidine, an effective sedative-analgesic agent commonly used in a variety of species.[1,9] It is not, however, a complete anesthetic, and therefore must be combined with other drugs to potentiate its effects. Two combinations that have been evaluated extensively in carnivores[9,12] and, to some extent, non-prosimian primates,[7] are medetomidine-ketamine and medetomidine-butorphanol-ketamine. Medetomidine has been shown to significantly reduce the dosage requirement for ketamine in many species, including great apes,[7] while the inclusion of butorphanol in the mixture has been shown to reduce the dosage requirement for medetomidine.[12] The combination, medetomidine-butorphanol-midazolam, eliminates the need for ketamine, and in dogs, has been shown to create an anesthetic state resembling that provided by inhalation agents.[13]

Twenty-three captive adult ring-tailed lemurs (ages 3-15 yr) undergoing routine annual exams at the Duke University Primate Center were used in the study. Animals were randomly assigned to one of three groups: 1) MK (n = 6), medetomidine (Domitor, Pfizer Animal Health, Exton, PA 19341), (40 or 60 µg/kg) three animals at each dose, + ketamine (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA 50501) (3 mg/kg) with atipamezole (Antisedan, Pfizer) (0.2 mg/kg) reversal; 2) MBK (n=9), medetomidine (40 µg/kg) + butorphanol (Torbugesic, Fort Dodge Animal Health) (0.4

mg/kg) + ketamine (3 mg/kg) with atipamezole (0.2 mg/kg) and naloxone (Abbott Laboratories, Abbott Park, IL 60064) (0.02 mg/kg) reversal; and 3) MBMz, (n=8), medetomidine (40 µg/kg) + butorphanol (0.4 mg/kg) + midazolam (American Pharmaceutical Partners, Inc., Los Angeles, CA 90024) (0.3 mg/kg) with atipamezole (0.2 mg/kg), naloxone (0.02 mg/kg), and flumazenil (Romazicon, Roche Laboratories Inc., Nutley, NJ 07110) (0.02 mg/kg) reversal. Individual animals were netted and weighed prior to calculation of dosages. Drugs were mixed in the same syringe and injected into the cranial quadriceps muscle using manual restraint. The animals were then placed in transport kennels and allowed to sit in a darkened room for 10 min while the drugs took effect. Once immobilized, the animals were moved to an examination area for instrumentation.

Physiologic parameters were recorded every 5 min. Indirect mean arterial blood pressure (MAP) and heart rate (HR) were measured oscillometrically (Dinamap Model 8300, Critikon, 4710 Eisenhower Blvd., Tampa, FL 33614) with a size 2 cuff placed just above the tarsus over the anterior tibial or dorsal superficial pedal arteries. A lead II electrocardiogram was recorded continuously (Physio-Control Lifepak 6S Cardiac Monitor, Physio-Control Corp., Redmond, Washington 98052). Oxygen-hemoglobin saturation (SpO_2) was assessed by pulse oximetry (Vet/Oxä 4403, Heska Corp., 1801-A Airport Rd., Waukesha, WI 53188), and end-tidal CO_2 ($ETCO_2$) and respiratory rate (RR) were measured by sidestream capnography (VetCap 7100, Heska Corp.). A femoral arterial blood sample was taken 30 - 60 min post-induction for measurement of blood gases and electrolytes using a portable i-STAT clinical analyzer and EG7+ cartridges (Heska Corp.). The following parameters were measured: pH, pCO_2, pO_2, hematocrit, sodium, potassium, and ionized calcium. HCO_3 values were calculated. Animals received reversal agents after 50 min of consecutive measurements or when they became sufficiently alert that data collection was no longer possible, whichever came first.

All three anesthetic combinations produced heavy sedation within 3-5 min after injection and complete immobilization within 10 min. Their duration of effect, however, varied dramatically. Median working time (time beyond initial 10-min induction) was 6 min (range: 3-16) for the MK group, and 19 min (range: 16-47) for the MBK group. Within 5 min of being instrumented, five of six animals in the MK group had sufficient voluntary movement and chewing activity that procedures had to be terminated. They were also very rigid and roused more easily than those in the other two groups. MBK animals were relaxed and sedate enough to perform quick procedures within the first 10 min of working time; however, they began to recover quite rapidly shortly thereafter. In marked contrast, the median working time for the MBMz group was 50.5 min (range: 20-58). Six of the eight animals in the group exceeded 50 min, at which time they were reversed. These animals had excellent muscle relaxation and more than adequate sedation to perform complete physical exams and dental work over the 50 min of working time.

The cardiorespiratory effects of each of the three drug combinations were very similar. Median HR immediately after instrumentation (t = 0) was 154 beats/min (range 100-172) for the MK group, 133 beats/min (range: 88-148) for the MBK group, and 118 beats/min (range: 94-137) for the MBMz group. Median HR remained constant within groups throughout the procedures. No arrhythmias were observed in any of the animals. Median MAP values at t = 0 were 115 mmHg, 106 mmHg,

and 120 mm Hg for the MK, MBK, and MBMz groups, respectively. MAP decreased steadily in all three groups during the first 20-30 min before stabilizing around 70-90 mm Hg. Median RR at t = 0 was 58 breaths/min (range: 35-90), 50 breaths/min (range 36-76), and 49 breaths/min (range 25-59) in the MK, MKB, and MBMz groups, respectively. Median $ETCO_2$ at t = 0 was somewhat higher in the MBK group relative to the other two groups; 50 mm Hg (range 28-55) for the MBK group compared to 39 mm Hg (range: 37-44) for the MK group, and 40 mm Hg (range 32-46) for animals receiving MBMz. Median $ETCO_2$ values remained consistent within a given group throughout each procedure. SpO_2 values at t = 0 were 94% (range: 93-99), 95% (range: 88-100), and 96% (90-98) in the MK, MBK, and MBMz groups, respectively. Median values for each group remained within the 94 - 99% range at each time point throughout the procedures. PaO_2 values were within the range of 80-111 mm Hg, 57-109 mm Hg, and 67-91 mm Hg in the MK, MBK, and MBMz groups, respectively, in good agreement with predicted PaO_2 values based on SpO_2 readings. Measured PCV, Na^+, K^+, and Ca^{2+} values were similar among all three groups, ranging from 41 - 53%, 146-153 mmol/L, 3.3-4.6 mmol/L, and 0.99-1.23 mmol/L, respectively. Measured pH and HCO_3^- values were also similar among groups, ranging from 7.29-7.4 and 20-27 mmol/L, respectively.

All animals in each of the three groups were given reversal agents irrespective of their level of sedation. Atipamezole and naloxone were administered by intramuscular injection and flumazinil by intravenous injection. Significant differences in the quality of recovery were observed between animals in the three groups. Animals in both the MK and MKB groups appeared dazed, showed uncoordinated, jerky muscle movements, and salivated excessively for up to 45 min post-reversal. In contrast, animals in the MBMz group exhibited normal posture, coordination, and grooming behavior within 10 min of reversal.

From a physiologic standpoint, all three drug combinations may be considered safe to use, and recovery times for each combination were much shorter than those reported for ketamine and tiletamine: zolazepam in arboreal primates.[4,6,10] From a practical standpoint, however, only the MBMz combination can be considered a reasonable substitute, as it was the only combination that provided the working time typically needed to perform routine field procedures. The short duration of action of MK in lemurs relative to larger primates,[7] is likely explained by the fact that smaller primates have a higher metabolic rate.[3] The effectiveness of MBMz can be explained by the synergy of three different receptor agonists ($alpha_2$-adrenergic, opioid, and $GABA_A$) working together to provide controllable (and reversible) CNS depression. Further experiments are required to evaluate the effectiveness of MBMz in the field.

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THE USE OF KETAMINE AS A PRIMARY IMMOBILIZING AGENT IN GORILLAS (*Gorilla gorilla*)

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Abstract

Effective, safe, efficient induction of anesthesia is a common goal for clinicians handling free-ranging or captive gorillas (*Gorilla gorilla*). For many years, prior to development of other agents, the drug phencyclidine was the standard. This was followed by ketamine, then tiletamine-zolazepam, and then the combination medetomidine-ketamine.[1-3] There is a paucity of literature detailing anesthetic regimens in gorillas and the few studies that have been published are recent. No published compilation of data regarding the use of ketamine in gorillas is available.

In February 1999, 19 gorillas were moved to the new Congo Gorilla Forest exhibit at the Bronx Zoo. All animals, except for three animals less than 2 yr old, were chemically immobilized for relocation. Although tiletamine-zolazapam had previously been used frequently for gorilla immobilizations at the Bronx Zoo, ketamine HCl was selected as the primary immobilizing agent to accomplish the moves. The data presented includes all immobilizations in which ketamine was used for induction or as the sole anesthetic agent for gorillas at the Bronx Zoo for these relocations and for other procedures over a period of 9 yr.

Materials and Methods

Bronx Zoo gorilla immobilization records from 1991 to present were reviewed. Five male gorillas (age: 2-41 yr) were anesthetized a total of 12 times and 12 female gorillas (age: 2-37 yr) were anesthetized a total of 23 times, for an overall total of 35 immobilizations in which ketamine HCl (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA 50501 USA) was used as the anesthetic induction agent. The animals were usually darted using CO_2-powered projectiles containing ketamine. Atropine (Amvet Scientific, Yaphank, NY 11980 USA) was often included (n = 22) in the initial dart at a dosage rate of 0.04 ± 0.02 mg/kg Five 4-yr-old animals received 5 mg diazepam orally, 2 hr prior to ketamine being administered via hand injection. All animals subsequently received oxygen via nasal tube, facemask, or endotracheal tube. Isoflurane (Aerrane, Baxter Caribe Inc, Deerfield, IL 60015 USA) was administered via the same route, if needed, to complete procedures. Propofol (Gensia Sicor Pharmaceuticals, Irvine, CA 92618 USA) was used to maintain anesthesia in one animal. Supplemental i.v. ketamine was used for maintenance of anesthesia and to facilitate intubation as needed.

During the translocations, 13 animals underwent cardiac evaluation via transesophageal ultrasound, reproductive evaluation including transabdominal and transvaginal ultrasound, and dental examination and treatment. Several animals underwent field radiography. Procedures performed during other anesthesias included CAT scan, sinus surgery, arthroscopic surgery, laceration repairs, and medical evaluations of ill animals.

Monitoring included a combination of thoracic auscultation, pulse oximetry, peripheral non–invasive indirect blood pressure measurement, rectal temperatures, and/or end tidal capnography.

Results

Mean dosage for immobilization of all animals was 10.9 ± 3.0 mg/kg. For females (n = 23) the mean dosage was 11.1 ± 3.8 mg/kg; for males (n = 12) it was 10.6 ± 1.7mg/kg. For animals 2-9 yr (n = 18) the dosage was 10.0 ± 3.4 mg/kg, for 10-19 yr (n = 5) it was 14.3 ± 3.5, and for 20-41 yr (n = 12) it was 10.9 ± 2.0 mg/kg.

Time from the initial dose of ketamine to the first effect was 2.3 ± 2.0 min. Time from initial dose to light anesthesia was 9.9 ± 7.0 min. Mean heart rate, respiratory rate and mean arterial blood pressure with ketamine alone were 118 ± 21beats per minute, 25 ± 12 respirations per minute, and 125 ± 20 mm Hg, respectively.

Discussion

Ketamine was chosen as the immobilizing agent for these gorillas due to its history of safety in many species. In particular, the number of gorillas aged 20 yr and above, with unknown or uncertain cardiovascular function, was an impetus to try to avoid agents known to cause cardiac depression or prolonged hypertension. The use of an agent that preserves laryngeal reflexes afforded an extra measure of safety until animals could be intubated. Additionally, the availability of ketamine in a concentration of 200 mg/ml made injection of appropriate doses to large individuals via dart more feasible than in the past. The anesthesias in this survey were all rated excellent for induction and recovery. All animals were calm during recovery, displaying minimal anxiety and only short duration ataxia.

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IMMOBILIZATION OF RED WOLVES (*Canis rufus*) USING MEDETOMIDINE AND BUTORPHANOL

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Abstract

The red wolf (*Canis rufus*) is a critically endangered species that was extirpated from the southeastern United States.[5] Over the last 2 decades, the U.S. Fish and Wildlife Service (USFWS), in cooperation with other organizations, has conducted captive propagation and reintroduction of red wolves, in an effort to restore them to a portion of their former range. In eastern North Carolina, this effort has met with some success, however intensive management techniques are necessary for monitoring and maintaining the health of this red wolf population. Safe, effective, rapidly reversible immobilization protocols are essential for implementing these management techniques.

For several years, the USFWS has used a ketamine-xylazine combination for immobilizing captive and free-ranging red wolves; however, rough and prolonged recoveries been a concern with the use of this regime. In 1998, an investigation was conducted with captive red wolves, in order to determine the cardiopulmonary effects of xylazine-ketamine, medetomidine-ketamine, medetomidine-ketamine-acepromazine, and medetomidine-ketamine-butorphanol.[7] Smooth, quick inductions were achieved, but the investigation also documented marked hypertension (diastolic arterial pressure (DAP) > 116 mm Hg)[6] with all four drug combinations. Recoveries using xylazine-ketamine were prolonged and several animals exhibited pronounced ataxia.[7]

The purpose of this investigation was to determine whether medetomidine and butorphanol would provide safe, effective, and reversible immobilization of red wolves without causing hypertension. This combination has been shown to induce profound sedation in domestic dogs and is fully reversible.[1] We characterized the sedative and cardiorespiratory effects of medetomidine-butorphanol in captive red wolves. Due to concerns that this combination may not provide immobilization of adequate depth or sufficient duration, two other protocols were also evaluated.

Twenty-four adult, captive red wolves were used in this investigation. Each subject was assigned to one of the following experimental groups:

MB = medetomidine (Domitor, Pfizer Animal Health, Exton, PA 19341 USA; 0.04 mg/kg, i.m.) + butorphanol (Torbugesic, Fort Dodge Animal Health, Fort Dodge, IA 50501 USA; 0.4 mg/kg, i.m.) with atipamizole (Antisedan, Pfizer Animal Health, Exton, PA 19341 USA; 0.2 mg/kg, i.m. 0.2 mg/kg, i.m.) + naloxone (Nalxone HCl Injection, Abbott laboratories, North Chicago, IL 60064 USA; 0.02 mg/kg, i.m 0.02 mg/kg, i.m.) reversal (n = 7);

MBD = medetomidine (0.04 mg/kg, i.m.) + butorphanol (0.4 mg/kg, i.m.), followed with diazepam (Valium, Hoffman- La Roche, Nutley, NJ 07110 USA; 0.2 mg/kg, i.v.) at t = 0, with atipamizole (0.2 mg/kg, i.m.) + naloxone (0.02 mg/kg, i.m.) + flumazenil (Romazicon, Hoffmann-La Roche Inc, Nutley, NJ 07110 USA; 1 mg., i.v. 0.04 mg/kg, i.v.) reversal (n = 9); and

MBK(30) = medetomidine (0.04 mg/kg, i.m.) + butorphanol (0.4 mg/kg, i.m.), followed with ketamine (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA 50501 USA; 2 mg/kg, i.v.) at t = 30, with atipamizole (0.2 mg/kg, i.m.) + naloxone (0.02 mg/kg, i.m.) reversal (n = 8).

The initial injection of medetomidine and butorphanol was made via i.m. hand-injection into the caudal hindlimb. Wolves were left as undisturbed as possible for 15 min, after which an initial blood pressure measurement was taken oscilometrically. Wolves were then transported to a central processing area where other initial measurements were taken (t = 0) including heart rate, respiratory rate, body temperature, end tidal carbon dioxide, and indirect hemoglobin saturation. These measurements were recorded at 10 min intervals. An initial arterial sample was taken (t = 0) from the femoral artery for blood gas analysis and repeat measurements were taken at t = 30 and t = 50 min. An i.v. catheter was placed and, in most wolves, small volumes (0-8 ml/kg) of lactated ringer's solution were administered over 30 min. In eight wolves, higher volumes (11-26 ml/kg) of cool fluids were administered as treatment for hyperthermia. Hyperthermic wolves were also treated with cold packs. At the end of each procedure, the catheter was removed, the wolf was transported to its recovery area, and atipamezole and naloxone were administered. Flumazenil was administered i.v. to MBD wolves before the catheter was removed.

Complete immobilization occurred within 15 min in 21 wolves and within 20 min for 23 wolves. One wolf was not completely immobilized until administration of diazepam. At t = 0, the wolves were heavily sedated and non-responsive to external stimuli with relaxed muscle tone. Wolves that received only MB generally appeared to be more lightly sedated than wolves that were administered additional agents. Four of 7 MB wolves and six of eight MBK(30) wolves were twitching and would occasionally lift their heads when stimulated by ear manipulation or dental scraping. MBK(30) wolves did not demonstrate this activity after ketamine was administered. No MBD wolves responded to manipulation, althoug one spontaneously recovered at t = 25 min.

Heart rates (HR) were similar between the three groups for the first 30 min of the procedure (Table 1). Heart rate decreased until t = 30 for all three groups. Three wolves (two MB and one MBD) had heart rates fall below 40 bpm. Median heart rate increased in the MBK(30) group after receiving ketamine and differed significantly from the MB and MBD groups at t = 40 and t = 50. Wolves

maintained normal cardiac sinus rhythm throughout the procedure, with the exception of one MB wolf, four MBD wolves, and one MBK(30) wolf that experienced periods of second-degree heart block. Respiratory rates were similar between the three groups at all time points, were regular, and did not show substantial change over time. Body temperatures were highest at t = 0, ranging from 37.6 - 42.2 °C, but declined steadily over time.

Systolic, mean, and diastolic arterial blood pressure values were elevated immediately following induction in all three groups (Table 1), but decreased over time. However, blood pressure increased in MBK(30) wolves following ketamine administration.

Median PaO_2 values were $\geq$ 70 mm Hg throughout the procedure, although 10 wolves had PaO_2 values < 70 mm Hg at t = 0. PaO_2 values increased in these latter animals at subsequent time points. Median S_aO_2 was $\geq$ 93% at all time points for all groups and median $PaCO_2$ values ranged from 25 - 50 mm Hg. Median arterial blood pH values were between 7.26 and 7.34.

Twenty-one wolves were fully recovered within 10 min of reversal agent administration. The remaining three wolves (one from each group) stood within 12 min, and appeared to have fully recovered by 17 min. MBK(30) wolves exhibited mild ataxia for 5-10 min. Median time to standing for all groups was 6 min and to full recovery was 7 min. Only one spontaneous recovery (one MBD wolf at t = 25 min) occurred.

Three wolves had periods of time when HR $\leq$ 40 beats per minute. In the subsequent year a trial was conducted to determine whether an anticholinergic could be administered with the initial injection, in order to ameliorate bradycardia without causing hypertension or tachycardia. Glycopyrrolate was the anticholinergic chosen as it less likely than atropine to cause tachyarrythmias. Wolves (n = 6) were immobilized with medetomidine and butorphanol in the same manner as the prior year. For the first four wolves, physiologic measurements were taken for 10 min after induction and then incremental doses of glycopyrrolate were administered through an i.v. catheter, in order to try to determine an appropriate dose that could be administered with the medetomidine and butorphanol. At t = 10 min, 0.0025 mg/kg glycopyrrolate was administered, then 0.005 mg/kg at t = 20 min, and 0.01 mg/kg at t = 30 min.

Median HR at t = 0 was 97 bpm and had dropped to 40 bpm by t = 10. Median HR was 44 bpm at t = 20, but increased to 93 bpm by t = 30. At t = 40, median HR increased to 143 bpm. Similar changes were observed in blood pressure. Median DAP was 121 mm Hg and 115 mm Hg at t = 0 and t = 10, respectively. Median DAP did not increase initially, with values of 121 mm Hg at t = 20 and 112 mm Hg at t = 30, but then increased to 143 mm Hg at t = 40.

An increase in HR was not observed until a dose of 0.005 mg/kg glycopyrrolate (total dose of 0.0075 mg/kg) had been administered, at which point HR increased moderately. When 0.01 mg/kg glycopyrrolate (total dose of 0.0175 mg/kg) was administered, there was profound tachycardia and pathologic hypertension. The glycopyrrolate appeared to have a threshold effect between 0.0075 and 0.0175 mg/kg. Therefore in the next wolf, a conservative dose of 0.008 mg/kg glycopyrrolate was

administered i.m. with medetomidine and butorphanol. Initial HR and blood pressure values for this animal were severely elevated at t = 0 (HR = 130 bpm and DAP = 158 mm Hg) and were sustained throughout the procedure. In light of this, the next animal was given a dose of 0.0025 mg/kg glycoypyrrolate i.m. with medeotmidine and butorphanol. While HR was not as dramatically elevated (HR = 90 bpm) at t = 0, this animal was severely hypertensive with SAP of 211 mm Hg and DAP of 160 mm Hg). This hyperension was sustained until the animal was reversed at t = 20 min. Because of the severe hypertension that was observed with low doses of i.m. anticholinergics, the anticholinergic study was discontinued.

Medetomidine-butorphanol provided good sedation and immobilization for handling and transport; minor procedures could be performed. The full effects of the MB combination lasted 30 – 40 min and the addition of either diazepam or ketamine prolonged the combination's effects and provided a deeper plane of sedation. However, wolves that received a low dose of ketamine had increased hypertension and post-reversal ataxia. The cardiorespiratory effects of the three drug protocols were similar, and were more favorable than for previously described combinations that included ketamine administration at the onset of immobilization. Blood pressure of red wolves anesthetized with MB and MBD were lower than those reported for wolves anesthetized with regimens that included ketamine.[2,4] All three protocols provided effective immobilization with minimal adverse effects, however the MB combination offered several advantages for short procedures, including complete reversibility (at any time), fewest drugs, and lowest cost. For longer procedures, MBD or MBK(30) could be used.

We cannot recommend the routine use of anticholinergics for preventing low HR in red wolves anesthetized with medetomidine and butorphanol. Tachycardia and hypertension were observed in animals that received glycopyrrolate and the use may create more problems than they solve. This is consistent with earlier reports of tachycardia and hypertension in medetomidine-induced dogs administered 0.04 mg/kg atropine i.m.[3] Tachycardia has also been noted in medetomidine-ketamine immobilized gray wolves that were administered 0.05 mg/kg atropine i.m.[2] Of course, clinican disgression may dictate that anticholinergics are necessary in certain situations. However in our experience, wolves with HR < 40 have generally shown no signs of physiologic distress, being normotensive, with pink mucous membranes, normal capillary refill times, and excellent recoveries. If, during an immobilization, it is perceived that a wolf with a low heart rate must be treated, it may be more appropriate to administer reversal agents and discontinue the procedure, rather than risk tachyarrythimas or hypertension that are likely to be caused by the administration of anticholinergics.

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Table 1. Median (and range) values for red wolves immobilized with medetomidine-butorphanol (MB), medetomidine-butorphanol-diazepam (MBD), and medetomidine-butrophanol with late supplemenation of ketamine (MBK30).

Time	MB		MBD		MBK30	
			Heart rate (beats per minutes)			
0	76	(40-102)	66	(42-114)	77	(44-108)
10	60	(40-79)	56	(45-96)	76	(40-86)
20	63	(38-80)	57	(49-84)	64	(48-82)
30	57	(32-64)	60	(48-70)	72	(46-80)
40	50	(38-82)	59	(36-84)	80[a]	(52-108)
50	56	(34-85)	51	(36-80)	88[a]	(52-94)
			Systolic blood pressure (mm Hg)			
0	166	(124-182)	150	(98-194)	129	(90-188)
10	148	(114-172)	166	(82-201)	156	(114-176)
20	150	(88-162)	160	(88-183)	142	(127-168)
30	146	(61-162)	143	(80-172)	143	(128-166)
40	141	(117-150)	143	(96-162)	158	(140-162)
50	138	(123-152)	135	(116-178)	160	(138-165)
			Diastolic blood pressure (mm Hg)			
0	124	(74-140)	124	(56-161)	114	(52-144)
10	114	(60-120)	120	(48-132)	112	(76-132)
20	106	(58-114)	114	(60-122)	100	(82-130)
30	104	(39-114)	107	(42-138)	107.5	(98-115)
40	101	(64-112)	94	(64-122)	117.5	(92-130)
50	97	(86-116)	105	(78-132)	110	(78-128)

[a]Statistically significant difference from other groups.

MULTIPLE ISOFLURANE ANESTHESIA IN A CAPTIVE BLACK RHINOCEROS (*Diceros bicornis*)

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Abstract

Inhalant anesthesia in rhinoceros has been reported on a couple of occasions. In all instances reported, euthanasia was performed[1] or the rhino expired in the perianesthetic period from surgical complications[2]. This report describes the successful maintenance and recovery of a black rhinoceros anesthetized twice for diagnostics evaluations of a nasal fracture and obstructive respiratory disease. A 4-yr-old male black rhinoceros weighing an estimated at 1000 kg was anesthetized twice and maintained on isoflurane and ventilated on both occasions for diagnostics evaluation of a fractured maxillary sinus. The animal was considered a high risk for anesthesia due to the known respiratory compromise from the fractured maxilla and obvious respiratory stridor and the possibility of pulmonary disease secondary to the trauma and resultant infection. On the first occasion, the rhino was premedicated with oral halperidol. This was followed by 40 mg detomidine and 20 mg butorphanol i.m. Induction was accomplished with 2 mg etorphine i.m. Standing immobilization occurred at this point and the animal was safe to approach. An additional 0.25 mg etorphine i.v. and 300 mg xylazine i.v. was utilized to facilitate sternal recumbency and intubation. A 28-mm endotracheal tube was placed with the aid of a 1.7-m colonoscope. Antagonism consisted of 125 mg of naltrexone given simultaneously i.v. and i.m. The rhino stood in 1 min but was slightly sedate for an additional 20 min.

Seventy-five days later the rhinoceros was again premedicated with 40 mg detomidine and 20 mg butorphanol i.m. followed by 2 mg etorphine i.m. Standing immobilization was again achieved and 1000 mg ketamine i.v. was used to induce recumbency and intubation. This time the rhinoceros went down in lateral recumbency. The respiratory rate dropped to 3/min before it was moved into sternal recumbency and intubated. Naltrexone was again given, 100 mg i.v. and i.m. plus 4000 mg of tolazaline i.m. and the rhinoceros stood within 5 min but was sedate for an additional 40 min.

On both anesthetic episodes respirations were assisted at 8-10 per minute with a 20-L bellow large animal ventilator. Isoflurane was kept between 2-3 % based on heart rate and rate and depth of spontaneous efforts to breathe and response to surgical or other procedural stimulation. Serial blood gas taken from an auricular artery, non-invasive blood pressure monitoring with the cuff on the base of the tail, oxygen saturation via pulse oximetry, and base apex electrocardiogram (ECG) monitoring were followed during the procedure when feasible. The rhino was maintained in sternal recumbency for the entire procedure. The elapsed time of isoflurane anesthesia for the first anesthesia was 172 min and 230 min for the second procedure. Recoveries were smooth and uncomplicated with the

animal exhibiting normal behaviors within 2 hr of each event.

For the first episode, body temperature was maintained at 100.9° F (38.3° C) for the duration of monitoring, heart rate averaged 44/min (range: 36-58), oxygen saturation averaged 92% (range: 100-84%). Blood pressures averaged 130/99/114 mm Hg and deviated very little during the procedure. Initial arterial blood gas taken prior to intubation revealed relative hypoxemia (PaO_2, 96 mm Hg) and mild hypercapnia ($PaCO_2$, 57.9 mm Hg). Once intubated and provided with ventilator support, both values essentially resolved with PaO_2 at 218 mm Hg and $PaCO_2$ at 44.8mg Hg. They remained near these levels for the duration of monitoring. Electrocardiogram tracing revealed a normal sinus rhythm.

For the second episode, body temperature was again maintained at 100.9° F (38.3° C) for the duration of monitoring. During the first 30 min following ketamine and while in lateral recumbency and not yet intubated, heart rates where elevated (average 57/bpm, range 49-76), respirations decreased to 3-4/min, oxygen saturations decreased (mean 83%, range 80-86%) while blood pressures where elevated (average 190/143/166). Compared to the first episode, initial arterial blood gas sampling obtained prior to intubation revealed hypoxemia (PaO_2, 72 mm Hg) and hypercapnia ($PaCO_2$, 44.2 mm Hg). Further sampling revealed an increase of PaO_2 (average 203 mm Hg, range 167-259) but the hypercapnia persisted and even worsened ($PaCO_2$, average 56 mm Hg, range 34.1-68.5). After this initial 30 min elapsed, heart rate returned to what was deemed a more acceptable level (average 43/bpm, range 35-52), blood pressures stabilized at an average of 136/79/104 mm Hg. Electrocardiogram tracing again revealed a normal sinus rhythm.

Several elements were key to the success of these anesthetic episodes. The first is that the rhino was tractable to the point of allowing the premedications to be given by hand. In fact, this animal had received 1 wk's worth of i.v. antibiotics prior to the second episode due to septicemia. The halperidol did not seem to affect the ability to allow this hand injection nor did it appear to enhance the overall quality of anesthesia so it was not included in the second procedure. It was essential to maintain the rhino in sternal recumbency to prevent a ventilation/perfusion mismatch (V/Q) on top of any pre-existing pulmonary compromise. The fact that the rhinoceros was in lateral recumbency combined with the intravenous ketamine may have combined to produce the prolonged hypercapnia seen in the second event. The V/Q mismatch may have been from the recumbency and produced an area of lung consolidation on the dependent lung that was not thoroughly expanded even with assisted ventilation. Venous admixture associated with lateral recumbency is well documented in horses and suspected in a white rhinoceros under isoflurane anesthesia[1]. Ketamine was chosen in the second instance based on personal preference and in an effort to avoid additional narcotics. In this case it may have been better to repeat the supplemental dosing as was done in the first episode based on the initial parameters collected following ketamine. Depth of anesthesia was judged primarily by depth and rate of spontaneous respirations and heart rate. Position of the eye was not useful in determining depth of anesthesia. Recoveries were quick and extremely smooth in both cases and in fact surprisingly fast in the first case. At no time during either event was there any muscle rigidity or hyperexcitability noted so common with ultra potent narcotics.

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HEALTH EVALUATION OF WILD SIBERIAN TIGERS (*Panthera tigris altaica*) AND AMUR LEOPARDS (*Panthera pardus orientalis)* IN THE RUSSIAN FAR EAST

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Abstract

From 1992 through 1997, 20 wild Siberian tigers (*Panthera tigris altaica*) and seven wild Amur leopards (*Panthera pardus orientalis*) were captured, darted, and immobilized 36 times and 10 times, respectively, for radio-telemetry studies in the Russian Far East.[2] Initially, all 27 animals were captured with Aldrich spring-activated foot snares (Margo Suppliers, High River, Alberta, Canada), then immobilized and radio-collared. Subsequently, six of the 20 radio-collared tigers were re-darted and immobilized from a helicopter for re-collaring.

Tigers and leopards were darted for immobilization using 3-ml plastic darts and 1.5 mm × 38 mm collared needles projected from a CO_2 powered rifle (Telinject USA, Saugus, CA 91350 USA). Both species were immobilized with an initial dose of 6.6 mg/kg ketamine (Fort Dodge Laboratories, Inc. Fort Dodge, IA 50501 USA) mixed with 0.66 mg/kg xylazine (Bayer Corporation, Shawnee Mission, KS 66201 USA).[1] Anesthesia was maintained with supplements of 1.1 mg/kg ketamine. Seizures during immobilization were observed 22 times in 15 tigers, and once in one leopard, and were managed with 0.07 – 0.2 mg/kg diazepam (Roche Pharmaceuticals, Nutley, NJ 07110 USA) i.v. Anesthesia was reversed in both species with 0.04 - 0.13 mg/kg yohimbine (Lloyds Laboratory, Shenandoah, IA 51601 USA). Blood and tissue samples were taken during each immobilization. Fecal samples were collected sporadically, and yielded no evidence of gastrointestinal parasites. Ticks (*Dermacentor* and *Ixodes* spp.) were found on six tigers and two leopards.

Packed cell volumes were determined in the field using microhematocrit samples spun in a bench top centrifuge. Serum samples were frozen, and transported to the United States for analysis using an automated analyzer (Hitachi 717 Automatic Analyzer, Roche Diagnostics, Indianapolis, IN 46250 USA). Serum chemistries for wild tigers were compared to results from captive tigers (Table 1). Sixteen of the 20 blood parameters tested between wild and captive tiger populations were significantly ($P \leq 0.05$) different. Serum chemistries for wild leopards were compared to results from captive leopards (Table 2). Eight of the 20 blood parameters tested between wild and captive leopard populations were significantly ($P \leq 0.05$) different.

Noticeable differences in hematocrit values between the wild and captive populations may be due to a number of things including the type of centrifuge used, centrifuge speed and cycle variations due to the use of a generator for wild samples, and environmental differences between captive and

wild cats. In particular this difference may reflect that most captures were done in the winter when there is limited water availability for the wild populations as compared to the captive population which always has water freely available.

Infectious disease serology tests were performed (Washington Area Disease and Diagnostic Laboratory, Pullman, WA 99165 USA) for feline leukemia virus antigen (ELISA), and antibodies to feline enteric Coronavirus/feline infectious peritonitis (FECV/FIP; IFA), feline immunodeficiency (ELISA), canine distemper virus (CDV; virus neutralization, and feline panleukopenia (IFA) viruses. Serology antibody tests for *Bartonella henselae* (Western Blot) were also performed (National Veterinary Laboratory, Inc., Franklin Lakes, NJ 07417 USA). Results are presented in Table 3.

These data supply the first baseline information on free-ranging animals for comparison to captive populations of two highly endangered felids. Contrasts in serum chemistry results, or seroprevelance of disease may be indicators of potential conservation threats from human impacts requiring further investigation. The antibody titers to CDV, FECV/FIP, *Bartonella henselae*, and panleukopenia detected in these cats may reflect the endemic presence of these viruses in the wild population, or may reflect exposure through predation on infected domestic animals. In either case these viruses may pose a significant threat to the wild populations,[2] and the presence of antibodies warrants further investigation.

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Table 1. Serum chemistry values for wild Siberian tigers in the Russian Far East, and captive tigers.

	Wild tigers					Captive tigers[a]					
	Mean	SD	Min	Max	*n*	Mean	SD	Min	Max	*n*	*P*
Glucose (mg/dl)	168	70.1	29	320	35	132	44	46	310	882	> 0.05
Creatinine (mg/dl)	2.1	0.8	0.9	4.2	35	2.7	0.9	0.6	5.2	867	> 0.05
BUN (mg/dl)	29.4	16.4	13	67	35	27	7	13	52	867	> 0.05
Uric Acid (mg/dl)	0.65	0.82	0	4.9	35	0.3	0.3	0	1.4	350	> 0.05
Total Protein (g/dl)	7.6	0.7	6.4	9.1	35	7	0.6	5.1	9.2	811	> 0.05
Albumin (g/dl)	4.2	0.5	2.2	4.9	35	3.7	0.4	2.4	5.2	725	> 0.05
Cholesterol (mg/dl)	191	41	130	261	35	228	52	0	391	843	> 0.05
Sodium (mEq/L)	153	3.8	141	160	35	150	3	138	163	835	< 0.05
Potassium (mEq/L)	4.4	1.4	3.2	12	35	4.2	0.4	3.3	6.3	838	< 0.05
Chloride (mEq/L)	116	4.2	102	124	35	120	4	103	133	819	> 0.05
Calcium (mg/dl)	9.6	0.7	8.4	11.9	35	10.1	0.6	8.4	12.2	865	> 0.05
Phosphorus (mg/dl)	7.9	10.6	2.8	10.7	35	5.8	1.3	3	10.4	852	> 0.05
T. Bilirubin (mg/dl)	0.36	0.3	0.1	1.4	35	0.2	0.1	0	0.8	790	> 0.05
AST (IU/L)	134	168	20	946	35	27	14	6	114	790	> 0.05
ALT (IU/L)	84	50	27	304	35	55	29	13	215	410	> 0.05
Alk. Phos. (IU/L)	48	47	0	182	35	40	41	0	217	821	< 0.05
LDH (IU/L)	899	1071	92	4550	35	264	237	17	1919	756	> 0.05
Amylase (U/L)	1199	314	765	1910	35	1364	1200	160	7847	89	< 0.05
T_4 (µg/dl)	1.1	0.6	0.4	2.5	35	2.6	1.5	0.5	6.3	13	> 0.05
HCT (%)	47	6.4	37	67	25	38.1	5.4	23.3	55	950	> 0.05

[a]MedArks, International Species Inventory System, Apple Valley, MN 55124 USA.

Table 2. Serum chemistry values for wild Amur leopards in the Russian Far East, and captive leopards.

	Wild leopards					Captive leopards[a]					
	Mean	SD	Min	Max	*n*	Mean	SD	Min	Max	*n*	*P*
Glucose (mg/dl)	102	48	51	166	6	126	41	57	276	242	< 0.05
Creatinine (mg/dl)	2.1	0.43	1.7	2.9	6	2.4	0.7	1	4.9	234	< 0.05
BUN (mg/dl)	36	10	20	47	6	33	9	16	76	243	< 0.05
Uric Acid (mg/dl)	0.6	0.4	0.4	1.3	6	0.2	0.2	0	0.7	142	< 0.05
Total Protein (g/dl)	8.6	0.4	8.2	9.2	6	7.4	0.7	5.5	9.6	229	> 0.05
Albumin (g/dl)	3.9	0.3	3.4	4.3	6	3.4	0.5	2.3	4.6	203	> 0.05
Cholesterol (mg/dl)	150	19	129	177	6	171	60	73	441	238	< 0.05
Sodium (mEq/L)	155	3.3	151	159	6	152	4	140	162	223	< 0.05
Potassium (mEq/L)	3.9	0.3	3.6	4.2	6	3.9	0.4	3	5.2	226	< 0.05
Chloride (mEq/L)	118	4.8	112	124	6	119	5	105	137	218	< 0.05
Calcium (mg/dl)	9.7	0.4	9.1	10.4	6	10	0.6	8.3	11.7	239	< 0.05
Phosphorus (mg/dl)	3.6	0.9	2.3	4.4	6	5.3	0.9	3	8.3	233	> 0.05
T. Bilirubin (mg/dl)	0.3	0.2	0.1	0.7	6	0.2	0.1	0	0.6	228	> 0.05
AST (IU/L)	197	136	61	300	6	41	26	11	205	236	> 0.05
ALT(IU/L)	69	16	51	84	6	52	37	11	205	179	< 0.05
Alk. Phos. (IU/L)	15	12	6	38	6	24	17	0	111	219	< 0.05
LDH (IU/L)	1716	1371	369	3888	6	144	139	29	1344	175	> 0.05
Amylase (U/L)	798	277	500	1062	6	1005	389	260	2850	58	< 0.05
T_4 (µg/dl)	1.1	0.6	0.5	1.3	6	2.7	0.7	1.3	3.8	25	> 0.05
HCT (%)	51.3	3.4	48	56	4	37	5.5	23.4	52	264	> 0.05

[a]MedArks, International Species Inventory System, Apple Valley, MN 55124 USA.

Table 3. Serologic tests performed on wild Siberian tigers and Amur leopards in the Russian Far East (number of positive animals and number of animals tested).

	Siberian tigers	Amur leopards
Feline leukemia antigen (ELISA)	0/18	0/6
Feline enteric coronavirus/feline infectious peritonitis antibodies (IFA)[a]	2/20	3/6
Feline immunodeficiency virus antibodies (ELISA)	0/20	0/6
Canine distemper virus antibodies (VN)[b]	0/20	2/6
Panleukopenia antibodies (IFA)	11/18	4/6
Bartonella henselae antibodies	0/17	2/5

[a]Immunofluorescent antibody test.
[b]Virus neutralization test.

SURVEY ON PRESENCE OF THE ENDOTHELIOTROPIC ELEPHANT HERPESVIRUS (EEHV) IN THAI CAMP ELEPHANTS

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Abstract

The outbreak of a fatal hemorrhagic disease in captive Asian elephants (*Elephas maximus*) in Europe[1] and the United States[2] has alarming consequences for the survival of not only the elephants of both species kept in zoos and animal parks but also for the survival of the already strongly reduced (locally endangered and diminishing) free-ranging wild populations, in particular of the Asian elephant.

Approximately 10 yr ago, more than 10 African elephants (*Loxondanta africana*) were imported to Thailand and kept together with Asian elephants in different zoos and safari parks all over the country. The African elephant is a potential source of the Herpesvirus lethal for Asian elephants. Today only one of these African elephants is still alive. This remaining individual is socialized with Asian elephants at the Korat Zoo. Currently, there are approx. 2300 captive Asian elephants and about 1,800 wild elephants in Thailand. The majority of the captive population is involved in tourism business as riding or performing elephants in traditional elephant festivals. The logging industry excluded elephants from work in 1990. A few of the former logging elephants are utilized as so called "begging elephants" and less than 100 elephants are kept in governmental zoos for educational purposes. In general, elephants play an important economic and cultural role in Thailand.

In March 2000 a joint project was started to investigate the possible presence of the endotheliotropic elephant Herpesvirus (EEHV) in the captive elephant population in Thailand. In addition, the analysis of the genetic diversity of the Thai elephants was initiated using the microsatellite-technique.

A team of veterinarians from the Institute for Zoo Biology and Wildlife Research, Berlin, the Wilhelma Zoological and Botanical Garden, Stuttgart, and the Mahidol University, Nakhonpathom collected different tissue samples and parasites of 83 elephants for histology, PCR analysis, and DNA sequencing in nine different locations. Twenty-one of these elephants had to be sedated by intravenous injection with 1-3 ml of 10% xylazine (Rompun TM, Bayer, Inc., Germany) to allow for diagnostic procedures, like ultrasound guided lymph node biopsies, or treatments, like foot care

or abscess lancing and flushing. Transrectal ultrasound investigations and semen collections were performed without sedation. All samples were immediately snap-frozen in liquid nitrogen and subsequently analyzed by PCR in Germany. To date, none of the elephants investigated was positive for EEHV. The project will be continued in the next years.

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ASSESSING THE POTENTIAL FOR DISEASE TRANSMISSION FROM AUSTRALIAN WHITE IBIS (*Threskiornis molucca)* TO HUMANS AND FOOD-PRODUCTION ANIMALS: A BEHAVIORAL AND EPIDEMIOLOGIC SURVEY

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Abstract

Over the last 15-20 yr, the population of Australian white ibis (*Threskiornis molucca)* on the Gold Coast of Queensland, Australia, has grown at an increasing rate, primarily due to the adaptation of these birds to urban scavenging. Access to suburban landfills has provided a virtually unlimited food resource for ibis in the southern coastal region of Queensland. With an increased presence of ibis in public recreational areas, local government and public health officials sought to determine the potential risk of transmission of zoonotic pathogens, such as S*almonella* spp., Japanese encephalitis (JE) virus, and avian influenza virus between ibis and people. Because ibis also frequent farms, the potential for transmission of diseases such as Newcastle disease, salmonellosis, or avian influenza between ibis and food production animals, such as chickens and cows, was considered as well. In order to assess the potential risk of disease transmission, a two-part study was conducted, coupling disease surveillance with a behavioral study focused on the interaction between ibis and people, and ibis and food production animals.

One hundred ibis were captured at a suburban landfill for the disease surveillance component of the study. Blood samples and choanal and cloacal swabs were collected. Cloacal swabs were cultured for S*almonella* spp. and blood serum was tested for antibodies to avian influenza, Newcastle disease virus, and generic flaviviruses, including JE virus, Murray Valley encephalitis (MVE), and Kunjin virus (KV). Fecal samples were cultured for *Salmonella* spp. and serotyped. Seven percent of the ibis samples grew S*almonella* spp., with the majority (4/7) growing *Salmonella virchow* (Table 1). Avian influenza (AI) antibody tests were performed using a competition ELISA. The seroprevalence of AI was 30.7% (27/88), 20.5% (18/88) weak positive, 10.2% (9/88) strong positive. Of sera tested 32.9% (29/88) were positive for NDV antibodies using the hemagglutination inhibition test. Of samples tested 2.3% (2/88) were positive for JE or other generic flavivirus antibodies using a competition ELISA test. The two positive samples were then tested using a plaque reduction neutralization test and one sample tested positive for Kunjin virus and negative for JE and Murray Valley encephalitis virus, while the second sample tested negative for all three viruses.

Disease surveillance was coupled with a behavioral study of ibis during daily feeding activity to assess the potential for disease transmission to people and food production animals. Four locations were selected for observations: a public park, a landfill, a tidal mudflat, and a poultry farm. Five

birds were randomly observed every 2 min for 5 hr over 4 days at each location (3000 bird observations per site). The park was chosen as a representative area where people and ibis frequently interact by means of people feeding ibis; ibis approaching people for food; and ibis having direct contact with food objects such as picnic tables, water fountains, and grills. Ibis may contaminate food objects with feces or mucosal secretions, which represents a potential means of transmission of zoonotic diseases. Young children may be at an increased risk of infection because they are more likely than adults to have contact with objects contaminated with bird feces and they require a smaller dose of pathogen to contract disease.[4] Playing in an area where bird droppings were observed has been associated with an increased risk of S*almonella* infection (OR = 10.5, P = 0.04).[2]

The public landfill represented a place where ibis aggregate in large numbers to feed. Because of the higher density of birds at landfills, there is a higher frequency of agonistic interactions between the birds (Table 2) relative to the other foraging sites included in this study. An increased level of interaction provides greater opportunity for communicable diseases to be amplified within an ibis population that feeds at a landfill. The tidal mudflat was included as a "wild-type" setting, where ibis feed and commingle at lower density, and forage for natural food such as invertebrates. At the mud flat, ibis interaction was less frequent than the other areas. A poultry farm was used for observation to assess the interaction between ibis and food production animals such as chickens (layers and broilers) and cows. Newcastle disease virus can be devastating to poultry operations, and as carriers of the virus, ibis may be a potential vector. Wild birds, in general, may be considered as a possible vector for transmitting salmonella to cows, chickens, and other farm animals.[3] On the contrary, cows and chickens may already carry salmonellosis, and salmonellas can survive for long periods of time in the environment (2 yr or more) in feces, dust, and litter, thus farms may be a potential source of S*almonella* infection for ibis.[1]

Although wild birds have frequently been implicated as reservoirs for human disease, human activity usually plays a part in facilitating the perpetuation of diseases in wild bird populations. One study that reported on gulls as vectors of *Salmonella* spp., demonstrated that gulls that feed at landfills or sewage treatment areas become infected with the same species of *Salmonella* found in humans.[5] While there have not yet been any reported associations between cases of S*almonella* infection in people and ibis in the Gold Coast region of Queensland (J. Bates, personal communication), exclusion of ibis from landfill sites would decrease the opportunity for ibis to obtain human pathogens from putrescible waste.

This report demonstrates that ibis may carry zoonotic pathogens, however, the potential for transmission of disease between ibis and humans can be greatly reduced through public education and simple management strategies such as not feeding ibis; adopting proper personal hygienic practices; and protecting food and water resources from contamination at public recreational areas. Similar precautions undertaken by farm managers may help reduce risk of disease transmission from ibis to domestic animals, since ibis may be involved in outbreaks of significant diseases such as avian influenza, Newcastle disease, or salmonellosis. Restriction of ibis from direct contact with chickens or chicken feces as well as livestock and their water resources may reduce the potential for

disease transmission between ibis and food production animals on farms.

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Table 1. *Salmonella* spp. cultured from cloacal swabs of free-ranging Australian white ibis.

Salmonella species	% ibis positive (n = 100)
S. birkenhead	1.0%
S. oranienburg	1.0%
S. typhimurium	1.0%
S. virchow	4.0%

Table 2. Comparative frequency data for agonistic behavior among Australian white ibis at different locations.[a]

Location	Bill-poke	Lunge with bill open	Lunge with bill closed	FS	Head bob with bill open	Head bob with bill closed	Any number of ibis competing for a single piece of food
Landfill	0.02	0.04	0.00	0.01	0.02	0.00	0.00
Park	0.00	0.01	0.01	0.01	0.00	0.00	0.02
Tidal mudflat	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Farm	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a]All behaviors were regarded as having been directed toward another conspecific.

HEALTH ASSESSMENTS OF FREE-RANGING RADIATED TORTOISES (*Geochelone radiata*) IN MADAGASCAR

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Abstract

Introduction

Establishing normative data for free-ranging animals is essential in order to make judgements on the effects of human activities, effects of captivity and especially when contemplating translocations, restocking or introductions of animals. Fauna in Madagascar overall is under extreme pressure from human activity. Herpetofauna is undergoing onslaught from professional collectors for the international pet trade and equally important for food to Eastern markets. Rather than habitat degradation, many populations of reptiles are disappearing for entry into the human food chain.

In March of 1998 personnel representing the Reptile Department, the Field Veterinary Program, and the Wildlife Health Clinical Care Department of the WCS, worked at Cap Sainte Marie in southern Madagascar. The objective was to assess the health status of free-ranging radiated tortoises (*Geochelone radiata*) and to develop a normative data base for them.

Methods

Cap Sainte Marie is an area located at the southernmost tip of Madagascar. Over a 2-day period 36 free-ranging radiated tortoises underwent health evaluations. Blood was collected from brachial blood vessels and processed using routine field methodologies.[2] Nasal samples (n = 15) were collected by flushing sterile water into each nostril and aspirating the residual fluid. Samples were placed into liquid nitrogen until being shipped on dry ice to the laboratory for mycoplasma PCR. Fresh feces from 13 animals were placed into tryptose-soy broth and frozen in liquid nitrogen for subsequent culture. Feces placed in formalin (n = 11) were processed using routine techniques for parasite ova determination (Dr. Ellis Greiner, Dept. of Pathobiology, CVM, U FL, Gainesville, FL 32610 USA) and immunoflorescent antibody (IFA) testing (Anilab, Baltimore, MD 21228 USA) for detection of *Cryptosporidium* spp. (n = 11).

Plasma and nasal flush samples were stored at -70° C and fixed blood smears remained in Madagascar until a CITES permit could be obtained and transport back to the United States arranged. Subsequently, plasma was analyzed for biochemicals (IDEXX Veterinary Services Inc. One IDEXX Drive, Westbrook, ME 04092 USA), minerals (Animal Health Diagnostic Laboratory,

P.O. Box 30076, Lansing, MI 48909 USA), and antibodies to *Mycoplasma* spp. via enzyme linked immunoassay (EIA) (Mycoplasma Research Lab, 1600 SW Archer Rd, Gainesville, FL 32610 USA), and antibodies to herpesvirus (EIA; Dr. Francesco Origgi, Dept. of SACS, CVM, U FL, Gainesville, FL 32610 USA).

Results

Hematology results are presented in Table 1. Hematocrit was only performed on five samples due to technical problems in the field and ranged from 20-24% (median = 22.0) Total solids ranged from 2.2-4.6 g/dl (median = 3.0). White blood cell counts were generally low, ranging from 296-8,712 cells/mm^3 (median = 1,760).

Plasma biochemicals and minerals are presented in Tables 1 and 2. Nasal flushes were all negative by PCR for *Mycoplasma* spp. The mycoplasma serologies were negative in 27/28 samples, with one being suspect. Herpesvirus EIA was positive in 3/6 (50%) tortoises.

Fecal analysis for parasites revealed pinworms in 11/11 (100%), *Eimeria* spp. in 2/11 (18%) and *Caryspora* sp. in 1/11 (9%) samples. IFA testing for *Cryptosporidea* sp. was positive for one of 11 (9%) samples Bacterial culturing of feces was performed on 13 specimens. Two animals (15%) were positive for *Salmonella* sp. serovar *kodjovi*.

Discussion

Overall the animals were in apparent good health. The hematologic and plasma biochemical data, when compared to published values for captive animals[3] were clinically unremarkable. Uric acid levels in this population were higher than captive animals which may be a reflection of the xeric nature of the habitat in which the wild-caught animals were captured.

The finding of *Eimeria* spp. in 18% of the animals may be significant. Intranuclear coccidia has been identified as a pathogen sometimes causing death in several species of chelonia.[1,2] It is possible that this protozoan may be the responsible organism. The finding of *Cryptosporidium* sp. is not unexpected and is consistent with findings in other free-ranging chelonians (unpublished data Raphael). The Salmonella serovar recovered from these animals is not known to be found in animals in the USA, and no serovars commonly found in captive animals in the USA were found in the free ranging animals.

Evidence of exposure to herpesvirus was unexpected as there were no signs of clinical disease on physical exam. The significance of this finding is unknown as there is limited information available on herpesvirus in free-ranging tortoises. The finding of one suspect positive mycoplasma serology sample with no positive PCRs and no clinically ill animals may indicate that the range of sensitivity of the test is not appropriate for this species or that the animal in question had very low titers to the organism.

The information gathered in this study should be considered baseline on a population that was relatively undisturbed by human perturbation. As such, it may be useful in future years as one prong for determination of the population health. The Cap Sainte Marie region is designated as protected, and there is presently a cooperative plan between WCS and Madagascar for establishing it as a National Park. Because of its remote location, the presence of government officers, and restricted access points it represents an opportunity to conserve and manage an intact patch of spiny desert and what appears to be extraordinary populations of radiated and spider tortoises, the landscape species of southern Madagascar.

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Table 1. Plasma biochemicals of free-ranging radiated tortoises (*Geochelone radiata*) (mean and range, *n* = 14).

Alk. Phosphatase (IU/L)	249.0 (6-354)
ALT (IU/L)	4 (0-4)
AST (IU/L)	109.0 (66-187)
CPK (IU/L)	1275.0 (261-329)
LDH (IU/L)	687.0 (242-960)
Albumin (g/dl)	1.5 (1-1.9)
Total protein (g/dl)	3.3 (2.7-4.3)
Globulin (g/dl)	1.8 (1.6-2.4)
BUN (mg/dl)	18.0 (2-56)
Cholesterol (mg/dl)	64.0 (60-140)
Glucose (mg/dl)	64.0 (47-93)
Calcium (mg/dl)	11.1 (9.0 -12.2)
Phosphorus (mg/dl)	3.5 (2.8 – 4.3)
Potassium (mEq/dl)	6.3 (5.7 – 11.0)
Sodium (mEq/dl)	146.0 (134 – 155)
A/G ratio	0.8 (0.6 – 1.1)
Uric acid (mg/dl)	0.9 (0.5 – 1.4)

Table 2. Plasma mineral concentrations of free-ranging radiated tortoises (mean and range, *n* = 13).

Calcium (ppm)	125.0 (78.6 – 155.0)
Phosphorus (ppm)	81.3 (62.7 – 114.0)
Ca/P	1.6 (1-2)
Copper (ppm)	0.359 (0.285 – 0.569)
Zinc (ppm)	2.84 (1.71 – 5.09)
Iron (ppm)	1.63 (0.42 – 3.03)
Potassium (ppm)	280 (243-401)
Magnesium (ppm)	45.3 (37 – 58.9)

MEDICAL MANAGEMENT OF CONFISCATED CHIMPANZEES (*Pan troglodytes*) IN UGANDA

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Abstract

Since 1997, the authors have worked together on developing a health care program for confiscated orphaned chimpanzees brought to the Uganda Wildlife Education Centre (UWEC) in Entebbe. The initial project in 1997 involved a chimpanzee health care workshop in which 13 chimpanzees were immobilized for physical examinations, dental work, vaccinations, tuberculin testing, and routine bloodwork. Participants included veterinarians involved with care of chimpanzees in Uganda as well as veterinarians affiliated with the Mountain Gorilla Veterinary Project in Rwanda and Uganda. Goals of the workshop were to provide health screening of UWEC chimpanzees before moving them to a new exhibit, and to provide hands-on experience with great ape immobilization and health care for Ugandan and Rwandan veterinarians.

In 1998, the second phase of this work was to move 23 chimpanzees from UWEC to Ngamba Island in Lake Victoria. The chimpanzees were released into a semi-free-ranging situation on the island. Prior to the move to this ecotourism site, 23 animals, including some from the original group, were immobilized for health examinations, dental work, tuberculin testing, vaccinations and bloodwork. Immobilization protocols using either tiletamine and zolazepam (Telazol,® Fort Dodge Animal Products, Fort Dodge, IA 50501 USA) or medetomidine (Orion Corp., Orion-Farmos, Expoo, Finland) & ketamine (Fort Dodge Animal Products) were used and arterial blood gases were compared between the two protocols by sampling all animals at initial immobilization time and every 10 min thereafter for 30 min.

Currently chimps are supplemented with food twice daily at one end of the island, which ensures that the animals emerge from the forest at these times and can be visualized by tourists standing on an observation platform. A holding area has been constructed in this area for new chimps being gradually introduced to the island group, as well as for temporary housing of animals needing medical attention.

In July, 2000, the authors collaborated with Drs. Jonathan Sleeman, Richard Wrangham, and Gladys Kalema in organizing a workshop for monitoring the health of wild chimpanzee populations at Kibale National Forest. Over 35 participants attended representing veterinary and park staff as well as behavioral researchers from Rwanda, Uganda, and Tanzania. Lectures and wet-labs were held discussing disease patterns among chimpanzee and human populations, utilization of urine and fecal samples, and necropsy protocols.

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HEALTH EDUCATION WORKSHOPS FOR LOCAL COMMUNITIES OF BWINDI IMPENETRABLE NATIONAL PARK, UGANDA

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Abstract

The local community surrounding Bwindi Impenetrable National Park has limited access to primary health care and practices inadequate personal hygiene. They come into close contact with mountain gorillas through tourism, research, and gorillas crop-raiding gardens surrounding the park. One of the habituated gorilla groups got scabies suspected to have come from the local community. To reduce the likelihood of mountain gorillas getting diseases from people, health education workshops on risks of disease transmission between humans and gorillas were presented for the first time to local communities bordering Bwindi to bring about a long-term improvement in local communities' health and hygiene. The workshops were conducted by veterinarians, wardens, and rangers from Uganda Wildlife Authority together with district health personnel, from January to May 2000.

Introduction

Bwindi Impenetrable National Park (BINP) is home to approximately half of the world's highly endangered mountain gorillas of an estimated number of 650, the others are found in the Virunga volcanoes in Rwanda, Democratic Republic of Congo (DRC) and Mgahinga National Park in Uganda.[5] A small part of Bwindi is in DRC. Bwindi Impenetrable National Park was gazetted in 1991,[1] which meant that people could only have access to the forest through controlled activities including tourism, research and multiple use, which includes access to forest products for honey, medicinal plants and basket weaving material. At this time, the level of poaching had been significantly reduced.[1] Parts of Bwindi are surrounded by fragmented patches of secondary forest owned by local people who contribute significantly to the long-term conservation of mountain gorillas.

The area surrounding Bwindi has one of the densest populations of people in Africa with an estimated 200 people per square km.[1] The local population around Bwindi is among the poorest in Uganda, a developing country, with a Gross Domestic Product per capita: purchasing power parity of $1060.[8] The nearest clinic to the local population is at least 20 km away for most people, who also lack basic hygienic amenities including clean water and pit latrines.[11] This has resulted in a large percentage of people having preventable diseases that can potentially be spread to gorillas, including scabies, diarrheal diseases, measles, and tuberculosis (TB).[10] TB is exacerbated by Human Immunodeficiency Virus infection/ Acquired Immune Deficiency Syndrome (HIV/AIDS) of which

Uganda, Rwanda, and DRC have amongst the highest prevalence in the world.[2,10]

Mountain gorillas are very closely genetically related to humans, and are therefore potentially at risk from getting human pathogens.[7,9] There is an apparently high prevalence of scabies in people bordering Bwindi Impenetrable National Park, who have a low personal income and practice poor hygiene.[4,6] A group of Bwindi gorillas had scabies which could have been transmitted to them from contaminated clothing or other fomites of infected people, during tourist visits or while outside the park boundary when raiding banana crops in people's gardens.[4] According to the district medical personnel the most commonly treated diseases in people around Bwindi are malaria, respiratory tract infections, diarrheal diseases, scabies, ringworm, TB, intestinal parasites, tropical ulcers, and eye infections including river blindness. If human health in the areas surrounding Bwindi is not improved, then gorilla health is put at an ever-increasing risk. Education is thought to be one of the most effective ways to bring about a long-term change in people's attitudes. Conservation education workshops have been carried out in most of the communities surrounding Bwindi through the Community Conservation department of Uganda Wildlife Authority, to bring about a long-term appreciation for gorilla and wildlife conservation. Health education workshops had never before been presented to the communities surrounding Bwindi who are largely unaware of the risks of human to gorilla disease transmission.

Materials and Methods

Bwindi Impenetrable National Park is surrounded by 19 parishes. The health education program was presented to an estimated number of 1,065 people from eight villages in five parishes, targeting areas where gorillas go out into people's gardens to raid crops, including Sarambwe village in DRC. The workshops were conducted using a participatory rural appraisal method that involved:

1. The education team giving objectives and an agenda of the workshop
2. The local communities giving their expectations of the workshop
3. The education team giving lectures on how human and gorilla health contributes to gorilla conservation and tourism, with references to gorilla behavior and ecology.
4. Local community members giving their recommendations of how the present situation should be improved to prevent human and gorilla disease transmission.

There was concern that the local community would not be receptive to the workshop as they may feel that the park authorities care more about the welfare of gorillas than the welfare of the local communities. Therefore the program was presented in such a way that the local community would see the benefits of improving their health to promote gorilla health and conservation. The discussion and lectures were supplemented by visual aids including flip charts, photographic slides and video films, and educational brochures given to everyone at the end of the workshop.

Results

A summary of the different groups and their responses to the workshops are presented in Table 1.

There were generally positive attitudes of the different groups of people towards the workshop content and this was in direct relation to the level of benefits received from gorilla tourism and conservation. These benefits included revenue sharing funds directed towards community development projects, employment of people as park staff, income generating businesses of restaurants and tourist accommodation, and multiple use activities. The local community groups that were unreceptive to the program had not experienced any of these benefits and saw gorillas as a threat to their livelihood. An incident of crop raiding by a tourist gorilla group during one of the programs negatively influenced farmers in Mukono parish adjacent to Buhoma tourist site, that had received the most tourism benefits. Discussion between communities and the education team led to a consensus and development of recommendations. A multidisciplinary team of people will be needed to follow up these workshop recommendations. The team should consist of community conservation wardens, veterinarians, and district health personnel with the support of their parent ministries. Recommendations made by the workshops included:

1. Improving the human gorilla response team for chasing gorillas from people's gardens composed of local community members trained by park personnel.
2. Improving health care for the local community by setting up mobile clinics to reach people who are far away from the clinics, and have to walk for long distances to receive medical attention. This will include setting up a clinic at Bwindi Impenetrable National Park.
3. Continuing health education workshops to promote a long-term change in local community habits by including this topic in the ongoing community conservation education activities of Bwindi Impenetrable National Park.

Discussion

The level of tourism and conservation benefits received from the park authorities appeared to influence the community's attitudes. Each group of people at these venues has received varying amounts of benefits from the park, including revenue sharing funds from tourism, employment, small income generating opportunities, and multiple use activities such as access to forest products for basket materials, medicinal plants and honey. Revenue sharing started off in 1993 with 12% of gorilla tracking fees (US$150 per foreign non resident per hour with a maximum number of six people per group per day) going to the local parishes. At full occupancy, this was US$78,840 per year shared equally among the 19 parishes surrounding the park. This was reduced in 1997 to 20% of park entrance fees (US$15 per foreign non-resident per visit) which at full occupancy would be US$13,140 per year. This amount of money has built schools (10), clinics (6), and roads (3) in the local parishes.

It was concluded that education workshops are better received if local communities value gorilla conservation, which appears to be linked to tourism or other conservation benefits. To be able to create a long-term positive change in local community attitudes through education workshops towards gorilla conservation and better human health, it is vital that the communities are receiving tourism and conservation benefits, especially the Sarambwe community in the DRC. To date one of the recommendations that has been followed up is the health education workshops now being

included on the community conservation program of Bwindi, to bring about a long-term positive attitude towards improving health and hygiene of local people who come into close contact with gorillas. This education model can be adapted for other local communities bordering national parks with endangered primates, especially primates that come into close contact with people.

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Table 1. Background and health education workshop recommendations of Bwindi local communities, January to May 2000.

	Group A	Group B	Group C	Group D
Background				
Parish	Mukono	Bujengwe	Nteko	Sarambwe
Venues/villages	3	3	1	1
Proximity to habituated gorillas	Adjacent to tourist gorilla groups	Not adjacent to habituated gorillas	Adjacent to gorilla group almost fully habituated for tourism	Adjacent to tourist or habituated gorilla groups
Tourism benefits	Revenue sharing funds	Revenue sharing funds	Revenue sharing funds	No funds
	Income generating activities (e.g., from providing tourist accommodation/food)	No income generating activities (e.g., from providing tourist accommodation/food)	No income generating activities (e.g., from providing tourist accommodation/food)	No income generating activities (e.g. from providing tourist accommodation/food)
	Employment as park tourism trackers, rangers, guides	Very little park employment	Very little park employment	Very little park employment
Conservation benefits	Little multiple use activities	A lot of multiple use activity; forest products for basket weaving, honey, medicinal plants	Little multiple activities	No multiple use activtities
Quality of education	Conservation education	Conservation education	Conservation education	No conservation education
Level of crop raiding by gorillas	Medium	Low	High	Medium
Attitude to workshop	Very receptive and enthusiastic	Receptive and curious, but felt that the park could give more tourism benefits	Quite receptive and curious, but felt that the park was delaying tourism to their area	Not receptive and mistrusting, felt that the gorillas were a threat to their livelihood
Recommendations				
Non medical	Human gorilla conflict response team should be enforced	Have a screening method for people coming close to gorillas	Human gorilla conflict response team should be enforced	The park should purchase their land adjacent to the park instead of resettling them with no compensation
Personal hygiene	Proper disposal of rubbish	Have enough recommended pit latrines	Dig recommended types of pit latrines	No recommendation
Medical	Put up a health unit nearby	Go for medical treatment as soon as you need it	Ministry of Health should concentrate on areas where gorillas and people have close contact	Have as many hospitals as churches

INITIAL REPORT ON THE CONSERVATION OF THE GIANT GOMERAN LIZARD (*Gallotia simonyi gomerana*), CANARY ISLANDS, SPAIN

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Abstract

In 1999, biologists discovered fecal material that provided evidence for the existence of a large lizard of the genus *Gallotia* on the island of La Gomera, Canary Islands. The existence of *Gallotia*, a genus restricted to the Canary Islands, on La Gomera was known from fossil records but was considered to have been long extinct. Following this discovery, an exhaustive search of the island from June to September 1999 led to the capture of six animals (two male, four females) from the Risco de La Merica in the Valle Gran Rey. The decision to remove these animals from the wild was based upon fears of extinction due to the increasing feral cat population. Canarian Gallotia lizards comprise of *G. galloti* (La Palma, Tenerife, El Hierro, La Gomera, Las Palmas), *G. intermedia* (La Palma, Tenerife), *G. stehlini* (Gran Canaria, Fuerteventura), *G. atlantica* (Gran Canaria, Lanzarote and Fuertaventura), and *G. simonyi madachoi* (El Hierro). The taxonomic classification of this new lizard, *Gallotia simonyi gomerana*, is pending official sanction. These lizards are listed in CITES appendix 1. Initially all lizards were maintained in individual vivaria within secure government offices on the island of Tenerife. Three animals (one male, two females) were subsequently moved to a purpose-built, outdoor breeding facility on the island of La Gomera. The Canarian authorities have considerable experience of maintaining and breeding *Gallotia* lizards from various islands and similar husbandry and nutritional practices were adopted for these animals.

In April 2000, one male developed hind limb paresis and the authors were requested by the Spanish Government to examine and assess the six specimens. The health assessments included husbandry and nutritional evaluations, physical examinations (including weight and length measurements), hematology, biochemistry, fecal microbiology and microscopy, and survey radiographs where electricity and equipment were available.

All the animals were microchipped and treated with oxfendazole and metronidazole due to the heavy burdens of unidentified nematodes and *Trichomonas* sp. protozoa on the fecal examination. The male with hind limb paresis was diagnosed with a spinal lesion at L8-L9. Despite initial improvement, the lizard died several months later. Post-mortem examination indicated severe mycotic hepatitis, 1cm diameter mycotic granuloma over T7-T8 with associated osteomyelitis of the spine. The remaining animals were considered clinically healthy although minor improvements in captive management were recommended.

It is hoped that through continued cooperation between the Canarian Government, conservationalists and veterinarians, a successful breeding program will increase the captive population and facilitate reintroduction to the wild once circumstances become more conducive to survival. Such plans have proved successful for other *Gallotia* species in the Canary Islands.

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VETERINARY PARTICIPATION IN AN EXPERIMENTAL RELEASE OF ELK (*Cervus elaphus*) INTO THE GREAT SMOKY MOUNTAIN NATIONAL PARK

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Abstract

Cervus elaphus, known as elk or wapiti, is the most widely distributed cervid in the world. Wapiti were historically abundant in the southern Appalachians but are thought to have been eliminated from the region by the mid-1800's. Recently, there have been several efforts to reintroduce this species to the southeastern US, largely due to the conservation efforts of the Rocky Mountain Elk Foundation. Beginning in 1999, the Wildlife Management Department, Great Smoky Mountain National Park (GSMNP) and faculty of the Department of Comparative Medicine began interacting on veterinary aspects of an experimental release of elk into the GSMNP, scheduled to occur in 2001. Through this relationship, elk health and management concerns were intercalated into the comprehensive experimental release plan.

Initial discussions focused on the important diseases of elk,[1] with emphasis on diseases not currently identified in the GSMNP or region. Diseases and parasites of concern included: Bovine tuberculosis (TB), chronic wasting disease (CWD), bovine brucellosis, *Parelaphostrongylus tenuis*, *Echinococcus granulosis*, *Fascioloides magna*, *Dermacenter albipictus*, *Psoroptes* spp., and *Otobius megnini*. The release plan addressed each pathogen of concern, via testing, treatment, or source herd health history.

A free-ranging enclosed herd of elk at the US Forest Service's Land Between the Lakes National Recreational Area (LBL), Kentucky, was selected as the source of the release animals. This herd had been established in 1996 and 1997 with animals from Elk Island National Park, Alberta, Canada. The LBL herd has had no cases of TB, CWD, or brucellosis**.** The Elk Island National Park herd has been certified TB and brucellosis free since 1972 and there is no history of CWD in that herd. All elk were tested for TB and brucellosis prior to transfer from LBL. Each elk was treated for parasites with ivermectin (Merial, Iselin, NJ 08830 USA) and cloruslon (Merial) while held at LBL prior to transportation to GSMNP. Chronic wasting disease and *Echinococcus granulosis* infections remain concerns regarding the animals released in GSMNP. Any elk that dies or is euthanatized will be removed from the study site and necropsied. Carcasses will be specifically screened for CWD and *E. graunulosis* infection.

Development of chemical capture techniques was the other major area of veterinary involvement. Carfentanil citrate was chosen as the principal immobilization agent; however ultra-potent opioids

had not been previously used by GSMNP staff. The primary topics veterinarians were asked to address were immobilization regimens and personnel safety. Following an initial immobilization trial, the following regimens were utilized: adults (body weight range = 124 to 269 kg, *n* = 19): 1.5 mg carfentanil (Wildlife Pharmaceuticals, Inc, Fort Collins, CO 80524 USA) and 45 mg xylazine (Miles Inc., Animal Health Products, Shawnee Mission, KS 66201 USA) and 0.05 ml either saline or hyaluronidase (Sigma Chemical, St. Louis, MO 63178 USA; approx. 150 IU) suspended in sterile water, i.m.; and juveniles (body weight range = 91 to 183 kg , *n* = 7): 0.9 mg carfentanil citrate, 30 mg xylazine, and 0.05 ml of either saline or hyaluronidase in sterile water, i.m. Agents were delivered by 1ml darts and powder-charge rifle (Pneu-dart, Inc., Williamsport, PA 17703 USA), Twenty-two immobilizations were considered good to excellent (minimal running after darting, first effect within 4.5 min, lateral recumbency within 8.5 min of darting). One adult and one juvenile required a second dart (all darts contained the same amount as initial darts) to be captured and one adult required three darts to be immobilized. Mean (± SD) time to first effect was 2.7 ± 1.0 min and mean time to lateral recumbency was 3.9 ± 1.8 min, for immobilizations rated good or excellent. All elk were reversed with naltrexone (Wildlife Pharmaceuticals; 150 and 100 mg, for adults and juveniles, respectively; half the dose i.v. and half s.c.) and i.v. yohimbine (Sigma Chemical; 30 mg and 20 mg, for adults and juveniles, respectively). All animals recovered from immobilization satisfactorily and there were no mortalities associated with the chemical restraint.

As of 2 May 2001, all elk are doing well and there have been no mortalities.

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DIAGNOSTIC TESTING FOR *Mycobacterium bovis* IN NONDOMESTIC HOOFSTOCK

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Abstract

Mycobacterium bovis is typically the agent of tuberculosis in hoofstock. In the past 20 yr, *M. bovis* infection has occurred in ungulates in at least 20 U.S. zoos.[14] The American Zoo and Aquarium Association and the American Association of Zoo Veterinarians have recommended testing for tuberculosis; however, prior to 2001, no standard testing protocols had been implemented. Federal regulations have only required testing prior to interstate shipment of federal eradication "program species", including domestic cattle, bison, and cervids. With few exceptions, diagnostic tests have been validated only in these program species. The National Tuberculosis Working Group for Zoo and Wildlife Species was formed to address issues regarding tuberculosis in zoos, circuses, and other animal collections and this group has recently drafted guidelines for tuberculosis surveillance, including recommendations for testing.[14]

Intradermal (ID) Testing

In zoos, intradermal (ID) tests are typically the primary screening tools for detecting *M. bovis* infection. However, ID tests have rarely been validated in non-program hoofstock and some reports suggest low sensitivity and/or specificity in non-domestic species.[3,9] It may be difficult to evaluate the response in some species because of skin thickness. Further data needs to be generated in order to make conclusions about the validity of ID testing in many non-domestic hoofstock species. Animals that respond to ID tests should be further evaluated by other techniques. ID tests are additionally problematic in that they require handling animals both for initial tuberculin injection and again for injection site palpation. Post-testing suppression of the immune response precludes retesting 10-60 days after ID testing (10-90 days in cervids).

The host's response to tuberculin is primarily cell-mediated, depending on previous exposure to antigen(s) and resultant differentiation of lymphocytes. Responses become more pronounced as infections become more extensive. False-negative ID tests may occur for many reasons and anergy can be seen in severely infected animals that lose the cellular response. Poor immune responses can be caused by stress, concurrent infections, a lack of antigenic stimulation, variability in antigen recognition, or immune-modulating therapy.[4] False-positives may occur in animals with exposure to non-tuberculous mycobacteria. The primary screening test in cattle and bison is the caudal fold test. While this test is an effective screening tool in cattle and bison, it is ineffective in cervids and

has not been validated in most other hoofstock species.

The single cervical test (SCT) requires ID injection of bovine tuberculin in the neck. It is the official screening test in cervids and is recommended for many non-bovid hoofstock.[14] A similar test can be applied in camelids, swine, and tapirs, however in these species, non-cervical sites have been recommended.[14] Extensive work has been conducted to validate both the SCT and the comparative cervical test (CCT) in cervids; however, most of this evaluation has been conducted on *Cervus elaphus* (red deer and elk) with few reports of validation in other species. In cervids, the SCT has had a high sensitivity (Se) of 86-97%, but a specificity (Sp) of only 70-78%.[5,16] In contrast, the CCT has a lower Se (70 - 84%), but a higher Sp (80-99%) in cervids.[5,10] This test is performed by injecting balanced bovine and avian tuberculins at separate sites; the two responses are then compared. The CCT's low Se may lead to an increase in false-negative results, so it is currently recommended that it be used only as a secondary/confirmatory test for all hoofstock.

Blood Testing

Whether testing for cellular or humoral immunity, blood testing for tuberculosis can be advantageous as it only requires handling the animal once. However, techniques for testing blood have had problems with sensitivity and specificity. Most blood techniques have not been validated in non-domestic hoofstock and some require special sample handling or preparation.

Lymphocyte Transformation

Lymphocyte transformation assays measure cellular immunity by quantitating the antigenic stimulation of T-helper lymphocytes.[2] Lymphocytes are separated from the blood by differential centrifugation, challenged with antigens, and then incubated. Following incubation, cultures are pulsed with a radioisotope-labeled nucleotide. After further incubation, the cells are harvested and incorporation of the radioisotope is measured. Lymphocytes previously challenged with the antigen, proliferate and incorporate elevated levels of the radioisotope. Blood samples must be carefully handled and typically need to be processed within 48 hr. In cervids, reported Se and Sp have been highly variable. Differences in results may depend on species tested, laboratory techniques, antigens, stress level of the animal, and/or statistic methods of evaluation.[2,4,6]

Gamma Interferon

The gamma-interferon test is a whole blood assay that detects specific lymphokines produced when lymphocytes respond to specific antigens. It is used in attempt to differentiate nonspecific lymphocyte transformation from antigen-specific stimulation. Lymphocytes must remain viable and functional, so it is important that the sample be handled gently and processed quickly (within 8-12 hr). Once the lymphocytes have been stimulated, the plasma supernatant is drawn off and can be frozen and stored. Research in cattle has suggested that this assay has superior Se and Sp to the caudal fold test.[17] However, the test uses specific monoclonal antibodies and its unclear how well gamma-interferon will be detected in other hoofstock species.

Enzyme-Linked Immunosorbent Assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) is a serologic test used to measure antigen or antibody. These tests only require serum, so sample collection, transport, and processing are simple and samples may be preserved indefinitely. Blood is collected and centrifuged. Serum is extracted and frozen (preferably at -70°C) until it is tested. An antigen is usually bound to a plate and the animal's serum is placed in contact. Plates are washed and the ELISA measures the amount of bound antibody. The development of antibody detection methods that are not species-specific has facilitated the use of ELISA in non-domestic species.[7,15] Many different antigens have been used for tuberculosis detection, including antigens from both *M. tuberculosis* and *M. bovis*. The multiple-antigen ELISA, using a combination of several antigens, appears to provide the best combination of Se and Sp. Animals may come into contact with non-tuberculous mycobacteria, such as *M. avium* or saprophytic mycobacteria, so antigens from these organisms have also been used in the multiple-antigen ELISA, in an effort to increase the test's specificity.

Serologic assays have had considerable variation in Se and Sp. One multiple-antigen ELISA demonstrated Se of 85% and Sp of 100% in red deer,[8] while an investigation in multiple cervid species revealed lower Se (66%) and Sp (56%).[7] There is some evidence that serologic responses are species-specific and that different antigens, concentrations of antigens, and/or different "cut-off" levels are necessary for different species. When our laboratory evaluated infected and non-infected bongo (*Tragelaphus eurycerus*), an infected individual had a much stronger serologic response than its non-infected cohorts. However, the infected bongo's response was much less than that typically seen in *M. bovis* infected cattle.

The primary host response to mycobacterial infection is cell-mediated and it is thought that humoral responses are often weak, generally occurring only in advanced disease. The utility of the ELISA for routine screening of non-domestic hoofstock is still unclear, however, even if only severely infected animals are detected, the ELISA will be helpful as severely infected animals are likely to be shedding large numbers of organisms and need to be detected rapidly. The ELISA may also be useful for detecting anergic animals that no longer have a cellular response, but do have a humoral response. There is some evidence that the ELISA may be useful after ID testing, as exposure to ID antigens increases humoral antibody levels in infected animals.

Blood Test for Tuberculosis (BTB)

The results of LT and ELISA may be combined, along with plasma fibrinogen, to produce a composite assay termed the blood test for tuberculosis (BTB). Developed in New Zealand, this test has been reported to have excellent Se (> 95%) and Sp (> 92%) in red deer.[8] Findings were not the same when the BTB was used on elk and other cervids in the United States, as it failed to identify infected animals on multiple occasions.[16] It is not currently available in the United States.

Ag85 Dot Blot Immunoassay

Actively proliferating mycobacteria secrete Antigen 85 complex proteins.[4] The presence of these products in an animal's circulation may indicate active mycobacterial infection. The Ag85 assay is performed by blotting diluted serum samples and bovine reference samples to nitrocellulose.[4] Ag85 is detected by dot immunobinding using monoclonal antibodies. Because this assay detects antigens, it should not depend on the immunocompetency of the animal, nor should host-specific assays be necessary. A report on Ag85 assay in non-domestic hoofstock (multiple species), described higher levels in infected groups than non-infected groups.[11]

Direct Testing

Mycobacterial Culture

Although culture is the current "gold-standard" for diagnosis of *M. bovis*, this test is often used only if there is a history or clinical suspicion of tuberculosis. Mycobacterial culture can detect as few as 100 organisms/ml,[12] however results may take up to 8 wk. Recommended specimens have included lung, lymph nodes, and abscesses, but it may not be feasible to sample these tissues in live animals. For living non-domestic hoofstock, the tracheal wash is the currently recommended sample.[14] Nasal washes or swabs may be collected, but will likely be negative unless large numbers of mycobacteria are actively being shed. Other methods may include thoracoscopy, bronchoscopy, or thoracotomy. Infected animals may be culture-negative if samples are inappropriately collected, processed, or handled. Samples from non-domestic hoofstock should not be shipped using sodium borate as this may cause false-negative results. Sodium borate has been recommended for shipping tissues from cattle, to prevent overgrowth of non-tuberculous mycobacteria on the outer surface. Cattle samples are usually large enough that sodium borate doesn't penetrate all of the tissue, but samples from non-domestic hoofstock are usually smaller; sodium borate may penetrate the entire sample, thereby killing all mycobacteria.

Histopathology and Acid-Fast Staining

Histopathologic analysis is routinely performed to help confirm a diagnosis of mycobacterial infection, particularly postmortem. Tissues are embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined by light microscopy. Sections with granulomas are stained by the Ziehl-Neelsen or Kinyon acid-fast technique.[2] The presence of acid-fast bacteria is suggestive of *M. bovis* infection, although other non-tuberculous mycobacteria (as well as *Nocardia* spp.) can appear as acid-fast. Mycobacterial culture is typically necessary to confirm *M. bovis*, although a presumptive diagnosis may sometimes be made if there are histologic changes consistent with mycobacterial infection and a history of *M. bovis* in the herd.

Histopathology is not possible on many samples (tracheal washes, small tissue samples, etc.) that are taken from the live animal, and acid-fast smears may be performed instead. However, smears have a low sensitivity, as the lower limit of detection is about 1000 organisms/ml.[12] Hence, false-

negatives are common with acid-fast smears. False-positives may be caused by the presence of *M. avium*, saprophytic environmental mycobacteria, or *Nocardia* spp.

Polymerase Chain Reaction

The polymerase chain reaction (PCR) is typically used on samples that are taken for culture, histopathology, or acid-fast smear. The PCR is a nucleic acid amplification technique in which specific sequences of DNA/RNA are replicated, allowing for detection of target sequences that otherwise would not be present in high enough numbers. DNA/RNA probes are then used for detecting amplified mycobacterial sequences. PCR methods can detect as few as 20-50 mycobacterial organisms/ml[1] and in cases where positive samples are contaminated with bacteria, recovery rates for the PCR appear to be better than those for culture. Clinical trials have shown PCR to be highly specific for *M. tuberculosis*-complex organisms and preliminary information on these tests are very promising, with high Se (93%) and Sp (100%).[13] While PCR appears promising, this technology should still be utilized in conjunction with culture.

Conclusions

There are many diagnostic tests available and the tests used will vary by species, institution, and situation. No test is 100% sensitive or 100% specific. There appear to be species variations in response to tests of immunity and further information is needed for informed evaluation of these tests in non-domestic species. Many institutions that do not have documented *M. bovis* infection will continue to use ID testing for screening. A battery of diagnostic tests is more likely to be used in places that are addressing documented *M. bovis* infection. Clinicians are encouraged to consider submitting samples for tuberculosis testing when feasible, so that we may better understand the tests' utility in managing the health of our non-domestic hoofstock populations.

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CLINICOPATHOLOGIC FINDINGS IN *Mycobacterium tuberculosis* CULTURE-POSITIVE ELEPHANTS (*Elephas maximus*) IN COMPARISON TO CLINICALLY NORMAL ELEPHANTS

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Abstract

Mycobacterium tuberculosis has been known to infect elephants for approximately 2,000 yr and currently infects approximately 3% of elephants housed in captivity in North America.[5] The United States Department of Agriculture (USDA) has set and updated guidelines for the control of tuberculosis in elephants covered under the Animal Welfare Act.[8] Diagnosis at this time is based strictly on a single required test, *Mycobacterium tuberculosis* culture. This is markedly different than diagnosis in humans which includes screening of high risk populations or individuals with clinical signs using tuberculin testing, thoracic radiographs, and sputum cytology which is followed by culture.[3]

Intradermal testing in Asian elephants (*Elephas maximus*) has had poor correlation with culture results.[6] Thoracic radiographs in a 6,000 to 12,000 pound animal are not realistic. Sputum cytology is recommended in the USDA guidelines but not required. Additionally, nucleic acid amplification tests (NAAT) currently used in humans are used to diagnose *M. tuberculosis* complex (the mammalian pathogens), which includes *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*. These different species are all included in the reported 95% specificity of NAAT when compared with acid-fast sputum smears and culture. These would not be applicable in the specific diagnosis of the species *M. tuberculosis* in elephants. Additionally, NAAT is currently only used in human medicine in combination with cytology.[3] ELISA testing in combination with gamma interferon testing is promising but not currently validated. It can be costly and is only run in a few laboratories across the country.[4]

An evaluation of standard blood work was undertaken to determine if there are any values that could be identified as consistently different between culture positive elephants and clinically normal elephants (Tables 1,2). These values, though not providing a definitive diagnosis of *M. tuberculosis*, may function to flag affected animals within a population.

Approximately 50 Asian elephants were routinely evaluated over a 2-yr time period. Appetite, behavior, and physical condition were recorded daily. Routine veterinary physical examination including blood work was performed annually for healthy animals and on an as needed basis for sick animals. A data set of healthy animals was established. Any animal that showed any signs of illness including decreased appetite, weight loss, clinical disease, or significant changes from baseline

blood work was excluded from the data set. The data set of 20 healthy elephants was then compared to samples taken from five culture-positive *Mycobacterium tuberculosis* elephants when they were actively shedding organisms. Parametric values were compared using a Student's t test for two groups. Nonparametric values were compared using a Wilcoxon Rank Sum. Albumin:Globulin (A:G) ratios were further evaluated for sensitivity and specificity using a Receiver Operating Characteristic (ROC) curve.[2] Albumin was determined using a bromcresol green method and total protein was determined using a pyramolol red method on a Hitachi 911 (Boehringer Mannheim, Indianapolis, Indiana, 46250 USA). Globulin was calculated by subtracting the two. Protein serum electrophoresis of the sample was performed using an Appraise Densitometer System (Beckman Coulter, Inc. Fullerton, CA 92634 USA).

Cytochemical staining of the leukocytes revealed that a bilobed cell type unique to elephants is lightly chloroacetate esterase positive and strongly peroxidase positive. This is consistent with recent previous literature.[1,7] This cell is therefore classified as a monocyte and is reported as such in the tables above. Misidentification of these cells may result in underreporting of monocytes and increased reporting of lymphocytes.

Values that were significantly lower in positive animals ($P \leq 0.05$) included A:G ratio, mean cell hemoglobin concentration (MCHC), and glucose. Values which were significantly higher in positive animals ($P \leq 0.05$) included platelets, band neutrophils, eosinophils, calcium, and bicarbonate (TCO_2). Two values, red blood cell numbers and segmented neutrophils, were equivocally higher.

The A:G ratio is a measure of general inflammation in the body. It was therefore of particular interest in these animals and was further statistically evaluated. A ROC curve is a plot of sensitivity versus specificity over all possible decision thresholds. A ROC curve describes compromises in sensitivity and specificity independent of the prevalence of disease.[6] When the A:G ratio, determined using the Hitachi 911, was analyzed with a ROC curve, the value of 0.7 was determined to be the most effective decision threshold. This value had a 100% sensitivity and a 77% specificity in the population examined. The area under the curve (AUC) is considered to be the overall summary measure in a ROC curve. An AUC of 0.5 is considered to be a useless test while an AUC of 1.0 is considered to be a perfect diagnostic test. The A:G ratio determined using the Hitachi 911 had an AUC of 0.91. The gel A:G ratio had an AUC of 0.55. Additionally, animals that were initially negative all had a decreased A:G ratio at the time of positive trunk wash in comparison to baseline A:G ratio values for those animals.

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Table 1. Hematologic and serum biochemical reference ranges for Asian elephants (*Elephas maximus*) with a negative TB status.

	n	Mean	Standard deviation	Reference range
A:G ratio	31	0.75	0.12	0.6-1.1
Gel A:G ratio	31	0.69	0.12	0.45-1.0
Total prot (g/dl)	31	8.1	1.11	5.4-10.1
Albumin (g/dl)	31	3.4	0.36	2.6-4.4
Plobulin (g/dl)	31	4.6	0.88	2.9-6.3
Pprot (g/dl)	17	8	0.66	6.7-9.3
Ael alb (g/dl)	31	3.3	0.43	2.1-4.4
ALPHA1 (g/dl)	31	0.6	0.11	0.4-0.9
ALPHA2 (g/dl)	31	0.5	0.16	0.3-0.9
BETA1 (g/dl)	31	1.4	0.46	0.6-2.6
BETA2 (g/dl)	31	1.3	0.25	0.9-2.0
GAMMA (g/dl)	31	0.9	0.31	0.5-1.4
Na (mEq/L)	20	125	6.32	109-141
K (mEq/L)	20	4.5	0.34	4.1-5.3
Cl (mEq/L)	20	90	3.17	83-97
TCO_2 (mEq/L)	20	24	1.86	20-28
Anion gap	20	15.5	4.4	7-24
BUN (mg/dl)	20	10.8	2.81	5-16
Creat (mg/dl)	20	1.4	0.2	0.9-1.6
Ca (mg/dl)	20	10.2	0.88	8.2-12.0
Glucose(mg/dl)	20	80	13.92	63-124
PO_4 (mg/dl)	20	4.3	0.82	3.3-6.1
Total bilirubin (mg/dl)	20	0.16	0.06	0.1-0.2
Triglycerides (mg/dl)	20	52	54	18-160
Alk phos (IU/L)	20	116	37.19	53-204
AST (IU/L)	20	13	4.6	7-25
GGT (IU/L)	20	8.6	3.36	5-18
RBC ($x10^6/\mu l$)	20	2.9	0.4	2.1-3.6
HGB (g/dl)	20	12.3	1.26	9.6-15.3
PCV (%)	20	35.7	3.79	27-44
MCV (fL)	20	125	7.42	111-138
MCHC (g/dl)	20	34.7	0.58	33.4-35.7
RDW	18	16.8	1.08	14.9-18.7
PLT ($x10^3/\mu l$)	18	513	98.43	342-719
WBC ($x10^3/\mu l$)	20	13.6	2.7	8.6-18.2
Fibrinog (g/dl)	20	400	137.17	100-600
bands/µl	20	0	0	0.00-0.00
segs/µl	20	3180	820.59	1800-4300
lymphs/µl	20	2650	1456.64	990-5000
monos/µl	20	7140	2751.96	1600-12600
eos/µl	20	220	172.94	0-580
basos/µl	20	50	60.88	0-150

Table 2. Hematologic and serum biochemical reference ranges for Asian elephants (*Elephas maximus*) with a positive TB status ($n = 5$).

	Mean	Standard deviation	Reference range
A:G ratio[a]	0.56	0.04	0.5-0.6
Gel A:G ratio	0.66	0.11	0.52-0.81
Total prot (g/dl)	8.5	0.26	7.9-8.9
Albumin (g/dl)	3.2	0.3	2.7-3.5
Globulin (g/dl)	5.3	0.07	5.2-5.4
Gel alb (g/dl)	3.4	0.44	2.8-3.5
ALPHA1 (g/dl)	0.7	0	0.7 (all five animals)
ALPHA2 (g/dl)	0.7	0.04	0.6-0.7
BETA1 (g/dl)	1.2	0.47	0.5-1.5
BETA2 (g/dl)	1.5	0.46	1.1-2.1
GAMMA (g/dl)	1.1	0.22	0.8-1.4
Na (mEq/L)	122	20.77	86-150
K (mEq/L)	4.3	0.37	3.9-4.8
Cl (mEq/L)	93	3.67	89-98
TCO_2[a] (mEq/L)	25.7	2.13	23-28
Anion Gap	15.9	8.03	7.5-25
BUN (mg/dl)	11.2	2.28	9-14
Creat (mg/dl)	1.4	0.32	1.0-1.7
Ca[a] (mg/dl)	11.3	1.58	9.9-13.9
Glucose[a](mg/dl)	65.6	11.76	52-83
PO_4 (mg/dl)	4.7	0.64	4.1-5.8
T bili (mg/dl)	0.2	0.15	0.1-0.4
Glyceride (mg/dl)	40	33.18	14-86
alk phos (IU/L)	109	24.34	86-138
AST (IU/L)	16.4	4.62	10-22
GGT (IU/L)	9.4	1.34	8-11
RBC ($x10^6/\mu l$)	3.1	0.19	2.85-3.30
HGB (g/dl)	12.68	1.65	10.5-14.7
PCV (%)	36.4	5.03	30-42
MCV (fL)	120.2	7.92	110-129
MCHC[a] (g/dl)	33.9	0.57	33.4-34.8
RDW	17.24	1.5	16.0-19.2
PLT[a] ($x10^3/\mu l$)	616.6	48.11	540-658
WBC ($x10^3/\mu l$)	16.48	6.09	10.0-25.9
Fibrinog (mg/dl)	460	230.22	200-700
bands/μl[a]	88	85.26	0-170
segs/μl	4712	3475.08	2300-10620
lymphs/μl	1564	934.2	670-2850
monos/μl	9690	2474.68	6690-12000
eos/μl[a]	434.2	206.91	200-700
basos/μl	52	116.28	36894

[a]Indicates values that are statistically different ($P < 0.05$).

DIAGNOSIS AND MOLECULAR CHARACTERIZATION OF ATOXOPLASMA IN PASSERINE BIRDS

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Abstract

Atoxoplasma are coccidian parasites that infect passerine birds causing significant morbidity and mortality, especially in young animals. While disease caused by *Atoxoplasma* or *Atoxoplasma*-like organisms has been reported in a number of species, including the highly endangered Bali mynah (*Leucopsar rothschildi*), no definitive diagnostic test for identifying *Atoxoplasma* exists. In addition, the lack of information about the host-species specificity of the organism and the number of *Atoxoplasma* sp. that exist causes significant problems with regard to preventive medicine and captive population management. In the past, the most reliable method for detecting *Atoxoplasma* infection has been demonstration of sporozoites in mononuclear leukocytes on blood impressions. Finding typical isosporoid oocysts after sporulation of fecal samples and histology are additional techniques for identifying suspected cases of atoxoplasmosis. The purpose of this study was to develop a polymerase chain reaction (PCR) based molecular diagnostic test for atoxoplasmosis, to begin molecular characterization of the organism, and to begin compiling data on the host-range of different species of *Atoxoplasma*. Since 1988 we have identified 85 cases of suspected atoxoplasmosis in 32 passerine species based on microscopic evaluation of blood impressions, fecal flotation analyses, and/or histologic examination. Eighty-two of these 85 animals died, and atoxoplasmosis was cited as the cause of death or directly implicated in the death of 50 (61%) of these animals. In order to provide a more definitive diagnosis of atoxoplasmosis and to facilitate a molecular epidemiologic investigation of these cases, we have recently implemented a PCR method that amplifies conserved small subunit rRNA gene regions of apicomplexan protozoa. PCR amplicons from frozen or formalin-fixed, paraffin-embedded tissues of nine species of passerine birds from the Zoological Society of San Diego with suspected *Atoxoplasma* infection have now been generated, cloned, and sequenced. These genetic sequences are distinct from other coccidia for which sequence information is available, but are most similar to *Isospora* and *Caryospora*. In each animal, a homogenous PCR product was obtained, indicating that infection in the individual was likely limited to a single species of *Atoxoplasma*. Our findings also suggest that at least two species of Atoxoplasma probably exist in passerines, and that transmission of the same *Atoxoplasma* sp. between different avian taxa may occur.

EVALUATION OF THE ORAL IRON CHELATOR DEFERIPRONE FOR THE TREATMENT OF IRON OVERLOAD IN AVIAN SPECIES

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Abstract

Iron storage disease, or hemochromatosis, has been documented in a number of exotic and domestic species, as well as in man. Various avian species are affected by this condition, most notably mynahs, toucans, birds of paradise, starlings, and hornbills.[2,3,7,8] It is characterized by excessive accumulation of iron in parenchymal tissues with associated functional or morphologic damage. Birds with iron storage disease may die with no premonitory signs, or may succumb to hepatic or myocardial failure.[2,7,8]

Early diagnosis and treatment of hemochromatosis is essential, as the degree of iron overload has prognostic implications. Therapeutic options consist of phlebotomy or iron chelation therapy.[1,6] Phlebotomy has been employed in avian species, however difficulties arise due to the small size of some patients, the stress of frequent handling, and the uncertainty of the maximum safe volume of blood that can be removed at frequent intervals in a potentially compromised patient.[8] Currently deferiprone (L1, Apotex Inc. Weston, Ontario, Canada), which is administered orally, and deferoxamine (DFO, Desferal®, Novartis Pharmaceuticals, Dorval, Quebec, Canada), which must be administered parenterally, are the only two iron chelators available for treating the disease.[1,6] Neither drug has been studied scientifically in avian species, although there have been two case reports of successful iron chelation therapy with deferoxamine.[2,7]

We conducted a multi-phase scientific investigation into the pharmacokinetic and clinical applicability of deferiprone for the treatment of iron overload in birds. The objectives of the phase of our research reported here were: (1) to evaluate the efficacy of deferiprone to decrease hepatic iron stores in two species after inducing experimental hemosiderosis, and (2) to assess for clinical, toxicologic or pathologic abnormalities associated with drug administration.

Materials and Methods

White leghorn chickens (*Gallus forma domestica*) and domestic king pigeons (*Columba livia)* were used, to allow interspecific comparisons of the actions of deferiprone. Experimental design was identical for each species. Birds were randomly allocated to one of three groups of 10 birds (Control (NIL-C), Iron Loaded-Deferiprone treated (IL-DFP) or Iron loaded (IL)). All birds were banded, weighed and blood sampled to establish baseline hematologic and biochemical values, and plasma iron parameters (Iron levels, total iron binding capacity (TIBC), unbound iron binding capacity

(UIBC), and transferrin saturation levels (TS%)).

The birds in the IL-DFP and IL group were administered intravenous iron dextran at 25 mg/kg to induce hemosiderosis. The IL-DFP group was gavaged with a deferiprone suspension at a dose of approximately 50 mg/kg twice daily. The IL group did not receive any deferiprone while the NIL-C group received neither iron dextran nor deferiprone.

The duration of treatment was 30 days. Five birds in each group were randomly selected for weekly assessment of plasma iron parameters. Biochemical and hematologic parameters were assessed in these birds at the midpoint during the treatment period and at the end. Plasma zinc, copper and magnesium levels were assessed at the endpoint of the study. Birds were also weighed on a weekly basis to monitor body weight and adjust the deferiprone dose. In the leghorns, egg production was also monitored.

At the conclusion of the trial, the birds were euthanatized, necropsied and tissues were collected for histopathologic examination (hematoxylin-eosin and Perl's staining). The livers were weighed, and then portions submitted for quantitative determination of iron stores.

Results

There were no drug-associated mortalities in the pigeons, while three chickens died in the deferiprone treatment group midway through the study. Although there were no significant differences in food intake, there were differences in weight gain in the IL-DFP group compared with both the IL and NIL-C groups for both species (Table 1). After the morning treatment, birds being treated with deferiprone did not initiate feeding behavior as quickly as the non-treated groups, while there were no observed differences in feeding behavior among the three groups after the evening treatment. The most significant clinical finding in the deferiprone treatment group in both species was the production of rust colored urates indicative of renal excretion of Fe-deferiprone complexes.

There were no clinically significant alterations in the hematologic or serum biochemical values in either the pigeon or chicken trial. A decrease in plasma zinc levels was observed in the deferiprone treated birds of both species, while there were no significant changes in plasma copper or magnesium levels compared to the IL or NIL-C groups (Table 2).

The plasma iron parameters are summarized in Table 3. In both species, there was a marked reduction in hepatic iron concentration in the IL-DFP groups compared with the IL groups that were not treated with the drug. In the pigeons, the hepatic iron concentration in the deferiprone treated group was even lower than the NIL-C control group (Table 4). Blood collection did not affect endpoint hepatic iron levels. Histopathology results are pending.

Discussion

This study demonstrates the clinical applicability of the orally active iron chelator deferiprone for

the treatment of iron overload in avian species. When administered at a dose of 50 mg/kg orally twice daily the hemosiderotic state induced by experimental iron loading was eliminated within the 30-day treatment period. The drug can be administered with food since only the rate, not the extent, of absorption is affected by food consumption.[1,6]

No statistically or biologically significant alterations occurred in the hematologic and biochemical parameters measured in this study. Our study concurred with others that plasma iron concentrations and transferrin saturation (TS%) were a poor indication of total body and hepatic iron status as indicated by IL and NIL-C groups.[2,9] However, the decrease in TS% in the IL-DFP groups correlates well with the mechanism of action of drug, which mobilizes iron from transferrin, as well as free iron and iron from ferritin and hemosiderin. The increase in UIBC is most likely a reflection of the formation of deferiprone-iron complexes.[6]

Although iron chelation was successful, administration of deferiprone was not without side effects. Deferiprone has a strong affinity for iron, but also binds with zinc, copper and aluminum.[1,6] Further diagnostics, including histopathology, tissue culture, and deferiprone plasma levels, are being carried out at the time of this abstract to investigate the cause of death in the three chickens. There was no significant difference in copper levels among the three groups in either the chickens or the pigeons; however a significant decrease in plasma zinc levels was noted in both species. Zinc is one of the most metabolically active trace minerals, however storage pools are small, and zinc deficiency can be induced rapidly in some species of animals. Zinc deficiency is responsible for decreased appetite, cyclic feeding, and reduced weight gain, and could be a plausible explanation for the decreased weight gain in the deferiprone treated groups in this study.[4,5] Zinc is also an important component of other physiologic processes including immunocompetence, electrolyte balance, fertility, cell replication and growth, and feathering.[4,5] Zinc levels should be monitored closely while birds are being treated with deferiprone, and supplemental dietary zinc may be indicated.

Gastrointestinal disturbances such as anorexia, nausea, vomiting and diarrhea have been reported in human patients on the drug.[1,6] Although vomiting and diarrhea were not noted in the deferiprone treatment groups, the slower initiation of feeding activity may be related to transient gastrointestinal upset, especially if the drug is given in the absence of ingesta in the upper gastrointestinal tract. There was no evidence of arthropathy in the treated pigeons or chickens, as has been reported in up to 39% of human patients in clinical trials.[1]

In conclusion, deferiprone is a promising orally active iron chelator for the treatment of iron overload in birds. The drug may have potential for use in preventing hemochromatosis. Further clinical studies in affected species are needed to determine the most effective way to use this drug while minimizing the side effects associated with administration.

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Table 1. Average weekly change in bodyweight.

Group	Chicken	Pigeon
IL-DFP[a]	-38.2 ± 74 g (-2.43% ± 0.07%)	-2.6 ± 8.6 g (-0.19% ± 1.31%)
IL[b]	1.7 ± 47 g (0.27 ± 2.79%)	4.28 ± 5.65 g (0.73 ± 0.94%)
NIL-C[c]	28.5 ± 23.0 g (1.9% ± 1.59%)	4.48 ± 5.10 g (0.74% ± 0.85%)

[a]Iron Loaded-Deferiprone Tx.
[b]Iron Loaded.
[c]Control.

Table 2. Mean plasma zinc concentrations (mg/dL).

Group	Chicken	Pigeon
IL-DFP[a]	0.19 ± 0.95	0.27 ± 0.06
IL[b]	0.32 ± 0.34	0.69 ± 0.39
NIL-C[c]	0.31 ± 0.18	0.39 ± 0.18

[a]Iron Loaded-Deferiprone Tx.
[b]Iron Loaded.
[c]Control.

Table 3. Mean plasma iron levels, TIBC, UIBC and transferrin saturation for 30-day trial period.

Parameter	Leghorn Chicken			Pigeon		
	IL-DFP[a]	IL[b]	NIL-C[c]	IL-DFP	IL	NIL-C
Plasma Iron (μmol/L)	34.6	59.20	69.25	21.70	25.90	20.38
TIBC (μmol/L)	78.9	83.22	71.9	32.25	27.55	26.51
UIBC (μmol/L)	44.13	24.02	2.65	10.55	1.65	5.61
TS%	48.81	77.86	95.40	68.20	93.5	79.77

[a]Iron Loaded-Deferiprone Tx.
[b]Iron Loaded.
[c]Control.

Table 4. Mean hepatic iron concentration after 30-day trial period (μg of iron/gram of liver- dry weight).

Group	Chicken	Pigeon
IL-DFP[a]	544.10 ± 118.06	1302.60 ± 417.54
IL[b]	1380.50 ± 609.57	6722.50 ± 1225.45
NIL-C[c]	547.00 ± 87.08	3360.00 ± 1496.69

[a]Iron Loaded-Deferiprone Tx.
[b]Iron Loaded.
[c]Control.

DETECTION OF BLOOD IN COCKATIEL (*Nymphicus hollandicus*) EXCREMENT: A COMPARISON OF LABORATORY METHODS

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Abstract

To date, the only reliable laboratory method for the detection of blood in avian feces has been cytologic examination. This test relies upon the presence of intact red blood cells, which might remain intact during gastrointestinal transit, but are likely to break down when transit time is increased. Although some authors have advocated the use of a variety of laboratory tests for fecal blood detection, no test methods have been validated for birds.[5] The purpose of this study was to evaluate fecal cytology, Hematest (Bayer, Philadelphia, PA), Hemoccult and Hemoccult SENSA (Beckman Coulter, Inc., Fullerton, CA) for the detection of blood and/or hemoglobin in cockatiel excrement.

Twenty healthy adult cockatiels (*Nymphicus hollandicus*) were used in this study. The health status of the birds was evaluated by physical examination, packed cell volume, fecal direct smear, fecal Gram stain, and Wright-Giemsa stained fecal cytology. The study was divided into two phases (in vitro and in vivo evaluation). Both phases involved standardizing cockatiel whole and lysed blood to 150 g/L of hemoglobin (Hb).

The in vitro phase tested excrement mixed with whole blood or lysed blood at 0.75, 1.5, 3, 6, and 12 mg of Hb per g of excrement. These test concentrations were based on a pilot study and reports of assay sensitivities in dogs and humans.[1-3] All four test methods (fecal cytology, Hematest, Hemoccult, and Hemoccult SENSA) were performed on excrement samples (n = 20) spiked with each concentration of whole, but cytologic examination was not performed on feces spiked with lysed blood. Two different observers read each test for all samples. One gram of feces from each bird was mixed with 0.24 ml of 0.9% saline and tested by all four methods to serve as controls.

The in vivo phase of this study included the same 20 cockatiels as the in vitro phase. For control birds (n = 20), 2.5 ml of 0.9% saline was administered via soft rubber 5 French oro-ingluvial (gavage) tube. Pooled excrement from each bird was collected for a 12-hr period prior to saline gavage and 8 hr post gavaging. In addition, excrement passed between 8 and 24 hr post gavage was pooled and collected for testing. All samples were tested with all four methods by the same two observers as the in vitro phase of the study. A small amount of 0.9% saline was added to moisten dry excrement in preparation for testing. One week later, gavage administration of whole blood was

begun. Dosages of 5, 10, 20, and 40 mg Hb per kg of body weight were each administered to all 20 birds. These dosages were chosen according to the methods of a similar study with dogs.[4] Droppings were collected and tested at the same time intervals as the controls. Each bird was given the lowest dosage first and advanced to the next higher dosage after 2 consecutive days of negative fecal blood tests (via fecal cytology and Hemoccult SENSA). One week after whole blood administration (the last 2 days with negative fecal cytology and Hemoccult SENSA tests), the birds were gavaged with lysed blood and the same collection and analysis of excrement was performed as previously described.

Preliminary results determined that Hemoccult and Hemoccult SENSA were easy to use and read. Bird excrement dries quickly if it is not collected immediately after passage. However, saline can be added to dried excrement to allow it to be used with the test methods in this study. It is important to keep in mind that dietary peroxidases have been shown to cause false positive results with Hematest, Hemoccult, and Hemoccult SENSA. Examples of foods that contain peroxidases include uncooked or undercooked red meats and some raw vegetables including broccoli, cauliflower, radishes, turnips, horseradish, and some melons.[6] It is hoped that upon completion of this study the results will determine which diagnostic test is most useful to diagnose occult bleeding and to justify further diagnostic testing. In addition, these tests may also be useful for indicating the need for therapeutic intervention in the treatment of ulcerative gastrointestinal disease.

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LEVELS OF FECAL CORTICOSTERONE IN RELEASED SANDHILL CRANES: AN INDICATOR OF POTENTIAL STRESS FOR REINTRODUCED WHOOPING CRANES

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Abstract

The use of fecal corticosterone assays in North American cranes has been limited to controlled laboratory trials performed with sandhill cranes (*Grus canadensis*),[3,4] a common surrogate species used in crane research targeted to benefit endangered whooping cranes (*Grus americana*). These studies have confirmed that fecal corticosterone reflects changes in serum corticosterone brought about by adrenocortical stimulation following administration of ACTH. We chose to apply this assay to a specific release project where the ability to repeatedly handle birds for health evaluations was limited and supplementary diagnostic information was needed to fully assess stress induced by the methods used to rear the cranes prior to release. The objective of our pilot study was to document and compare ~3-mo trends of fecal corticosterone (FC) concentrations in two cohorts of sandhill cranes raised for release to the wild.

During summer and early fall 2000, sandhill cranes (Group 1, n = 14) reared in isolation from humans were transferred from Maryland to Wisconsin and trained to fly after ultralight aircraft as a prelude to an aircraft-guided 1250 mile migration to Florida (http://bringbackthecranes.fws.gov/).[2] A smaller group of sandhill cranes (Group 2, n = 8) were reared using similar techniques in Wisconsin but did not experience directed ultralight aircraft training; these birds were released using a single bird release method.[1] Fresh feces were collected from Group 1 (n = 47) individuals at arrival in Wisconsin in late June, and then anonymously from their pens approximately every 2 wk until migration in the first week of October. Feces were available slightly earlier from Group 2 (n = 49), and with individual identification until the last samples acquired in late September. Feces were frozen within 2 hr at -20°C, and thawed later for analysis. All samples were subject to radioimmunoassay for determination of corticosterone levels (ng/g) using established protocols.[4] Levels >200 ng/g were considered increased over baseline.[4]

Increased FC levels (median 292.4 ng/g, range 59.9-650.7 ng/g, n = 12) were observed in several Group 1 individuals upon arrival in Wisconsin, likely reflecting the stress of shipment. The lower FC levels observed in some cranes may have been due to sampling of feces produced prior to a hormonal increase. Corticosterone elevation in feces may not occur for 2 hr after adrenocortical stimulation.[4] FC levels in Group 1 birds returned to baseline levels that were sustained throughout

the remainder of the study (median 93.1 ng/g, range 61.2-263.6 ng/g, n = 32), despite increasing contact time with the ultralight aircraft and a shifting dominance hierarchy within the group. FC levels in Group 2 remained consistent throughout the study period (median 111.3 ng/g, range 22.8-401.0 ng/g, n = 49). There were occasional individual perturbations; including two cases that could be attributed to handling for banding and radiotagging 3 hr prior to fecal collection. There were no statistically significant differences in FC levels between the two groups when samples obtained close in time were compared. Significant disturbances, such as shipping, restraint for health examinations, and banding and radiotagging procedures appear most linked to increases in FC in sandhill cranes rather than stress invoked by the rearing and release methods used.

We intend to extend this initial study and apply FC monitoring to whooping crane reintroduction efforts. Knowledge of how the unique manipulations used for reintroduction of this endangered migratory species may subtly affect health is paramount to understanding the dynamics of the birds' behavior and survival during migration and after release.

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KLINEFELTER SYNDROME (39 XXY) IN AN ADULT SIBERIAN TIGER (*Panthera tigris altaica*)

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Abstract

Siberian tigers (*Panthera tigris altaica*) are a critically endangered species. Poaching and loss of habitat have drastically reduced the number of wild tigers. Captive reproduction is integral to conserving the species.

Klinefelter syndrome is any chromosomal anomaly with multiple X-chromatin genotypes.[5] In humans, the most common anomaly is 47 XXY.[3,4] In domestic felines, 39 XXY is commonly demonstrated by male calico and tortoise shell cats, of which more than two-thirds are sterile.[1] Testicular hypoplasia, azospermia, hyalinization and fibrosis of the seminiferous tubules, and clumping of Leydig cells are demonstrated during clinical and histopathologic evaluations.[1,4]

A 13-yr-old intact male Siberian tiger, weighing 195 kg, housed as a single adult at the Kansas City Zoological Park (KCZP), was immobilized for annual examination during the winter of 1999. As part of the examination, a routine skin biopsy was obtained, and forwarded to the Center for the Reproduction of Endangered Species for processing (C.R.E.S., Cytogenetics Division Zoological Society of San Diego, P.O. Box 120551, San Diego, CA 92112 USA). In addition, testicular measurements and physical examination of the reproductive tract were performed.

Fibroblast cultures of the biopsy were established by tissue dissociation in 0.5% collagenase B (Boehringer Mannheim). Metaphase chromosomes were obtained from heparinized blood and fibroblasts following general protocols.[3] Standard and G-banded karyotypes indicated an abnormal diploid chromosome number as the result of an additional sex chromosome. Contrary to the expected 2n = 38 XY, this tiger demonstrated 2n = 39 XXY.

The tiger demonstrated normal libido, often initiating mating with various enrichment items in his exhibit. However, numerous evaluations of ejaculates revealed no evidence of spermatozoa.

Approximately 1 yr later, the tiger was euthanatized because of multiple intervertebral disc disease (IVDD), confirmed with myelography. Blood obtained prior to euthanasia for a complete blood count and select serum profile revealed no abnormalities other than frequent "drumsticks"[1] observed in neutrophils. Histopathologic examination of formalin-fixed tissue specimens from the reproductive tract revealed a paucity of seminiferous tubules and expansion of the fibrous

interstitium in the testes. Remaining tubules were devoid of spermatogonia and contained moderate numbers of sustentacular (Sertoli) cells. An increase in interstitial fibrous connective tissue was noted in sections of the prostate and epididymis.

No other histologically significant findings of the reproductive tract were demonstrated. A degenerative myelopathy, compatible with IVDD was demonstrated in the spinal cord.

In domestic cats, 39 XXY is most commonly observed in male calico and tortoise cats. The coat colors (black and orange) are controlled by allelic genes on the X chromosome and usually coexist only in individuals with two X chromosomes.[2] Normal male cats have only one X chromosome, which carries the gene for orange.[2] The second X chromosome in XXY individuals allows for the expression of black.

The relationship of the tiger's normal coat colors (black, white and orange) to the XXY syndrome is undetermined at this time. Genetic lineage of this tiger is currently being evaluated.

In conclusion, we believe this finding in an SSP managed, critically endangered species mandates all institutions holding Siberian tigers to obtain skin biopsies for karyotype evaluation. Hopefully select breeding based on genetic lineage will eliminate this abnormality from the population and lead to the continued success of reproducing Siberian tigers in captivity.

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ANESTHESIA OF EASTERN GIANT ELAND (*Taurotragus derbianus gigas*) AT WHITE OAK CONSERVATION CENTER

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Abstract

Giant eland (*Taurotragus derbianus*) are a problematic species to anesthetize because they are very large and prone to hyperthermia, capture myopathy, tympany, and fatal aspiration pneumonia resulting from regurgitation. Two anesthetic drug combinations were compared at White Oak Conservation Center, Yulee, FL USA in order to improve current anesthesia techniques. Anesthesia using a combination of A3080-medetomidine-ketamine (reversal with naltrexone and atipamezole) was compared to a combination of carfentanil-xylazine (reversal with naltrexone and yohimbine). The carfentanil combination is superior to the A3080 combination because it causes less respiratory depression. Regurgitation can occur using either combination, so endotracheal intubation is highly recommended.

Introduction

There are two species of eland, the common eland (*Taurotragus oryx*) and the giant eland (*Taurotragus derbianus*) and they are the largest of the antelope species (an adult bull can weigh up to 910 kg[12]). The two species are very different in their responses to anesthetic drugs (S. Citino, personal communication). Anesthesia of common eland has been reported in the literature, but no reports for giant eland were found.[2-5,9,12] Giant eland are a problematic species to safely anesthetize because they are very large, have a tendency to run after being darted (which can lead to problems associated with hyperthermia and exertional myopathy if the induction period is prolonged) and are prone to tympany and regurgitation (leading to fatal aspiration pneumonia). An anesthetic protocol that results in a rapid and smooth induction with little excitation would be ideal as it would reduce the risk of hyperthermia and capture myopathy. In addition, drugs that lessen the likelihood of regurgitation would be of benefit.

A3080 is a synthesized fentanyl derivative with rapid, pronounced opiate agonist activity and it has a much shorter duration of action with only slightly less potency than carfentanil.[7,9,10] It has been used with success in elk, moose, roan antelope, impala, buffalo, kudu, elephant, waterbuck, and common eland.[1,6-8,10,11] In one study, induction times in elk using A3080 were significantly more rapid than with carfentanil and therefore A3080 may be beneficial in reducing hyperthermia and exertional myopathy.[8] Another study showed no difference in induction times between A3080 and

carfentanil in impala when similar mg/kg dosages were used, but also found induction times with A3080 were dose dependent.[6,7] Like other opioids, A3080 provides little muscle relaxation and is typically used in combination with drugs that provide muscle relaxation (e.g., alpha$_2$-agonists).

Methods

Five procedures using a combination of A3080-medetomidine-ketamine (reversal with naltrexone and atipamezole) were compared to 13 procedures using carfentanil-xylazine (reversal with naltrexone and yohimbine) (Table 1). All animals were clinically healthy and were being anesthetized for either electroejaculation, transportation, horn trimming, or hoof trimming. The females weighed 300-350 kg and the males weighed 400-500 kg.

Times to first effect and recumbency, total anesthesia time, time of reversal, depth of anesthesia, environmental temperature, humidity, and body weight were recorded for each anesthesia. Every 5 min, heart rate, respiratory rate, body temperature, direct blood pressure (A3080 immobilizations only), and SpO_2 were recorded. Within 5 min of induction and at every 10 min, an arterial blood sample was collected from the auricular artery and analyzed for pH, $PaCO_2$, PaO_2, HCO_3^-, TCO_2, base excess, and SaO_2. If possible two blood gas analyses were performed prior to oxygen supplementation by insufflation (10 l/min) or by positive pressure ventilation using a high flow demand valve (160 l/min). Serum creatine kinase and plasma lactate levels were measured following induction and immediately before reversal.

In all cases using A3080 and four of 13 cases using carfentanil, intubation was performed to protect the airway against regurgitation and aspiration (endotracheal tube sizes 16-24 mm).

Results and Discussion

Dosages of drugs used are summarized in Table 1. Mean heart rate, respiratory rate, SpO_2, PaO_2, and $PaCO_2$ trends for the two anesthetic protocols are shown in Figs. 1-3.

Induction was rapid with both anesthetic protocols and induction times were not significantly different. A3080 did not result in faster induction in this study, but this may have resulted from the use of a lower A3080 dose than the carfentanil dose used. With both protocols, some animals dove head first into the ground during induction. In two cases using the A3080 combination, mild regurgitation occurred during induction. This also occurred in one case using the carfentanil combination. Moderate to severe regurgitation occurred during anesthesia in all A3080 cases, though all these cases were held in lateral recumbency for electroejaculation. It was thought that the alpha$_2$-agonist, medetomidine, may have been contributing to the regurgitation, because previous experience with xylazine has shown that when used as the sole agent for sedation in giant eland, it can result in heavy sedation with regurgitation. Regurgitation also occurred in four of the 13 carfentanil immobilizations.

Respiration was severely depressed by the A3080 combination (Fig. 1) with all animals requiring

intermittent positive pressure ventilation (IPPV) through an endotracheal tube using 100% oxygen and a high flow demand valve. Animals anesthetized using the carfentanil combination maintained respiration and were only supplemented with oxygen by insufflation through an endotracheal tube or intranasally. SpO_2 values correlated well with SaO_2 values. SpO_2 were initially only fair (range 82-87) with both combinations, but improved (range 92-99) and were maintained with oxygen supplement (Fig. 2), but in the case of the A3080 immobilizations were probably only maintained due to the IPPV. PaO_2 and $PaCO_2$ trends (Fig. 3) for the A3080 and carfentanil anesthesias cannot be compared because giant eland given A3080 required IPPV with 100% oxygen, while the carfentanil-xylazine anesthetized eland maintained active respiration throughout and were only supplied oxygen via nasal or endotracheal tube insufflation.

Mean heart rates were lower with the A3080 combination than with the carfentanil combination (Fig. 1). This may have resulted from a reflex response to the peripheral vasoconstriction and increased blood pressure caused by the medetomidine in combination with the A3080. Moderate hypertension was seen initially in the A3080 anesthetized eland, but within 50 min all animals were normotensive, suggesting that the A3080-medetomidine combination may have also had a direct effect on lowering heart rate. Temperatures maintained in the range of 37.5-39.5C with both immobilization combinations and probably reflected the rapid inductions with minimal pacing / running times. CK and lactate levels were within acceptable limits. Recovery following reversal was smooth with both combinations and times to standing were similar.

Carfentanil-xylazine is superior to A3080-medetomidine-ketamine in giant eland because it causes less respiratory depression. Regurgitation can occur with both anesthetic combinations, so it is highly recommended to intubate giant eland in order to protect the airway as soon as possible following induction. Further investigation of alternative drug combinations is required to try to eliminate the problem of regurgitation.

One case of A3080-low dose medetomidine-azaperone-ketamine anesthesia and one case of A3080-azaperone-ketamine anesthesia (Table 1) were performed to see if reducing or removing the $alpha_2$-agonist dose from the anesthetic combination would reduce the incidence of regurgitation. Both cases regurgitated.

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Table 1. Immobilization regimes and their effects in eastern giant eland (*Taurotragus derbianus*).

Anesthesia agents	Time to first effect (sec)	Time to recumbency (min)	Reversal agents	Time of reversal (min)	*n*
A3080[a] = 14.4 ± 1.9 g/kg M[c] = 8.6 3.1 g/kg K[e] = 0.86 ± 0.13 mg/kg	72 ± 27	6.9 ± 2.7	N[b] = 430 ± 60 g/kg Ati[d] = 38.9 ± 11.7 g/kg	2.0 ± 0.8	5
C[f] = 5.8 ± 0.9 g/kg X[g] = 70.7 ± 22.3 g/kg	138 ± 57	8.3 ± 4.0	N = 592 ± 100 g/kg Y[h] = 103 ± 20 g/kg	2.7 ± 1.2	13
A3080 = 12.5 g/kg M = 1.9 g/kg Aza[i] = 0.38 mg/kg K = 0.91 mg/kg	60	3	N = 379 g/kg Ati = 7.6 g/kg	2	1
A3080 = 15.7 g/kg Aza = 0.52 mg/kg K = 0.84 mg/kg	120	4	N = 472 g/kg	2	1

[a]A3080 (10 mg/ml, Wildlife Laboratories Inc., Fort Collins, CO 80524 USA).
[b]Naltrexone hydrochloride (50 mg/ml, Trexonil, Wildlife Laboratories Inc.).
[c]Medetomidine (20 mg/ml, Wildlife Laboratories Inc.).
[d]Atipamezole hydrochloride (5 mg/ml, Antisedan, Pfizer Animal Health, Exton, PA 19341 USA).
[e]Ketamine (200 mg/ml, Creative Compounds, Wilsonville, OR 97070 USA).
[f]Carfentanil citrate (3 mg/ml, Wildlife Laboratories, Inc.).
[g]Xylazine (20 mg/ml, Xylazine-20, Butler, Columbus, OH 43228 USA).
[h]Yohimbine hydrochloride (5 mg/ml, Antagonil, Wildlife Laboratories Inc.).
[i]Azaperone (100 mg/ml, Kyron Laboratories Pty. Ltd., Benrose 2094, South Africa).

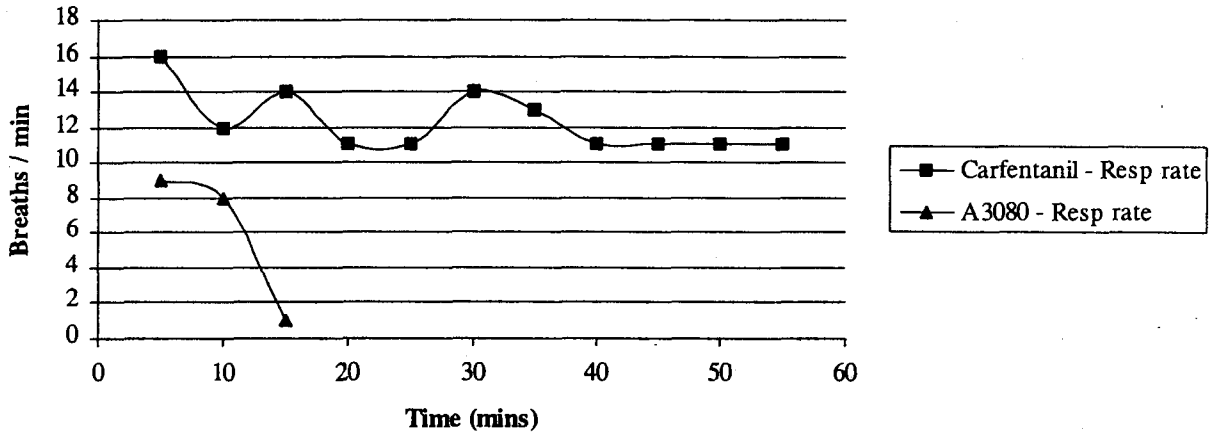

Figure 1. Mean respiratory rate for A3080-medetomidine-ketamine and carfentanil-xylazine anesthesia in eastern giant eland (*Taurotragus derbianus gigas*).

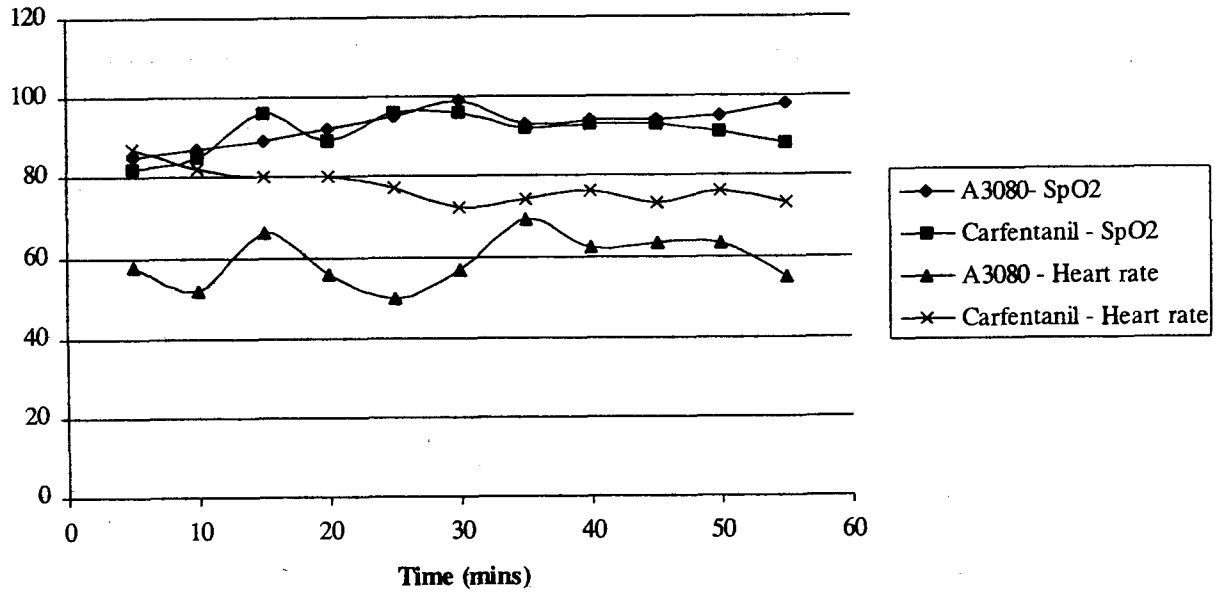

Figure 2. Mean heart rate and SpO_2 values for A3080-medetomidine-ketamine and carfentanil-xylazine anesthesia in eastern giant eland (*Taurotragus derbianus gigas*).

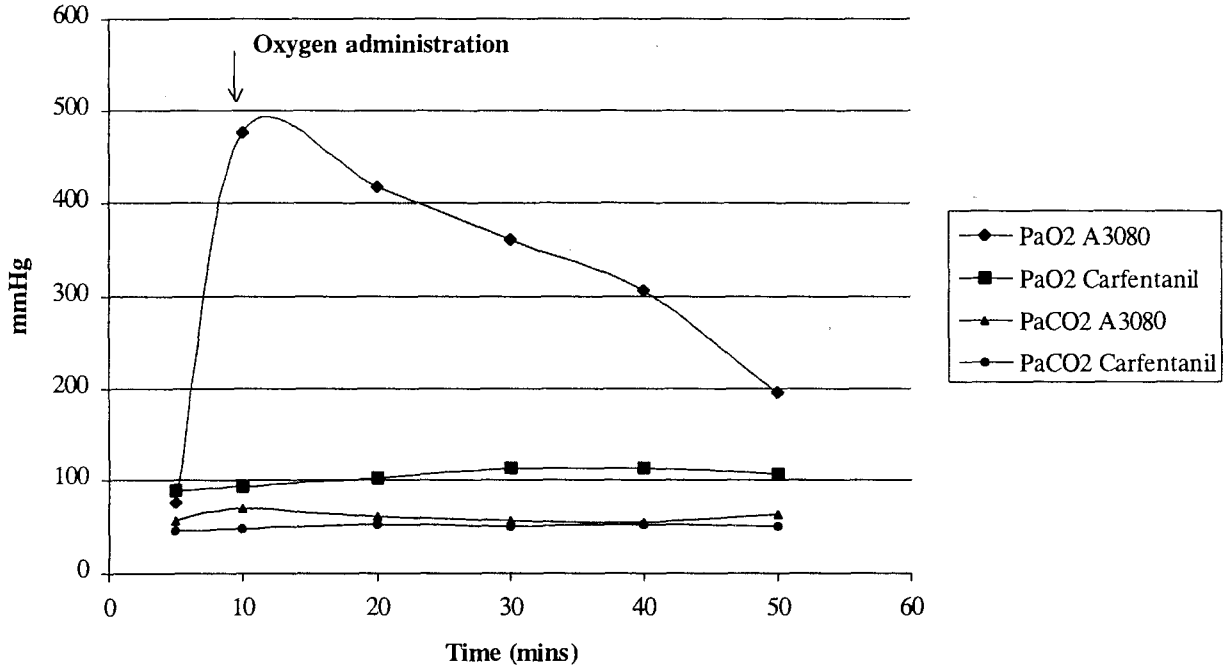

Figure 3. Mean PaO_2 and $PaCO_2$ values for A3080-medetomidine-ketamine and carfentanil-xylazine anesthesia in eastern giant eland (*Taurotragus derbianus gigas*).

IMMOBILIZATION OF CAPTIVE PUDU (*Pudu pudu*) WITH KETAMINE-MEDETOMIDINE-BUTORPHANOL AND REVERSAL WITH ATIPAMEZOLE AND NALOXONE

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Abstract

The pudu (*Pudu pudu*) is a native species of southern Chile. The pudu is listed as vulnerable in the Red Book of terrestrial vertebrates of Chile. Since this species is highly susceptible to stress it is important to have a safe anesthesia protocol when immobilization is required for managing medical and/or management problems and other investigations. Combinations of ketamine, medetomidine and butorphanol have been reported to be useful for routine sedation of small hoofed species.[1] Ten captive pudu were immobilized with ketamine - medetomidine - butorphanol (KMB) in December of 2000 and January of 2001 at the National Zoo in Santiago, Chile. All measurements were recorded and are represented in this report as the average ± one standard deviation for each parameter reported. The body weight (BW) in kg of the study population was measured 1 wk prior to conducting the series of anesthetic procedures reported in this study (Table 1). Each of the animals in the study group were hand injected intramuscularly with a combination of KMB mixed into a single syringe. Each animal was either caught in a hoop net or manually restrained. Once restrained, the KMB injection was given quickly, and the animal was released to reduce stress during induction. Two slightly different protocols were evaluated (groups 1 and 2). Results of KMB effects for each group are summarized in Table 1.

Groups 1 and 2 consisted of five animals each. Each group one animal received KMB at a dose rate of 5 mg/kg of ketamine HCl, 0.07 mg/kg of medetomidine HCl, and 0.3 mg/kg of butorphanol tartrate. Each group 2 received KMB at a dose rate of 2.5 mg/kg ketamine HCl, 0.07 mg/kg of medetomidine HCl, and 0.3 mg/kg of butorphanol tartrate. The induction time (IT) for both groups 1 and 2 was defined as the time post-KMB injection (PI) to the onset of recumbency sufficent to facilitate manual restraint without significant struggling or escape attempts. Once thus sedated, each animal was monitored for 40 min. In all cases, at the point where animals were first restrained the following parameters were measured; temperature (T) was measured in degrees Celcius using a rectal thermometer, heart rate (HR, beats/min.), respiratory rate (RR, respirations/min), and relative hemoglobin saturation (SpO_2) was measured by placing an oximeter probe on the tongue. At 40 min after KMB injection each animal was given atipamezole at 0.35 mg/kg and naloxone at 0.1 mg/kg were administered to reverse the effects of sedation. In all cases 50% of the total atipamezole and naloxone doses were given intramuscularly, and remaining total dose of each antagonist was then given. Recovery time (RT) for groups 1 and 2 was defined as the time in minutes from

administration of antagonists (PA) to standing posture.

The quality of immobilization was good with both protocols, with adequate muscle relaxation and analgesia in both groups to conduct routine medical examination routines (exam, blood collection, administration of minor noxious stimuli). Significant differences ($P < 0.05$) were seen between the protocols for body temperature and heart rate. Pulse oximetry values were strongly suggestive of hypoxemia, which can be addressed in most species by administration of 100% oxygen at 2-4 l/min through a soft rubber cannula inserted into one nostril. Induction time was not affected by ketamine dose, but mild residual ataxia was seen after reversal in group 1, suggesting that the higher dose of ketamine was responsible for the residual ataxia observed. These data demonstrate that KMB produced adequate chemical immobilization in pudu to safely accomplish routine medical/management procedures in the captive pudu examined. Recovery after administration of atipamezole and naloxone was rapid and smooth. Post-recovery sedation was not observed in any of the animals in groups 1 or 2.

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Table 1. Average ± one standard deviation for each of the parameters recorded for KMB-treated animals in group 1 and 2.

	Group 1	Group 2
BW(kg)	9.8 ± 0.6	9.8 ± 0.6
IT (min. PI)	4.9 ± 0.5	4.0 ± 0.9
T (°C)	39.5 ± 0.7	38.9 ± 0.7
HR (b/min.)	105.1 ± 15.7	85.7 ± 20.8
RR (resp/min.)	68.9 ± 15.9	68.8 ± 23.1
SpO_2 (%)	77.4 ± 6.8	79.2 ± 2.8
RT (min.)	3.7 ± 0.5	2.2 ± 0.6

REVERSIBLE IMMOBILIZATION AND ANESTHESIA OF FREE-RANGING BROWN BEARS (*Ursus arctos*) WITH MEDETOMIDINE-TILETAMINE-ZOLAZEPAM AND ATIPAMEZOLE: A REVIEW OF 575 CAPTURES

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Abstract

Tiletamin-zolazepam (TZ) is the drug of choice for immobilization of brown bears (*Ursus arctos*), black bears (*U. americanus*), and polar bears (*U. maritimus*).[4,5] Effective doses in free-ranging individuals are 7-9 mg/kg.[4,6,7] TZ has a wide margin of safety, and has no major cardiopulmonary or thermoregulatory side effects in bears.[3,6-8] The main disadvantage of TZ is extended recoveries. There is no reversal agent for tiletamine, and the use of a benzodiazepine antagonist in animals immobilized with high doses of TZ is not recommended. However, in combination with medetomidine (M), the effective dose of TZ in polar bears can be reduced by as much as 75%, and atipamezole (A) can then be used to shorten the recoveries.[1] The physiologic effects of MTZ have been studied in polar bears and black bears, and this drug combination is well tolerated by healthy individuals.[2,3] The purpose of the present study was to evaluate MTZ and A for reversible immobilization and anesthesia of free-ranging brown bears in Scandinavia.

During an 8-yr-period, 1993-2000, a total of 575 captures of 241 individual free-ranging brown bears were accomplished in Sweden and Norway with combinations of M (Domitor® 1 mg/ml or Zalopine® 10 mg/ml, Orion Pharma Animal Health, Turku, Finland) and TZ (Zoletil® 500 mg dry powder/vial, Virbac International, Carros Cedex, France). The bears were captured for radiotagging, biometry, and biologic sampling as part of an ongoing ecologic and demographic study on Scandinavian brown bears. Bears were darted from a helicopter using DAN-INJECT® equipment (DAN-INJECT ApS, Børkop, Denmark). Captures were made during spring and early summer (April-June). All bears received 5 mg A (Antisedan® 5 mg/ml, Orion Pharma Animal Health) per mg M, administered half i.v. and half s.c. or half i.m. and half s.c., for reversal.

Analyses of drug doses and induction times were carried out for 342 (59.5%) of the 575 capture events, including only animals that were completely immobilized for at least 40 min after a single dart injection and that did not require additional dosing during this period of time, and excluding

animals that were not observed going down (n = 28) and animals that could not be weighed (n = 25). Mean induction times varied from 3.3 to 6.5 min for different classes of bears. Summary statistics are presented in Table 1. The bears were grouped in three age classes: 1-yr-old, 2-yr-old, and adults (animals = 3 yr). A *t*-test (two-sample, two-tailed, unequal variance) was used to compare males and females in each age class, and P-values < 0.05 was considered significant. No significant gender difference were found for 1-yr-old bears, and data for females and males were pooled. In 2-yr-old bears, females received significantly higher drug doses than males. However, there was no significant difference in induction time. In adults, the induction time was significantly longer for males than for females, although there was no significant difference in drug doses.

A total of 72 surgical implantations of intraperitoneal radiotransmitters (Telonics Inc., Meza, AZ, USA) were performed under MTZ anesthesia in 70 individual bears (35 1-yr-old females, 20 1-yr-old males, six 2-yr-old females, 10 adult females, and one adult male). Muscle relaxation was good, and depth of anesthesia was adequate for surgery. Two animals, a 2-yr-old female and a 7-yr-old female with yearling cubs, underwent a second surgery after 1 and 3 yr, respectively, in order to change the implant. No pathologic lesions caused by the initial surgery or implant were seen in these individuals.

Three bears (0.5%) died during immobilization. None of these animals were undergoing surgery. Necropsies showed cardiovascular and respiratory collapse as the primary cause of death in a 2-yr-old male in poor body condition (33 kg; 0.095 mg M/kg + 11.9 mg TZ/kg total drug dose). In a 1-yr-old female (21 kg; 0.031 mg M/kg + 3.1 mg TZ/kg) and a 3-yr-old male (101 kg; 0.020 mg M/kg + 2.5 mg TZ/kg) severe hemorrhagic enteritis was found, but no specific infectious agent was isolated.

Rectal temperature was measured in all bears. In addition, animals undergoing surgery were monitored by use of pulse oximetry. Physiologic data will be presented elsewhere. Due to time constraint and logistics, data on recovery from anesthesia were collected only during the initial phase of this study. However, each bear was located the same day to ensure that the animal had moved away from the capture site. Radiotracking revealed no mortalities related to the capture event or the surgical intervention. No permanent separation of family groups (female with yearling cubs) occurred.

During the course of this study, standard immobilizing doses of MTZ for various age and sex classes were established. The following recommendations are currently applied: 0.5 mg M plus 125 mg TZ in 1-yr-old bears (15-45 kg); 1 mg M plus 250 mg TZ in 2-yr-old and 3-yr-old bears (45-70 kg); 2 mg M plus 500 mg TZ in adult females and subadult males (70-120 kg); 3 mg M plus 750 mg TZ in small adult males (120-180 kg); 4 mg M plus 1000 mg TZ in large adult males (180-240 kg). If the bear is not down in 15 min, a full dose is repeated. All bears are receiving 5 mg A per mg M, administered half i.m. and half s.c., for reversal.

In conclusion, medetomidine-tiletamine-zolazepam is an excellent drug combination for immobilization and anesthesia of free-ranging Scandinavian brown bears. Induction is rapid, the

safety margin is wide, there are no major adverse clinical effects, muscle relaxation is good, and analgesia is adequate for abdominal surgery. By use of atipamezole the recovery times can be shortened.

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Table 1. Summary of drug doses and induction times in free-ranging Scandinavian brown bears (*Ursus arctos*) immobilized for at least 40 min after one single dart injection with medetomidine (M) and tiletamine-zolazepam (TZ) 1993-2000. Data are given as mean ± SD.

	n[a]	M[b] (mg/kg)	TZ[c] (mg/kg)	Induction time (min)
1-yr-old[d]	106	0.035 ± 0.014	4.8 ± 1.7	3.3 ± 1.9
2-yr-old females	21	0.027 ± 0.007	4.4 ± 2.5	4.1 ± 2.0
2-yr-old males	24	0.023 ± 0.006	3.9 ± 1.2	4.4 ± 2.9
Adult females[e]	125	0.021 ± 0.007	4.8 ± 1.6	5.3 ± 3.4
Adult males[e]	66	0.020 ± 0.007	4.7 ± 1.8	6.5 ± 4.2

[a]Number of immobilizations.
[b]Domitor® 1 mg/ml or Zalopine® 10 mg/ml (Orion Pharma Animal Health, Turku, Finland).
[c]Zoletil® 500 mg dry powder/vial (Virbac International, Carros Cedex, France).
[d]Pooled data for males and females.
[e]Animals, 3 yr old.

ONE ELISA FOR ALL ARTIODACTYLIDS: ASSESSMENT OF A PROTEIN G CONJUGATE FOR DETECTION OF ANTIBODY IN NONDOMESTIC HOOFSTOCK

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Abstract

The Johne's disease serum antibody enzyme-linked immunosorbent assay (ELISA) has been a useful herd infection screening tool for domestic agriculture species. This protein-G conjugate test provides valuable diagnostic information and is both fast and inexpensive. Since the ELISA assay has been validated only for cattle in the United States, screening for *M. paratuberculosis* infection in U.S. captive and free-ranging non-domestic species has not benefitted from ELISA technology.[1] Johne's disease surveillance thus has been limited in most cases to fecal culture.

The ability of protein G to bind immunoglobulin in well-characterized laboratory species (rat, mouse, human, etc.) has been shown,[2] but its ability to bind immunoglobulin from most non-domestic hoofstock species has not been well described.[3] To determine if this antibody detection system can be used effectively for multiple non-domestic species, the binding characteristics of the protein G conjugate was assessed for 12 non-domestic hoofstock species and two domestic control species (bovine [positive control], chicken [negative control]; elk, muntjac, white tail deer; bison, sheep, bontebok, impala, llama, kudu/nyala, addax, antelope, oryx).

Partially purified immunoglobulin was obtained through high performance liquid chromatography (HPLC) of sera from six to eight healthy adult individuals belonging to each of the 14 species . Two methods (size-exclusion chromatography and SDS-PAGE) were used to confirm the presence of antibody and to quantify recovery. The binding characteristics of the protein G-horseradish peroxidase detection system utilized in the ELISA were compared for each sample. This was accomplished by measuring the optical density of a uniform amount of immunglobulin from each of the 106 individual samples when tested against seven concentrations (2.0-5.0 g/ml) of solid phase protein G (the antigen).

Discussion and Conclusions

Preliminary data shows protein G is capable of binding immunoglobulins from each of the non-domestic species assessed although the binding patterns were not uniform. Table 1 demonstrates immunoglobulin binding (expressed in mean optical density units for a species) for a uniform amount of immunoglobulin as the protein G antigen was increased from 2.0-5.0 g/ml. As expected, protein G did not bind immunoglobulin from any samples for the negative control species (chicken) at any antigen concentrations. The pattern for the positive control species (bovine) was also as expected: there was a step-wise increase in bound immunglobulin as antigen concentration was

increased. Interspecies immunoglobulin binding variability can be seen most clearly for the muntjac samples. When muntjac results are compared with, for example, white-tail deer (another cervid species) the latter showed even greater binding in the same step-wise pattern than was seen with bovine sera, while immunglobulin from muntjac samples bound poorly at each antigen concentration. Binding within species varied as well, a finding that will need to be considered when the algorithms used to interpret assay results are developed. For instance, statistically significant intraspecies variation was found for all species except bison, elk and white-tail deer ($P = 0.05$). The data did not support an ability to predict binding patterns based on taxonomic categories.

These findings suggest that the protein G conjugate may be useful in detecting antibody from many hoofstock species. This conjugate may therefore be useful not just for detecting antibody due to *M. paratuberculosis* infection but as a surveillance tool for finding antibody to many types of infection that produce antibody (by substituting different antigens in the solid phase portion of the assay). The performance of the ELISA is now being assessed using a repository of sera from confirmed cases of *M. paratuberculosis* infection representing a variety of species. Methods for interpretation and testing protocols are also being developed.

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Table 1. Antibody binding (mean optical densities).

Antigen	Control species	Family Bovidae species									Family Cervidae species			Other
(µg/ml) *	Chicken *n* = 8	Bovine *n* = 5	Bison *n* = 8	Bontebok *n* = 6	Kudu/nyala *n* = 7	Sheep *n* = 8	Addax *n* = 8	Impala *n* = 8	Oryx *n* = 8	Antelope *n* = 8	WT deer *n* =8	Elk *n* = 8	Muntjac *n* = 8	Llama *n* = 8
2	0.05	0.14	0.18	0.58	0.75	0.1	0.12	0.09	0.38	0.10	0.26	0.08	0.07	0.24
2.5	0.05	0.23	0.40	1.04	1.02	0.25	0.31	0.18	0.55	0.20	0.47	0.11	0.10	0.47
3	0.05	0.38	0.65	1.29	1.21	0.47	0.51	0.27	0.64	0.34	0.71	0.18	0.14	0.67
3.5	0.05	0.61	0.90	1.44	1.39	0.78	0.72	0.43	0.77	0.56	0.93	0.32	0.17	0.81
4	0.05	0.7	1.08	1.55	1.49	0.90	0.90	0.57	0.84	0.74	1.08	0.43	0.21	0.93
4.5	0.05	0.88	1.20	1.6	1.56	1.12	1.03	0.68	0.97	0.95	1.22	0.58	0.25	1.05
5	0.06	0.94	1.33	1.66	1.63	1.18	1.17	0.82	1.11	1.13	1.33	0.69	0.29	1.11

The number following the species name indicates number of individual samples (e.g., for chicken [negative control], eight were tested, for bovine [positive control], five were tested).
The OD value is the mean of the individual samples per species (e.g., 0.14 is the mean OD for five bovine samples tested at an antigen [protein G] concentration of 2.0).

DIAGNOSIS AND TREATMENT OF ACUTE RENAL FAILURE IN AN EIGHT-MONTH-OLD GIANT PANDA (*Ailuropoda melanoleuca*)

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Abstract

This report describes acute renal failure in an 8-mo-old female giant panda (*Ailuropoda melanoleuca*). Acute renal failure was diagnosed following an episode of gastrointestinal signs. An elevated blood urea nitrogen (BUN) was present on bloodwork and renomegaly was evident on palpation as well as on ultrasound examination. Kidney biopsy revealed marked tubular necrosis, mineralization, and dilatation. Scintigraphy revealed poor renal function. Her condition improved following a period of intensive therapy however she died 5 mo later. Main gross pathologic changes included renal calcification, liver enlargement, pulmonary edema, gastrointestinal hemorrhage, and mandibular bone deformity.

Case Report

A female giant panda, was born in August of 1999 at the China Research and Conservation Center for the Giant Panda (CRCCGP) in the Wolong Nature Reserve, Sichuan Province, China. Her dam had given birth to triplets and she was raised through a combination of hand-rearing and maternal care up to 6 mo of age. The panda was removed from her dam on 20 February of 2000 as part of a standard protocol to encourage adult females to come into estrus in the spring. Her body condition was not as good as other cubs and she tended to have more problems during this time.

The panda developed diarrhea in mid-March. She was treated with antibiotics, including gentamicin, and the diarrhea resolved. At the beginning of April, she had an episode of decreased activity, diarrhea, and vomiting which resolved within 48 hr. On 9 April she looked uncomfortable, had vomiting, loose stools, serous nasal discharge, and pruritus. She was treated for suspected respiratory and gastrointestinal disease for 3 days. There was little response to therapy. A blood sample was obtained on 13 April. Her BUN was greater than 140 mg/dl and she was acidotic. A hand-held chemistry analyzer was used therefore only a limited number of chemistries were available. On 14 April she was noted to have many small red macula on her skin as well as hair loss and continued pruritus. She was anesthetized for fluid therapy four times during 14-18 April. Physical examination revealed enlarged kidneys on abdominal palpation. Ultrasound examination confirmed renomegaly as well as ascites. Her condition improved following intravenous fluid therapy and therapy was discontinued after 18 April. On 24 April, the panda had vomiting, diarrhea, and was weak. She had developed significant alopecia on her head and abdomen by this time.

The animal was transferred to the People's Hospital of Sichuan in Chengdu for more aggressive diagnostics and treatment. Renal scintigraphy, renal biopsy, and repeat ultrasound examinations were performed there. Kidney biopsy revealed marked tubular necrosis, mineralization, and dilatation. Tubular basement membranes generally appeared to be intact. Remaining non-necrotic tubules were lined by attenuated or hyperplastic, regenerating epithelium. Bowman's spaces were often dilated and some glomeruli were small and atrophic. The interstitium was moderately to markedly expanded by edema, with mild accompanying fibrosis. Ultrasound examination reconfirmed bilateral renomegaly. The left kidney measured 8.4 × 6.4 cm. The right kidney measured 7.5 × 5.0 cm. These values were thought to be about twice the size of a normal kidney for an animal this age. Scintigraphy revealed reduced renal perfusion as well as reduced urine production. In addition to elevations in her BUN and creatinine she was also anemic. Urinalysis revealed a specific gravity of 1.004 (normal range: 1.015-1.028) as well as glucosuria and mild proteinuria.

The treatment plan consisted of intravenous fluid therapy, improving immune function, controlling secondary infections, improving renal circulation, treating the anemia, and adjusting her nutrition. She was treated initially with daily intravenous fluids. As her condition stabilized intravenous fluids were administered every 2 days. Her appetite, activity, and fecal consistency gradually improved though she was still weak relative to a normal juvenile giant panda. A second scintigraphy procedure was performed. Renal perfusion and urine production were improved. Her BUN returned to slightly higher than normal level however her creatinine remained elevated (7.68-6.49 mg/dl).

After 20 days at the People's Hospital, the panda was returned to CRCCGP in Wolong. Her bloodwork was monitored every other day for the next 2 mo. Urinalyses were performed daily. Because she was eating and her bloodwork was relatively stable fluid therapy was not continued. During this time (as well as when she was at the People's Hospital) she was treated with. Chinese caterpillar fungus mixture, Ketosteril, Epiao, as well as oral vitamins and iron supplements. Chinese caterpillar fungus mixture is used frequently in people in China to improve immunity and renal function. Ketosteril is a Chinese medicine containing amino acids (isoleucine, leucine, phenylalanine, methionine, L-lysine, L-threonine, L-tryptophan, L-histidine, L-tyrosine) used to treat renal disease. Epiao is similar to erythropoetin. The dose of Epiao was 75 IU/kg twice weekly. Her anemia initially improved during the first month of treatment but became ineffective during the second month of treatment and was discontinued.

During the next several months her condition was fairly stable. While her vitality improved it was below that of a normal juvenile giant panda. She had several episodes of gastrointestinal disease as well an episode of respiratory disease in September. During this time her BUN ranged between 18 - 36 mg/dl. Creatinine levels continued to be greater than 5 mg/dl. The anemia persisted. Her condition started to deteriorate in October. She developed severe abdominal distension, diarrhea, decreased activity, poor appetite, and hair loss. On October 19th, she developed acute hemorrhagic gastroenteritis and anorexia. She did not respond to treatment and she died on 21 October.

At necropsy the main pathologic changes were observed in the kidneys, liver, gastrointestinal tract,

and mandibular bone. Both kidneys had extensive calcification. Little normal renal tissue was present. The liver was enlarged; liver weight was 1220 g (normal adult liver weight at necropsy ~ 875 g). Severe mucosal hemorrhage was observed in the stomach and intestines, especially in the duodenum. The mandible was shorter than the maxilla. Pulmonary edema was also present. Histologic examination of tissues is pending.

Discussion

This is the first time a case of acute renal failure in a juvenile giant panda has been described. The panda's clinical signs prior to death are compatible with renal failure. While the exact cause is unknown some differentials include congenital disease, toxic insult, immune-mediated, or an infectious etiology.

One of the panda's siblings died at 2 days of age. It was the smallest of the triplets and was suspected to have a congenital problem involving the urinary system based on gross necropsy evaluation. The third triplet currently has higher creatinine and BUN values and it's body condition is not as good compared to other juvenile giant pandas. However, changes consistent with a congenital problem were not evident on the kidney biopsy. Renal failure secondary to gentamicin toxicity is another possibility. This cub received only one dose. Gentamicin has been used frequently at CRCCGP in other young giant pandas without problems. It is possible that this female already had an underlying condition making it more susceptible to gentamicin toxicity such as the episode of gastrointestinal signs prior to developing acute renal failure. Cubs are given access to an outside exercise yard for periods during the day. The panda was observed on occasion eating dirt as well as some wild plants. It is possible that she ingested a toxic compound via this route. No evidence of an infectious agent was seen on the kidney biopsy. Cultures were not performed due to limited diagnostic availability at CRCCGP. It is possible that her episode of vomiting and diarrhea prior to the onset of acute renal failure predisposed her to renal insult. The skin lesions noted are of interest however diagnostics were not performed. It is not know at this time if and how these lesions are related to the renal disease.

BREVETOXICOSIS IN DOUBLE-CRESTED CORMORANTS (*Phalacrocorax auritus*) ALONG THE FLORIDA GULF COAST

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Abstract

Epidemics of morbidity and mortality in double-crested cormorants (*Phalacrocorax auritus*) along Florida's Gulf Coast have been known to occur sporadically for at least 30 yr. During these epidemics, the Clinic for the Rehabilitation of Wildlife (C.R.O.W.), located on Sanibel Island in Lee County, has admitted a substantial number of cormorants with a consistent presentation of clinical signs. Blooms of the marine dinoflagellate, *Gymnodinium breve*, are often called red tides and have long been associated with marine animal mortality in the Gulf of Mexico.[1-4] In order to correlate epidemics in cormorants to exposure to brevetoxin, we compared the timing of admittance of cormorants to C.R.O.W. with epidemic-specific clinical signs to *G. breve* blooms around Sanibel Island from 1995 through 1999. We also report the common clinical and pathologic features found in cormorants admitted to the clinic from six epidemics occurring during this period.

The clinic admitted 360 out of 613 cormorants with the common clinical sign of severe cerebellar ataxia. The ataxia was characterized by a broad based stance, truncal incoordination, hypermetric gait and intention tremors of the head. Stimuli, such as patient handling, often resulted in exaggerated responses and hyperactivity. Positional vertical nystagmus was also noted in the majority of ataxic cormorants. The ataxia typically resolved after 2 to 4 days. Histopathologic findings in 10 cormorants from an epidemic in 1997 were generally non-specific and insignificant.

Admittance of cormorants with epidemic-specific clinical signs was significantly positively correlated with increased concentrations of *G. breve* (cells per litre) in local water ($P < 0.05$). The cross-correlation coefficient was also significant when *G. breve* levels lagged cormorant admittances by 2,4,6, and 8 wk. The time delay between *G. breve* blooms and cormorant admittance is consistent with the clinicopathologic features of this disease, which suggest that cormorants most likely experience a chronic and cumulative detrimental effect from exposure. Overall, from 1995 through 1999, there were six major blooms of *G. breve* around Sanibel Island and six major epidemics in cormorants. The high proportion of cormorants presenting with brevetoxicosis-specific clinical signs to this one rehabilitation center suggests that brevetoxin exposure is a substantial cause

of morbidity and mortality in cormorants in this area of southwest Florida.

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THE USE OF PSYCHOACTIVE DRUGS IN GREAT APES: SURVEY RESULTS

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Abstract

A survey was sent to zoologic institutions housing gorillas questioning the use of psychoactive drug use to control aggression. Survey results, as well as a brief discussion of the types of drugs used for behavior modification are presented.

Introduction

In recent years, psychoactive drugs have been a rapidly expanding component of modern veterinary practice for domestic animals as well as laboratory animals.[1] It is important to remember that the use of these drugs is extra-label and the pharmacologic, toxicologic, and behavioral effects seen may vary greatly from animal to animal. Because many of these drugs have side effects on multiple systems a complete physical examination is recommended before commencing treatment. A complete behavioral analysis should also be performed and a set of problem behaviors to be targeted clearly identified. Oversimplification of a complex behavioral problem may lead to errors that may be dangerous.

All psychoactive drugs are thought to have their effects through five main neurotransmitters in the brain. These neurotransmitters are acetylcholine, dopamine, norepinephrine, serotonin, and γ – aminobutyric acid (GABA). It is important to know which neurotransmitters are affected by each drug class in order to understand the possible related side effects.[6] Acetylcholine is produced from choline and is inactivated by anticholinesterase. Cholinergic receptors have numerous behavioral and physiologic effects that should be understood before initiating treatment with this class of drugs. Many psychoactive drugs affect acetylcholine producing anticholinergic side effects. These may include dry mouth, dilated pupils, GI stasis, and cardiac side effects, which may be of particular concern in captive gorillas.[3] Dopaminergic neurotransmission can occur by at least five subtypes of dopamine receptors, located both pre and post-synaptically. Dopaminergic antagonists may produce behavioral changes without the side effects of cortical depression. Norepinephrine is the precursor of epinephrine and also a central neurotransmitter. Norepinephrine agonists are stimulating and increase arousal through several mechanisms, including the reticular activating system. Serotonin (5-hydroxytryptamine) is produced in the brain from tryptophan. There are at least nine serotonin receptor types, both pre- and post-synaptically, and all with different behavioral effects. Serotonin is thought to be an important neurotransmitter in the regulation of sleep, pain, aggression, thermoregulation, appetite, and sexual behavior. Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter produced from glutamate and is widely distributed throughout the brain. It is thought to have numerous behavioral and metabolic effects.

Psychoactive Drug Groups

Most psychopharmaceuticals used fall into the following four groups, antidepressants, mood stabilizers, antipsychotics (neuroleptics), and anxiolytics.

Antidepressants are generally organized into three classes: the tricyclic antidepressants, the selective serotonin-reuptake inhibitors, and the atypical antidepressants.[6] The antidepressants tend to have a wide range of behavioral effects and can be used to treat other psychologic disorders as well as depression. *Tricyclic antidepressants*: The tricyclic antidepressants are labeled for use in human medicine to treat depression, but also have been noted to have additional usefulness in treating panic disorders and agoraphobia.[6] They have long half-lives, a narrow therapeutic index and there is a considerable risk of toxicity when overdosed. These antidepressants appear to block norepinephrine and serotonin presynaptic neurotransmitter receptors in the brain, reducing norepinephrine and serotonin turnover, thereby increasing their actions. Tricyclic antidepressants can have numerous side effects including cardiovascular side effects, which can be of particular concern in great apes.[3] Other side effects seen include anticholinergic effects. *Selective Serotonin-Reuptake Inhibitors (SSRI):* have a wide therapeutic index with a low incidence of side effects. In human medicine, they have been used to treat depression, obsessive-compulsive disorders, eating disorders and panic disorders.[6] The SSRI enhance central serotonin by blocking presynaptic input at serotonin receptors. They may also act through an increased output or increased postsynaptic receptor sensitivity. They rarely produce sedation and the most commonly seen side effects are gastrointestinal signs, including anorexia, nausea and diarrhea. Anxiety, agitation and insomnia have also been seen in humans.[6] *Atypical Antidepressants:* contains drugs that are different from both tricyclic antidepressants and selective serotonin-reuptake inhibitors.

Mood stabilizers include compounds that are dissimilar in make-up that have been used in human medicine to treat bipolar disorders, reduce impulsivity, emotional reactivity and aggression.[7] These drugs show some promise in great ape due to their effectiveness on aggression, however careful monitoring of blood levels and side effects are needed.

Antipsychotics (neuroleptics or major tranquilizers) have been used in human medicine for many years.[6] They tend to have marked effects on behavior with a low risk of toxic side effects. These drugs all act as dopamine antagonists and block dopamine receptors in the basal nuclei and the limbic system. They can produce behavioral quieting and decreased emotional reactivity with or without some sedation. The antipsychotics are subdivided into low-potency and high-potency drugs. Low potency drugs tend to require higher doses and therefore tend to have more side effects such as increased sedation, cardiovascular effects and anticholinergic effects but with a lower chance of producing extrapyramidal side effects. The high-potency drugs require relatively lower doses, have fewer side effects but have an increased incidence of extrapyramidal side effects. Some side effects seen when using antipsychotics include sedation, anticholinergic effects, and α-adrenergic blocking effects. In humans on high-potency antipsychotics there is also a risk of extrapyramidal motor side effects such as Parkinsonism, dystonic reactions, akathisia and rarely neuroleptic malignant syndrome and tardive dyskinesia.

Anxiolytics have antianxiety properties and include benzodiazepines, azapirones, barbiturates, and antihistamines. Most of their use in great apes appeared to be as pre-medications before stressful events such as anesthesia, transport or introductions. The benzodiazepines and azapirones were the most commonly used and will be discussed. *Benzodiazepines:* In human medicine, drugs in this category have been used to treat anxiety, panic disorders, epilepsy, and insomnia.[7] Because benzodiazepines can have paradoxic effects due to disinhibiting suppressed behaviors, their use to treat aggression should be carefully weighed. Benzodiazepines are believed to act by binding γ-aminobutyric acid (GABA) receptors in the central nervous system. Their anxiolytic properties seem to result from GABA like activity in the cerebral cortex and the limbic system. Some side effects that may be observed when using benzodiazepines include sedation, cortical depression, and muscle relaxation. There is little effect on the respiratory or cardiovascular system although they may potentiate respiratory depression produced by other sedatives. Benzodiazepines have amnesic properties and may interfere with learning conditioned responses. *Azapirones:* Buspirone was reported in great apes and is a non-sedating antianxiety drug that takes approximately 1 wk to initial effect. It has an antianxiety effect gained by blocking serotonin presynaptic and postsynaptic receptors. Buspirone causes down regulation of serotonin receptors and also acts as a dopamine agonist in the brain. This drug lacks sedative, muscle relaxant or anticonvulsant activities and does not impair motor function. *Other Drugs:* that have been used in veterinary medicine to treat behavioral problems such as barbiturates, antihistamines, narcotic antagonists, and progestin hormones.[7] Beta-blockers reduce signs of fearfulness in humans and have helped in one gorilla case. Androcur and Deslorin implants have also shown promise in aggression problems (Table 1).

Survey Results

A summary of all of these survey results is presented in Table 1. Doses, dosages, and results are listed as given. All animals listed are adults unless otherwise specified.

Conclusions

While the use of behavior modifying drugs may aid in the resolution of a problem, environmental management and behavioral modification are also needed in most cases. Veterinarians must understand the ethical issues surrounding how and when to use these drugs in order to maximize their effectiveness. Survey results indicate that as a whole, psychoactive drugs used in great apes to try to curb aggression have not been successful. This could be due to several things including but not limited too; misdiagnosis of behavioral problem type, multifactorial causes, incorrect dosing, inconsistent observations, and short treatment times. Certainly, more research is warranted in this field.

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Table 1. Results of survey.

Drug	Dose given	Dosage range	Animal	Drug class	Reason given	Response	Side effects seen
Androcur			Male chimp, Male spider monkeys	Anti-androgen	Aggression	Good	None
Alprazolam (Xanax)	0.25 mg s.i.d.	0.0024mg/ kg (combined with Prozac 0.2 mg/kg)	Adult female gorilla	Anxiolytic	Anxiety	Good	None
Atenolol	50-75 mg s.i.d.	0.48-0.7 mg/kg s.i.d.	Adult female gorilla	β blocker	Anxiety	Mixed	None
Buspirone (Buspar)		0.1 mg/kg b.i.d.	Adult male gorilla	Anxiolytic	Anxiety	Good	None
Clonazepam (Klonopin)	1-1.5 mg b.i.d.		Adult female gorilla	Anxiolytic	Self-mutilation	Fair	None
Deslorin implants			Lion tailed macaques	GnRH antagonist	Aggression		? Long term effects on fertility
Diazepam (Valium)	2.5-10 mg s.i.d.-b.i.d.		Bonobos	Anxiolytic	Sedation	Mixed	Titrate to effect
	20 mg b.i.d.		Female gorilla		Anxiety		Drowsiness at higher doses
	1.25-2.5 mg		All ages of gorillas		Pre-med		Depression
	30 – 60 mg		Gorillas		Intros	Mixed	
		0.25 mg/kg	Gorillas		Premed/ sedation	Good	
		0.2-0.6 mg/kg	Gorillas all ages		Premeds	Good	
Fluoxetine (Prozac)	10-40 mg s.i.d.		Adult female gorilla	Anti-depressant	Self mutilation	Poor	Got worse
		0.3-0.6 mg/kg s.i.d.	Juvenile male gorilla		Anxiety	Good	
		0.12 mg/kg starting	Male gorilla				
	20-80 mg s.i.d.				Aggression/combined with Haldol	Poor	Lethargy and diarrhea

Table 1. Results of survey (continued).

Drug	Dose given	Dosage range	Animal	Drug class	Reason given	Response	Side effects seen
Haloperidol (Haldol)		0.1 mg/kg	Gorilla	Anti-psychotic	Anxiety		No effect
	1-4 mg t.i.d. (increased over 4 days)		Female Gorilla		Anxiety	Slight improvement	
	3.75 mg b.i.d. then s.i.d.		Male Gorilla		Post-op	Calm but picked sutures	Drowsiness
	2.5 mg i.m.	Combined with diazepam	Female gorilla		Post-op	Good	Drowsiness
	15-20 mg		Male gorillas Juvenile male gorilla		Intros	Calm but still aggressive No effect	
	10mg				Intros		
	0.5 mg b.i.d.		Female gorilla Male gorilla		Anxiety	Good	
	10 – 50 mg s.i.d.	0.06 mg/kg to start			Aggression	Poor	Lethargy at high dose
Lorazepam (Ativan)	3 mg b.i.d.	0.2 mg/kg	Female Gorilla	Anxiolytic	Anxiety, group intro	Good	Lost fear, too playful
Midazolam (Versed)	5-10 mg 12.5 mg 70 mg		Gorillas Gorillas Female gorilla	Anxiolytic	Pre-med Pre-med Pre-med	Mixed	Excitation
Naltrexone	50mg s.i.d.		Female gorilla	Narcotic antagonist	Self mutilation	Poor	None
Paroxetine (Paxil)	20 mg s.i.d.	0.2 mg/kg	Female gorilla	Anti-depressant	Anxiety	Good	None
Quatiapine (Seroquel)			Male gorilla	Anti-psychotic	Aggressive to females	Fair	None
Risperidol	4mg s.i.d. x 14 days, then increased by 2 mg q 4 days to 12 mg b.i.d.		Male gorilla	Anti-psychotic	Aggression	Mixed	Sedation, GI

Table 1. Results of survey (continued).

Drug	Dose given	Dosage range	Animal	Drug class	Reason given	Response	Side effects seen
Sertraline (Zoloft)	50-100 mg s.i.d.		Female Gorilla	Anti-depressant	Self mutilation	Good	Drowsiness, anorexia
	100 mg s.i.d.		Female gorilla		Self-mutilation	Good when combined with Clonazepam	
		0.5-2.2 mg/kg s.i.d.	Male gorilla		Aggression	Poor	
Thoridazine (Mellaril)	200 mg s.i.d.		Male gorilla	Anti-psychotic	Facilitate breeding	Mixed	Drowsiness at high doses
	80 mg b.i.d. x 7 days, 90 mg b.i.d. x 7days, 150 mg b.i.d. x 7 days (maintenance at 100 mg b.i.d.)		Male chimp		Aggression	Good, switched to generic liquid for better acceptance and cheaper	Drowsiness at higher dose
	50-350 mg s.i.d. 200 mg s.i.d.		Male gorilla				
	100 mg b.i.d.		Male chimp		Aggression		
Zuclopent-hixol Dihydro-chloride		0.2-0.4 mg/kg b.i.d.	Gorilla	Antipsychotic	Intros and transport	Very good	

GREAT APE ESCAPES: OBSERVATIONS ON THREE CASES RESOLVED WITHOUT INCIDENT WITH THREE SPECIES OF GREAT APES

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Abstract

Great apes are the most intelligent animals in zoologic institutions. Due to their intelligence and strength, apes pose significant risks to employees, themselves, and the public when escapes occur. In some instances, dramatic action is necessary to resolve dangerous situations.

Three cases of great ape escapes are presented here which all occurred at the Kansas City Zoological Park (KCZP). All three were resolved without injury to the animal, public or staff. Animal escape protocols were generally followed and successful. With each escape, revision of these protocols influenced the successful resolution of future escapes.

Case 1

In 1995, a 31-yr-old female chimpanzee (*Pan troglodytes*) escaped over a 4.5-m wall and into the public area 12 days after introduction into the new 3-acre chimpanzee exhibit at the KCZP. A call from a keeper in an adjacent area alerted the primate keeper that a chimpanzee had escaped onto a boardwalk adjacent to the exhibit. A small crowd of patrons took pictures of her sitting within 2 m of children and adults. The chimpanzee was calm and made no aggressive movements until she saw the keeper approach, whereupon she charged the keeper. The keeper struck the chimpanzee and escaped into an adjacent brushpile. The keeper radioed the staff, which descended upon the enclosure. Patrons were locked in the closest restrooms and security guards protected entrances. Patrons in the rest of the zoo were escorted out of the zoo. Upon seeing zoo staff approaching, the chimpanzee jumped back into the exhibit. The chimpanzee troop was taken off exhibit and an inspection of the outdoor facility revealed that the escaped chimpanzee had climbed a pole supporting hot-wires, sat on top of the pole and jumped approximately 4.6 m to the top of the wall, whereupon she pulled herself over. A piece of acrylic was subsequently bolted to the wall and no further escapes have occurred.

In this case, an exhibit design flaw was corrected. This incident also prompted the purchase and usage of deterrent sprays carried by all animal staff. In addition, all staff is required to respond to any animal escape with appropriate poles, etc., as self-protection to distance or help fend off an attack. Stockpiles of adequate poles are located in strategic locations in the zoo for use during animal escapes.

Case 2

In 1997, a 14-yr-old lowland gorilla (*Gorilla gorilla*) escaped into the keeper area of the gorilla building. The keeper had forgotten to secure an overhead door and while working in one empty stall, saw a shadow pass by, whereupon the keeper looked out of the stall and witnessed the kitchen door close. The next set of doors at the time was not secure from the inside, but the gorilla proceeded to explore the kitchen area instead. The keeper opened the nearest stall door and threw the kitchen door open, whereupon the gorilla rushed down the hallway. The keeper ran from the stall, past the kitchen door, and manually held the door closed while the gorilla attempted to open the doors. The keeper subsequently called for help. Patrons were escorted from the forest exhibit, and the rest of the zoo. Veterinary staff immobilized the gorilla by darting him in the hallway through a darting portal. All controls to hydraulic doorways/exhibit, etc, were located in the same hallway the gorilla escaped into. The gorilla ran onto exhibit, whereupon he became immobile. The building was secured while additional staff monitored the anesthetized animal with binoculars. The gorilla was tuberculin tested, blood drawn, and placed in a stall to recover. Since that time, the doors have been reinforced, the outside doors must be key-locked, and expanded metal grates have been installed across windows. The controls to doorways, exhibit, etc, are still located in the hallway.

In this case, keeper error led to the escape. However, the escape pointed out several design flaws with the holding area, security doors, darting portals, and windows. Staff response was immediate. Firearms and deterrent spray were available. Most of the staff responded with some type of pole, etc. Two perimeters, one inside the building and one outside were established prior to immobilization. The resolution of this escape was an entire success.

Case 3

In 1998, a 12-yr-old male Bornean orangutan (*Pongo pygmaeus pygmaeus*) escaped from his outdoor enclosure by leaning a tire against a wall, reaching and subsequently grounding the hot-wire, and pulled himself over the wall. A zoo-grounds worker radioed staff that a member of the public reported being chased by an orangutan. When asked to confirm what the public was seeing, the grounds worker stated it was a large, orange colored animal which was sitting in a chair when the visitor opened an exhibit door. The orangutan chased the visitor, who ducked inside the door of the exhibit and waited until the orangutan stopped pounding upon the door, whereupon they contacted the grounds person. The keeper confirmed the escape, and staff responded immediately, forming a double-staff perimeter in constant visual contact with the animal. The ARCS committee, which maintains firearms in cases of animal escapes, was properly positioned and in constant visual and radio contact should lethal force have been necessary. The animal health staff drove by the orangutan and darted him, whereupon the orangutan chased the vehicle, climbed across the windshield, and subsequently became recumbent in the farm garden of the zoo. The orangutan was physically examined and returned to the inside holding area.

In this case, staff responding to the escape were prepared with poles, deterrent spray, visual and radio communication. Staff did not predict the risks associated with the growth of this young

orangutan to adult size, which contributed to his escape. The dry moated area was deepened, and tires were removed.

Conclusions

In none of the cases were the staff, public, or animal injured. Immediate response by security staff, animal response team and veterinary staff resolved all three cases without incident. In these cases, human error and design flaws contributed to the escapes.

In conclusion, great ape escapes, as with all animal escapes, can be effectively resolved. A calm, logical approach to each situation is mandatory for successful resolution of a great ape escape. Every effort to prevent escape is an obvious but not always predictable goal.

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A TIGER (*Panthera tigris*) ATTACK ON A KEEPER: A VETERINARIAN'S PERSPECTIVE

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Introduction

Discussions of attacks by captive wildlife on keepers and other zoo personnel are often limited. This is understandable, given the emotional impact of such events, potential political repercussions from supervisors and co-workers who learn of such discussions, and the complexity of legal actions. Nevertheless, development of strategies for minimizing the occurrence of such events and improved responses to such events requires discussion of animal attacks. The intent of this presentation is to provide one perspective on one attack by a tiger (*Panthera tigris*) on a keeper that occurred at the author's previous place of employment. The goal is to provide information for consideration, and derive general messages that can be applied to prevent crises in other situations. It is important to recognize that the perspective offered and the responses to this event were not necessarily the only perspectives or the optimal responses.

Prelude: The Stage is Set

Injuries and death due to attacks by captive wildlife on people are always possible if dangerous wildlife are kept in captivity, and human error is always a potential factor contributing to such attacks. A few of the issues that have precipitated attacks and similar crises at various institutions in the past include:

Facility Design Flaws

Facility design flaws do not appear to be a factor in this attack. However, design flaws have been recognized in other facilities where attacks have occurred. The failure of such concerns to be addressed during the design phase of these facilities can be attributed, in part, to failure to establish a cooperative, inclusive work culture.

Keeper Training and Compliance with Policies

Appropriate training and daily work policies should be the starting point for prevention of an attack. A number of policies and standards of practice were in place at the facility when the tiger attack occurred. Keepers were not authorized to work without a supervisor present until they had demonstrated an appropriate level of proficiency and were following the policies for the area. A policy of note was that keepers were not required to work with the tiger if they did not feel that they could handle it on a given day, with no questions asked. Similarly, a supervisor could relieve a keeper of their tiger responsibilities with no questions asked. The inside tiger holding area consisted of a keeper work area with two tiger cages on either side, and a squeeze cage connecting the two

sides. This resulted in a "C" shaped arrangement of connected cages, and keepers were expected to always keep at least two locked doors between themselves and the tigers while they were working in tiger cages. Keepers were also expected to work alone after their training period, with the intention of preventing situations where a failure to communicate would permit one keeper to accidentally open a door that would allow a tiger direct access to another keeper. Keepers were required to carry a pepper spray product (Counter Assault, Kalispell, MT 59901 USA) when entering keeper work areas or holding areas for the tigers. Adherence to standard daily policies would have prevented this tiger attack.

Dangerous Animal Escape Protocol Developed and In Place

It is recognized that even strict adherence to daily policies will not prevent all crisis situations with potentially dangerous captive wildlife. Therefore, zoologic institutions develop plans for escapes and other crises. Such plans should be developed with the input of all relevant personnel, including animal care staff, supervisors, maintenance and facility personnel, and top administration. Once a plan is in place, regular training and practice is required to facilitate the institution's response to a crisis. For dangerous wildlife such as tigers, appropriate response team selection and training for rifle certification and darting is essential. Attention to adequate insurance and liability coverage for the staff is prudent. It is important to communicate such plans to local law enforcement personnel so that response efforts can be coordinated effectively during a crisis. In addition, establishing a good working relationship with the media in advance of a crisis is highly desirable. Deficiencies in the above can undercut an institution's response to a crisis, and had variable impacts for dealing with the tiger attack in this instance.

Animal Temperament

All individuals of a potentially dangerous species should be considered a risk to human health and not under-estimated. It must be recognized that an individual animal has opportunities to contemplate escape and attack opportunities on a full-time basis. In contrast, staff attention is divided among other animals and responsibilities in a professional setting. Attention is also devoted to personal responsibilities and concerns. Consequently, the opportunity for a dangerous animal to capitalize on staff mistakes exists. The individual tiger in this attack was perceived by staff to have an aggressive nature. In this instance, human error coupled with an aggressive animal lead to an attack that could have had a fatal outcome.

Work Culture

Common issues of concern for many zoologic institutions include interpersonal conflict, effective and appropriate communication, and staff morale. Interpersonal conflict and dissension among staff in the section of the zoo where the tiger attack occurred was a problem, in general. Conflict between the victim of the attack and a supervisor was a problem, in particular. Personality conflict, significant differences in opinion, and an inability to work together in a productive manner undoubtedly contributed to the occurrence of the attack by distracting employees and creating

negative attitudes.

The Accident

On the day of the accident, the keeper (victim) entered a tiger cage for routine cleaning, and no doors were in the closed and locked position between the tiger and the keeper. The tiger recognized this opportunity, traveled unimpeded between cages to the cage where the keeper was located, and attacked. A volunteer in a nearby office heard some unusual sounds and contacted another keeper. This keeper (the rescuer) entered the keeper work area and found the victim in a tiger cage sitting up with the tiger chewing on the victim's shoulder. The rescuer told the victim to close her eyes, and then sprayed the tiger with pepper spray. The tiger reacted to the spray, released the victim, moved to another cage, and the rescuer pulled the victim out of the tiger holding area while closing two locks on the way out. An ambulance was subsequently called, the tiger area supervisor (Supervisor A) for the day arrived, and supervisors and keepers from other areas of the facility arrived to provide physical assistance and comfort the victim. One of the victim's few memories of the incident was another keeper holding her hand and speaking with her.

At the time that the victim was being assaulted by the tiger, the veterinarian was off grounds speaking on the telephone with a supervisor (Supervisor B) in another area of the zoo. The veterinarian heard human vocalizations over the zoo's radio in the background that were not recognized until later that evening as the victim vocalizing in an attempt to attract attention. Subsequently, the veterinarian heard the rescuer state in a distressed voice that "X was doing Y to Z and would not stop." Supervisor B promptly stated that she had to hang up and depart. As the veterinarian was not certain what animal was doing what action to another animal or human, he telephoned an area of the zoo where he was certain that somebody would answer the telephone. The person answering the telephone was not capable of relaying information about the radio conversations that were concurrently heard by the veterinarian in the background. The veterinarian departed from home and arrived at the zoo approximately 15 min later. The media had already arrived, and the veterinarian parked along the street and ran toward where the media appeared to be congregating.

Upon arrival at the tiger facility, Supervisor A met the veterinarian. He explained that a tiger attack occurred, an ambulance had taken the victim to the hospital, the victim appeared to be stable, and that Supervisor B had been unable to load darts as per the established escape protocol for immobilizing escaped tigers. The curator was intent on having the tiger darted, as the local police were approaching him about shooting the tiger. The tiger was intermittently visible through a window in the keeper work area, but was contained in the tiger holding area and not a threat to human safety. It was therefore possible at this time to address employee needs, such as ensuring that the rescuer was not left alone and received adequate medical care for irritation due to pepper spray.

The emotionally charged atmosphere around the tiger enclosure hampered discussion of the cause of the incident and the appropriate response. The tiger was contained and not an immediate threat to human safety. Therefore, it was possible for the veterinarian to request a meeting with the

Curator, and Supervisors A and B in a nearby room. During this time, one of the maintenance supervisors took the initiative to inform the police that the tiger was contained and that the police needed to leave zoo grounds. At that time, it was uncertain whether the attack occurred due to failure to lock doors, failure of the enclosure to contain the tiger, or other explanations. Due to this uncertainty and concern for human safety, the veterinarian and curator failed to agree on the need for immediate darting strategies. Due to the disagreement and the highly energized atmosphere, the veterinarian departed with the intent of bringing an air pistol and alternate darting system to the scene. This also gave the veterinarian the opportunity to 'step back' and reevaluate the situation. While outside of the building containing the tiger enclosure, the veterinarian observed the potential for the tiger to be shifted into an outside enclosure. Therefore, a plan was established with Supervisor A to shift the tiger outside and then close the shift door. Before this was done, radios were checked, and all participants had at least one spotter with a working radio to monitor for errors and hazards. The tiger was easily shifted outside and contained in the outside enclosure without the need to use chemical immobilization.

After the tiger was safely located in an outside pen, it was possible to examine the inside enclosure. At that time, it was possible to establish that enclosure failure had not occurred. Keepers were able to initiate normal closing procedures, and cleanup of the tiger area was delayed until the following day. The veterinarian was able to address emergency darting equipment needs, at this point, to ensure that it would be possible to respond to a subsequent emergency. The veterinarian conducted a visual examination of the tiger and established that the tiger did not suffer adverse effects from the pepper spray. The veterinarian was also able to initiate a review of the tiger's medical records to determine rabies vaccination status and identify other potential concerns for the keeper's health. A Web-based search for literature on the microbial flora most likely to contaminate the keeper's wounds and appropriate antibiotic prophylaxis was conducted. This was provided to the victim's health care providers later that evening. Further discussion with the victim's clinicians occurred over the next few days regarding the risk of rabies and other diseases. Within 2 hr of the attack the media had contacted the veterinarian by telephone and pager by systematically calling telephone numbers with the zoo's prefix and hearing the contact information provided by the veterinarian's voicemail message.

The Aftermath

The day following the attack, a crisis counselor was brought in to assist staff that were involved in the incident. The inclusion of a crisis counselor in the institutions crisis response was critical for addressing staff emotional needs. The facility's USDA inspector subsequently discussed the incident with the tiger's curator, keepers that were involved in the incident, and the facility's Director. Some discussion occurred about the incident among senior staff. The curator indicated several times after the attack that the veterinarian responded to the attack differently than the curator, but never elaborated on his feelings or opinions of the incident. The keeper is still disabled and has significant pain from her injuries.

Discussion

A common theme to many attacks by captive wildlife in zoo and aquarium settings is the failure of the institution to resolve interpersonal conflict and work culture issues. This results in failure to communicate and resolve animal management and safety issues, including facility design and daily animal management concerns. Poor morale and decreased productivity and carelessness can arise. Subsequently, human error may occur and provide the animal with an opportunity to attack or escape. Such an event can contribute to a worsening of the work culture and personal interactions. Thus, building consensus should be an important goal of zoo personnel management, and will prevent the occurrence of many crises. This may be accomplished with the assistance of management consultants (especially those specializing in work culture issues), regular staff meetings, staff retreats, and strategic planning where problems are identified and goals are set.

A significant challenge is the identification of appropriate disciplinary responses to violations of policy. This is a difficult issue to address, as the work culture and the personal response of the violator need to mesh for disciplinary actions to succeed. A particular challenge is how to address "close calls" and other violations that require self-reporting by employees.

As tragic as the tiger attack was, such crises are an opportunity to learn, grow, and improve institutional deficiencies and policies, as well as human safety and animal welfare issues. This incident indicates the value of having well trained staff with good communication skills, stable personalities, an ability to think and respond quickly to novel events, and strong ethical standards, as many individuals rose to the occasion and provided appropriate responses to the animal crisis and the personal needs of the victim and the rescuer.

This incident illustrated the potential for conflicts in decision making authority to arise. It is a challenge to completely address such issues in advance, as an individual's ability to respond to a crisis can be difficult to anticipate. Some facilities address such issues by including flexibility in the decision-making process based on the personnel available and the particular circumstances at hand. Other institutions appear to have a more rigid hierarchy. Nevertheless, it is important to have a chain of command in place for any given situation, and that this be clear to all participants. In this instance, the veterinarian had control of decisions regarding the tiger by virtue of his expertise in the equipment and drugs required for chemical immobilization.

In the effort to glean lessons for future incidents from this event, it is important to remember that the safety of the responders was not compromised after the rescuer and victim left the tiger area. In part, this was a luxury provided by the fact that the tiger was safely contained in the tiger holding area. This provided an opportunity to establish spotters, verify that radios were working, and consider response options. This scenario will not be available in many instances. Although this was a novel situation that had not been practiced, it does not negate the value of regular drills, role-playing, and discussion for developing the traits needed to quickly assess and respond to novel circumstances. In this instance, the inability to successfully employ the dart system prevented the unnecessary darting of the tiger when it was in a contained situation, although the emergency

response plan called for darting. However, this failure indicated a failure in the advance preparation for such events.

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DEALING WITH THE PRESS IN TRYING TIMES

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Abstract

Escapes, accidents, tragedies, deaths, and controvertial decisions at an institution may require the zoo veterinarian to emerge as a spokesman. Dealing with the press and other media, even under the best of circumstances, can be an uncomfortable experience.

Using the approach that you are conversing with a concerned, but uninformed acquaintance or neighbor helps establish in your own mind a working frame of reference. YOU are the expert, a caring, skilled medical professional, whether you happen to feel that way or not at the time.

Do your homework! Know the facts. Be informed. Present the material in a straightforward manner.

Avoid being condescending. Use terms, situations, and examples that the writer can relate to. Explain the situation as you would to your parents. Journalists may not have 8-10 yr of advanced medical training, but most are intelligent, perceptive people. Some may have an agenda, but most do not and are honestly just doing their job.

Be consistent in what you present. Keep your message simple. Pare down extraneous information. Cluttering up the conversation with a dozen differential diagnoses to impress someone with your vast knowledge will only serve to confuse them. Your message will become lost.

Having a primary working diagnosis with one or two differentials will allow the reporter to focus the coverage better, but allow you the flexibility to shift the emphasis of your workup as new information becomes available.

Don't be afraid to admit that you don't know, but keep in the forefront that you have laboratory tests pending, histopathology under examination, and have been and are consulting other "experts" in the field from other institutions.

Have strength of conviction in your arguments. If you don't believe what you're saying, you certainly won't be able to convince anyone else of your stand on an issue.

Demonstrate compassion and empathy for the creatures in your care. You may be the medical professional who bases decisions on scientific evidence, factual data, and medical reality. You are also the kid who became a veterinarian because you liked animals. None of us is going to get rich being a zoo veterinarian.

Lastly, arm yourself with honesty, credibility, and competence. Unless you have established a reputation for these traits with your own keepers and staff, a perceptive journalist will sense the disparity. The most precious compliment paid is from the keeper who thanks you for all you've done…and the animal died. With support like this, dealing with the press in trying times becomes immensely easier.

LETHAL WEAPONS: A VETERINARIAN'S PERSPECTIVE

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Portions of this paper first appeared in the American Zoo and Aquarium Association's 1996 Annual Conference Proceedings**.**

Abstract

It is common for zoos to exhibit animal species that are potentially dangerous. These animals could inflict serious injury or cause death should they come into direct contact with people. Although modern zoo exhibits are designed to safely contain the animals and reduce the risk of injury to zoo staff and visitors, history has shown that no exhibit is perfectly designed. An especially difficult task is to prevent the public from getting too close or even entering a dangerous animal exhibit.

Highly publicized tragedies with circus elephants have also shown that zoo professionals can be asked for assistance in responding to emergency situations. These tragic events are uncommon and unlikely to occur in an American Zoo and Aquarium Association (AZA) accredited institution. However, even in a well-managed facility it is prudent for an organization with dangerous animals to have in place an emergency protocol listing standard operating procedures to respond to animal escapes. At the least, such documentation might lessen an institution's liability should an event occur. A recent survey indicated that 16 of the responding AZA accredited institutions had experienced dangerous animal escapes and 13 "had to discharge firearms kept on site during a dangerous animal situation."[1]

This commentary discusses the very rare occurrence of when a dangerous animal must be shot. This is an act of last resort when a human life is in imminent danger and the animal must be killed. In this situation attempts at tranquilization and recapture may prolong the exposure of the human victim and increase the chance of serious injury or death. If humans are not at risk but the dangerous animal must still be killed, then attempts at sedation can be made. After chemical immobilization of the animal, a qualified veterinarian can administer euthanasia solutions such as pentobarbital sodium or potassium chloride.

Many zoos have well-written "Dangerous Animal Escape Guidelines." The Honolulu Zoo has over the last several years updated and modified its own guidelines following the excellent protocols of other zoos such as the San Diego Zoo and the Houston Zoo. In 1994, a tragic event with a circus elephant in Honolulu prompted additional changes in our zoo's protocol. We offer this information for your review and consideration, and not as a recommendation of how all zoos should operate.

In the past, many zoos with dangerous animals have assigned staff members to a shooting team with killing weapons. These staff members often have been trained with the assistance of the local police

department (usually the Special Weapons and Tactics or SWAT team). Although these police officers are highly trained and qualified, they may lack the experience, knowledge, and weapons to deal with large dangerous exotic animals. Ironically, one of the best sources of information in this area comes from a group that zoo professionals usually don't associate with, namely big game hunters.

While it may be uncomfortable for some zoo professionals to consult with and study the methods used by hunters, it must be remembered that the knowledge gained is vital to the understanding of dealing with an escaped dangerous animal. In these litigious times, it would seem prudent not to ignore important information that is common knowledge to one group of people. Additionally, if such a definitive judgment has to be made to kill an animal to save a human life, it is incumbent upon the individual making that decision to carry out that action in the most expedient manner to lessen the suffering of the animal. It would seem logical that the zoo veterinarian be intimately involved with this process. The zoo veterinarian has the education, training and the authority to euthanatize animals. The zoo veterinarian has the experience of judging how and when to dart an escaped zoo animal. All of this accumulated knowledge should be incorporated into the zoo's firearms protocol.

Even before obtaining a weapon, a review of current local statutes should be done to ascertain the extent of liability for staff members on the shooting team. This is especially important if a dangerous animal escapes from the zoo grounds and must be pursued beyond the zoo's perimeter fence. Without some acknowledgment or written authorization from local law enforcement agencies, zoo staff could be subject to criminal penalties, even while taking justifiable steps to protect the public. The 1996 Lautenberg Amendment to the federal Gun Control Act of 1968 prohibits anyone convicted of a misdemeanor crime of domestic violence from shipping, transporting, receiving or possessing firearms or ammunition. The penalty for this felony offense is a fine of $250,000 and/or 10 yr in prison. The amendment specifically does not exempt government agencies, including law enforcement and the military. In order to comply with the law, members of the shooting team may need to register yearly with the local police department, who will conduct a criminal background search through the Federal Bureau of Investigation.

The selection of the shooters from zoo staff is possibly the most important decision of this procedure. The good judgment of the shooter is, I believe, the most critical factor to be considered. Your local police department could provide you with a psychologist to administer psychologic screening, as done with police recruits if you choose to have formal documentation of the selection process. Obviously, proficiency with firearms in terms of shooting accuracy is necessary, but this can be addressed as a training issue. We have utilized the services of National Rifle Association (NRA)-certified instructors and a former army drill instructor with the 82nd Airborne Division.

To cover firearms safety training we have sought instruction from the city shooting Range Master and from the State Hunter Safety program. We have conducted walk through training scenarios of zoo grounds with the safety instructors, safari hunters, and SWAT team officers.

The choice of weapons should be determined by the kind of dangerous animal they would be used against. Here again, the premise is that human life is in imminent danger. In this instance, we feel the objective would be to kill the animal as quickly and as safely as possible. We believe the goal would be to shoot one bullet into the animal's midbrain. Another zoo, judging this goal to be very difficult to achieve initially, has a protocol that instructs the shooter to first disable the animal (e.g., bull elephant) in the leg, before targeting the brain.

In order to achieve our objective of stopping the animal with one shot, we have accepted the recommendations of big game hunters. These recommendations are detailed to the point of suggesting the kind of rifle, the type of cartridge, and the weight of the bullet. A good reference for this type of information is *Lyman's Guide To: Big Game Cartridges & Rifles* by Edward Matunas.[21] A comprehensive discussion of weapon selection and response in a zoologic institution can be found in the paper by Baker in the American Association of Zoo Keepers publication *Resources For Crisis Management in Zoos and Other Animal Care Facilities*.[2] Basic knowledge about projectile ballistics can be gained by studying articles by Michael Pavletic.[24] There are many important references that span a variety of information on weapons and their use.[1-31]

For the largest African mammals (elephant, rhinoceros, hippopotamus, buffalo), big bore cartridges are recommended. These include 500 grain solid bullets of the 458 Winchester magnum or the 416 Rigby calibers. Good bolt-action rifles, such as the Pre-1964 vintage Winchester models are recommended. Since the shooting distances are generally short, riflescopes are not generally recommended, although the newer "Red Dot" type scopes could be considered. This type of scope is of low magnification, has adjustable size and brightness of the dot, and is unaffected by parallax.

For bears and large cats, the 375 H&H magnum cartridge was often recommended. This was especially so if the Nosler "Partition" or Swift "A frame" bullets were used. Again a good bolt-action rifle should be considered.

A 12-ga shotgun with 00 buckshot or slugs would be advantageous to have as a backup gun to cover the shooter or to use in close, confined, or heavily planted areas. Zoos commonly have 12-ga shotguns as a backup or sometimes as their primary weapon. As was learned with the escaped circus elephant in Honolulu, a 12-ga shotgun even with magnum slugs is incapable of quickly stopping an adult elephant. However modern shotgun slugs, such as the French Sauvestre "Sledgehammer" slug, claim to be effective against Cape buffalo.

Another difficulty to be considered is the need to securely store the weapons, yet have ready access to them. A good commercial gun safe, such as those from HOMACK Company, is well constructed, easily secured to walls, and quick to open. If the safe is located in a building that is already monitored with an alarm system, door contacts could easily be wired into the system.

Advanced polymer recoil pads such as the "Kick Killer" pad from Action Products provide shoulder protection from the extreme recoil when firing high-powered rifles.

Training and qualification requirements of the shooters are ambiguous and inconsistent among zoos. Each zoo will have to determine its own level of proficiency. Some zoos train their shooters with their local police SWAT teams. The Honolulu Zoo determined that the shooting team would not be able to match the expertise of the SWAT team in terms of accuracy. Also, the cost of qualifying with the SWAT team's training schedule would have been very expensive. Each cartridge of the 458 Winchester magnum costs us about $2.75 plus shipping. Our protocol requires shooters to qualify and then re-qualify every 6 mo. We require a shooter to hit a high percentage of shots into a certain sized target at a set distance within a prescribed time period

Unless your police department provides a complete training program, documentation of weapons safety training may be difficult to substantiate. Hunter safety classes may provide good general safety guidelines, however most of the instruction concerns hunting. The NRA can provide a basic rifle/shotgun safety course, if you have access to an NRA-certified instructor.

A particularly difficult area to obtain information deals with targeting or aiming points. A general knowledge of anatomy and advice from local hunters may not be adequate for formal training. We sought to find published recommendations of aiming points to use as documentation in training. Here again, big game hunters provided the best and perhaps the only source of information. James Mellon's book, *African Hunter*, was one source we found with this kind of photographic aiming point recommendations.[22] I caution readers of this text that the pictures and content of this book are very graphic and depict the killing of animals that contemporary zoo professional would not condone. Similar information can be found in *Mahohboh* by Ron Thomson and *Safari* by Elmer Keith.[18,31]

Hopefully, there will not come a time when we will have to use these weapons. However, acknowledgment of the possibility that a dangerous animal may have to be killed to save a human life mandates that those in authority be prepared to act responsibly. The proper response requires good judgment on the part of trained zoo staff, adequate equipment in the form of appropriate weapons and bullets, and documentation of formal training.

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THE VETERINARY ROLE AS FIRST RESPONDER TO A MEDICAL EMERGENCY IN A CRISIS MANAGEMENT SITUATION

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Abstract

The use of firearms to contain dangerous animals is an established wildlife management technique in zoologic institutions. Historically, the use of firearms in crisis management situations involving dangerous animals has been noted to be statistically significant. Recent international events indicate that a viable threat potential exists for human injury or mortality during a crisis event. This clearly indicates that veterinary personnel may be required to act as medical first responders to a firearm-incurred injury until paramedics arrive on site. As a result, emergency medical response training is recommended. It is anticipated that injury rates will continue to increase in proportion to event occurrence and that further research is indicated. The role of law enforcement in zoologic crisis situations should be examined and defined.

Introduction

During the past 2 yr a new variant has developed within zoologic crisis management situations. In several instances the lives of emergency responders and bystanders have been placed at risk by an inappropriate use of firearms while attempting to resolve crisis events. In one event a veterinarian was killed and in two unrelated events people were injured by gunfire. As a result, it has become necessary to examine the data and circumstances associated with the events in question. It is the intent of this examination to ascertain what procedures would be necessary to prepare, prevent, or respond to a crisis situation when firearms are used inappropriately during a dangerous animal situation.

Event Data

On 14 March 2000 in Warsaw, Poland, a veterinarian was killed, when police officers opened fire on an escaped tiger. This event data in and of itself is cause for concern. However, three other unrelated incidents have occurred since the first and all closely parallel each other in key areas. While all events involved the use of firearms, it should be clearly noted that it is not the purpose of this paper to dissuade the use of firearms in a crisis management situation. The use of firearms to contain dangerous animals is an established wildlife management technique in zoologic institutions. Historically, the use of firearms in crisis management situations involving dangerous animals has been noted to be statistically significant.

The North American Crisis Management Survey is the best resource of statistic data in this regard. The survey was sent to 187 zoologic facilities in North America and 79 responses were received,

resulting in a response percentage of 42.25%. When asked, "Does your facility have an Emergency Response Team or Crisis Management Team?" 69.62% answered Yes, while 30.38% answered No. When asked, "Does your facility have an emergency procedure for escaped animals, and dangerous animals?" The answers were respectively 94.94% Yes, 3.80% No, and 87.34% Yes, 11.39% No, to dangerous animals. When asked, "Does your facility conduct scenarios or practice drills for a possible animal escape situation?" respondents indicated 62.02% Yes and 37.97% No. In regards to facilities having animal escapes, 77.21% Yes and 22.78% No, and of those who answered in the affirmative of escapes also indicating 20.25% dangerous animal escapes. With the applicable baseline, firearms usage can be examined. As to whether firearms are maintained on site for dangerous animal situations, respondents indicated 73.42% Yes and 25.32% No. When asked, "Has your facility ever had to use firearms kept on site for a dangerous animal escape situation?" 17.72% Yes and 75.95% No. Respondents clearly indicate while escapes do occur, they also have contingency planning, keep firearms on site, and are prepared to use them in the event of a crisis that involves dangerous animals. Thereby establishing a historic context.

In order to better understand the threat potential of inappropriate firearms usage, it is necessary to review the crisis management events in chronologic order:

7 March 2000, Warsaw, Poland

Three Bengal tigers (*Panthera tigris tigris*) were intentionally released from their circus cages by animal activists. Two of the tigers were recaptured before leaving circus grounds. However, one remaining tiger escaped into city streets after injuring a circus employee. This tiger was then pursued for approximately 2 hr by police and an assisting veterinarian through the busy streets of Warsaw. The tiger was finally cornered on a wasteland near housing blocks in the Warsaw suburb of Tarchomin, located in the eastern part of the city. The veterinarian then fired a tranquilizer dart, but was chased and attacked before sedation could occur. Police officers then fired a volley of shots and the veterinarian was struck in the head. The 47-yr-old veterinarian, identified as Ryszard Karczewski, died later in hospital. The tiger was shot to death by policemen armed with rifles and pistols. The Rzeczpospolita daily newspaper received an email from a group identifying itself as the Polish League for Protection of Animals. The message claimed that, "Once per week we will release animals from a circus or a zoo." In review of the event, Warsaw Police Chief Antoni Kowalcyzk acknowledged that the police appeared to have mishandled the chase, but said that they were ill equipped to handle such an unusual occurrence

3 April 2000, Sao Paulo, Brazil

Jose dos Santos and his son Jose were touring the animal cages during a break interval of a performance by the Vostok Circus in the northeastern city of Recife. Inadvertently, they approached too close to a cage. The 6-yr-old boy was pulled from his father by a lion (*Panthera leo*) and into a cage with four other lions in front of hundreds of spectators inside the tent. As the lions devoured the boy, police arrived on the scene and in an attempt to scare the animals away, sprayed the top of the cage with machinegun fire, wounding two bystanders with bullet fragments in the process. The

boy's body was recovered several hours later when police officers shot four of the five lions dead. A circus worker indicated that the lions had not been fed in 5 days. Police are investigating to establish whether the circus owners breached safety regulations by allowing the public to walk directly up to the cages. The lion tamer has been charged with manslaughter.

4 August 2000, San Simao, Brazil

Six circus lions (*Panthera leo)* were being housed at the zoo after local authorities determined that the lion's traveling cages were not sufficiently secure. After escaping from their cage at the zoo, the lions roamed this town in southeastern Brazil until they were shot and killed by police firing machineguns and shotguns.

12 August 2000, Boise, Idaho

While the zoo manager of Zoo Boise was conducting an unscheduled behind the scenes tour of the Amur tiger exhibit, a tiger (*Panthera tigris altaica*) escaped through an open cage door. The tour occurred during the zoo's 12th annual "Feast for the Beast" fundraiser. As the tour group entered the building they lined up against the wall of the keeper service hallway. Friend's of Zoo Boise board member, Jan Gould, who was located at the end of the line, was attacked by the tiger that pinned her by the head and shoulders. Sgt. Rich Schnebly of the Boise Police was accompanying the group and attempted to distract the tiger with a thrown bucket. When this failed he fired his pistol three times forcing the cat finally back into the cage and allowing the door to be closed. However, in the process of firing his sidearm, one of the shots ricocheted and struck Ms. Gould in the leg. She was then transported to Saint Alphonus Regional Medical Center for treatment of the gunshot wound and lacerations of the head, neck, and shoulders. Zoo Boise veterinarians and staff members sedated and thoroughly examined the tiger and found no evidence of injury or gunshot wounds.

To review, four of the events involve dangerous animals (felids), police, and firearms usage. In three of the events, people were either injured or killed. And, in all cases there was a questionable tactical use of firearms, not only in application, but the types of firearms that were utilized during the situation.

Event Resolution

In light of the aforementioned events, it is clear that zoologic facilities and wildlife managers should take preemptive measures. The threat potential for human injury or mortality in the process of resolving a crisis management situation is present. As a result, It is recommended that animal professionals factor into their emergency contingency plans and scenario based drills the following points to facilitate an effective resolution to a crisis event involving dangerous animals and the use of firearms:

- Veterinarians inherently have a higher degree of medical knowledge than other staff members in a zoologic institution. It is also likely that the veterinarian and their medical staff will be involved

in a crisis event. As a result, the veterinary staff would be the logical choice for training in first responder treatment in CPR and emergency first aid, such is offered by the American Red Cross.

- A dialogue should be developed between veterinary professionals and medical providers who have experience in the treatment of gunshot injuries, violent trauma, and triage techniques. This exchange of information would be applicable not only in isolated cases, but invaluable in the event of a natural disaster, due to the potential for widespread human and veterinary casualties.

- Major cities worldwide indicate that they are experiencing problems in first responder times due to urban growth, call loads, and associated traffic problems. All of which lengthen response times to the incident site and could jeopardize survival of critically injured parties. Facilities that do not maintain Emergency Medical Technicians (EMTs) on staff should consider sending a member of the veterinary staff through a recognized and certifying emergency medical technician course.

- Trauma equipment kits for human usage should be maintained in multiple locations throughout a facility, with special emphasis on access for the veterinary staff. Kits should offer treatment options for a wide range of injuries, but focus on severe trauma treatment. To date, the best examples for zoo usage are produced by Zee Medical and Certified Safety Manufacturing (CSM).

- Crisis management situations that involve dangerous animals require resolution of the animal aspect of the crisis before treatment of human injuries can begin. This only increases the need for having at least two members of the veterinary staff trained in treatment of human injuries. Especially when considering that more than one person could be injured at a given time or place.

- When veterinary professionals find themselves under time constraints, consideration should be given to sending a member of the veterinary staff for training. This would include veterinary technicians, veterinary assistants, veterinary keepers, and interns on extended assignment.

- Several facilities have chosen to out-source and contract EMTs for their facilities on a full time basis. This has the merit of being sensitive to time constraints. However, this also requires a significant outlay of funds from the annual budget to finance this particular approach. Historically, only the larger, more affluent facilities have been able to take this course of action due to fiscal considerations. Still, it does have certain advantages over permanent positions with full benefits.

- A member of the veterinary staff who has already received training in CPR and emergency first aid through the American Red Cross would be eligible to pursue further training towards an instructorship. This would in turn provide the facility with a staff member who not only has advanced training, but who also can act as an in-house training resource for other staff members.

- Veterinary staff members should be familiar not only with the aspects of animal sedation utilizing a dart projector, but also with all aspects of the weapons response for a zoologic institution. Conversely, staff members involved in emergency response procedures should be cross-trained on dart projectors, dosages, and applications. This could be facilitated by the veterinary staff or

outsourced to a private agency such as Safe Capture International, Incorporated.

- A senior or associate veterinarian should be involved with any dialogue that occurs at a zoologic facility in regards to development of a law enforcement protocol agreement. This agreement should not only clearly delineate the role of emergency responders, but local law enforcement as well. Specific attention should be paid to language that addresses law enforcement presence on grounds and pursuit by staff of an escaped specimen once it physically leaves the grounds of a facility. Spheres of influence and liability should be clearly determined.

Discussion

The problems associated with resolving crisis management situations are not only complex, but multifaceted as well. Local governments no longer see performance measurement as an option, but as a requirement. They have identified the four key areas, which influence first response medical care in the field: response time, effectiveness, workload, and cost. Unfortunately, these areas are at odds with the previously mentioned: urban growth resulting in higher demands, coupled with higher population density, higher call loads, and traffic problems which translate into delays in the field to reach injured parties.

Yet, at the same time the public has shown an accelerating trend and aversion to supporting tax initiatives that would empower public officials to raise revenue in order to provide more effective vital services to their communities. While at the same time professional organizations such as the National Fire Protection Association (NFPA) are considering adopting national standards for minimum response times.

When considering the issue of gunshot wounds themselves, the annual medical cost in the United States is close to $2.3 billion, with staggering associated injury and mortality rates. Conversely, this has created a wealth of treatment data for the medical professional to draw upon. Still, this should be tempered by the realization that while most medical providers do treat gunshot injuries, they are unlikely to have encountered a wound inflicted by a high-velocity safari grade bullet that was designed specifically to penetrate, expand, and cause maximum tissue destruction. As a result, a well trained vet staff is an asset.

Conversely, the relationship between zoologic facilities and law enforcement agencies should be reexamined. According to the North American Crisis Management Survey (1996), when facilities were asked if they have an escaped animal protocol agreement with local law enforcement, 41.77% responded yes. Also, when asked if a law enforcement agency assists with firearms training, 44.30% responded yes. While local law enforcement agencies are a convenient resource for zoologic facilities, the question should be raised, are they sufficiently equipped or trained to respond to a dangerous animal crisis situation? Moreover, are they familiar with the ethology of dangerous animals or the safari-grade weaponry that will be needed in a tactical situation to resolve a crisis similar to the aforementioned examples in a proficient manner?

Numerous zoologic facilities in North America maintain safari-grade firearms and ammunition on grounds. As a result, more often than not, they maintain equipment superior to their law enforcement counterparts. The reason for this is two-fold. The need for advanced weaponry is understood by zoologic professionals in light of the historic relationship between conservation and big game hunters, which is in turn tempered by their knowledge and training in dangerous animal management. This in turn supports the willingness of institutions to commit significant financial resources for firearms, ammunition, and training. Simply put, it is preferable to invest in a resolution rather than face the consequences of liability, litigation, and years of long-term damage to public relations and marketing. From a fiscal management point of view alone, it is the cost effective choice for a zoologic facility in modern society.

Conclusion

As crisis managers we should be aware that precedence necessitates a need for proactive measures. Still, we must keep in mind all of the hypothetic components that could lead to gunshot injury and act accordingly to prevent the incident from occurring in the first place. This can be facilitated through study of current literature and previous experiences,[1-21] evaluation, and training. The basic factors of animal behavior cannot be dismissed such as primary signs of aggression or the "fight or flight" response with their associated distances and resulting actions, such as an animal attack resulting in either conspecific or human injury. Law enforcement agreements should be reconsidered in light of the previously mentioned events, as zoologic professionals appear to be the better equipped and trained personnel for resolving dangerous animal situations. The use of firearms in a crisis is a viable option, but it should be tempered by the experience, safety, and skill of the responders.

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A CLOSE CALL: SALIENT POINTS OF A SERIOUS ELEPHANT KEEPER INJURY BY AN ADULT AFRICAN ELEPHANT (*Loxodonta africana*)

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Abstract

An experienced elephant keeper was seriously injured by an adult female African elephant (*Loxodonta africana*) under his care on 3 November 2000. He sustained several broken bones and his recovery period will be minimally 8-10 mo. He narrowly escaped with his life. The salient points surrounding this incident are given below.

The keeper was working the elephant through a 2 to 3 foot-wide gap in a hydraulic door and was pulled through that gap into the stall with the elephant. The keeper was working alone with no back-up.

There is a Toledo Zoo Elephant Management Policy for elephant handling and management in place, drawn up by the Elephant Management Committee (consisting of the elephant manager, deputy director, senior veterinarian, mammal curator, and executive director) and approved by the Toledo Zoological Society Board of Trustees. It was in place at the time of the injury.

The Toledo Zoo has had a free contact training system for its elephants since 1982, with no prior serious injuries. The two-keeper policy for handling the animals has been in place for 10 yr. This policy was relaxed by the elephant keeper staff due to internal personnel problems between some of the elephant keeper staff creating conflict.

The elephant that inflicted the injury has been in the program at the Toledo Zoo since 1986. She is 19 yr old and weighs approximately 8100 pounds. She is one of two adult females currently housed at the zoo.

The female elephant in question had been successfully artificially inseminated the month before the injury occurred and was pregnant at the time of the injury. The keeper had treated the surgical wound below her anus (a result of the insemination procedure), and was offering a treat to the animal by placing it in her mouth through the door opening. She clamped down on his arm and he was pulled into the stall, where he sustained several serious injuries, before escaping.

The Toledo Zoo has had a hydraulic elephant restraint device (ERD) in place for several years, but it malfunctioned frequently and was installed in an outside sand yard, so was only usable in warm weather conditions. Due to its unreliable performance it had not been used for any procedures since its installation.

Events and Policy Changes Made as a Result of the Incident

The incident was not reported to the United States Department of Agriculture (USDA) until approximately 1 mo later when another incident (sloth bear death) was being investigated. A USDA inspection occurred from December18-20, 2000, and the only noncompliant item cited in relation to the incident was 2.40 (b)(1) under Veterinary Care, stating that the ERD could not be used by the attending veterinarian in its present location (outside) and condition. As a result, plans for enclosing the ERD and servicing it for use are now being implemented.

It was also clarified by the USDA investigator that incidents involving human injury by elephants only fall under USDA Animal Welfare regulations in cases involving potential injury to members of the visiting public (vs. employees).

The remaining elephant keeper staff and the mammal curator met and immediately after the incident and reviewed the facts of the incident, identified mistakes and problem areas, assessed the elephant for signs of aggressive tendencies not seen before, and through consensus agreed to continue with a free contact system of elephant handling. This information was not distributed or discussed with other members of the Elephant Management Committee specifically until 2 mo later. The policy now stands that a meeting of the Elephant Management Committee and a member of the Human Resources department will convene within 1 wk of an elephant keeper injury to review the incident, and that all statements, memos, and recommendations related to such incidents will be routed to all members of the Committee as they are generated.

The Elephant Management Policy was reviewed and the two- keeper system is now mandatory. No elephant contact will occur at all unless the second qualified keeper is within safe response distance.

The Elephant Management Committee will now meet quarterly instead of once per year.

The Elephant Safety Assessment Team, consisting of the Elephant Manager, elephant keeper staff, a veterinarian, and the Zoo's safety coordinator will meet twice per year. Using the AZA Guidelines for Elephant Management and Care, they will evaluate the safety of the elephant program and report findings and recommendations to the Elephant Management Committee.

DEATH OF A SLOTH BEAR (*Melursus ursinus inornatus*) AT THE TOLEDO ZOO: WHEN THE CHECKS AND BALANCES FAIL

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Abstract

A series of events involving misinformation, poor communication, and interpersonal conflict within and between the mammal department and curator, management, and veterinary staff at the Toledo Zoo led to the inadvertent denning up of a healthy 18-yr-old female sloth bear without food or water for a period of almost 3 wk in preparation for cubbing. This led to her death by dehydration on 4 December 2000. Internal inquiries as well as a United States Department of Agriculture (USDA) investigation and inspection ensued. Details of the event and aftermath are discussed below.

Introduction

The Toledo Zoo has maintained and reproduced several bear species over its 100-yr history. Sloth bears, however, were a relatively new species to this institution, the first sibling pair arriving from Poland in 1997. The second female ever brought to Toledo Zoo per Species Survival Plan (SSP) recommendations for breeding arrived in April 1999. She had cubbed successfully at her previous institution in 1996.

The Toledo Zoo has long established standard denning protocols used successfully for other hibernating species of bears (namely American black bears, *Ursus americanus*, and polar bears, *Ursus maritimus*) for the cubbing period. These include isolating the pregnant female from her exhibit and exhibit- mates in a dark, quiet, secluded holding area with minimal keeper contact or disturbance. This is meant to simulate natural denning/hibernating conditions, minimize disturbance during this time, and reduce the chance that such stress will cause cannibalization of the cubs by the mother. Food and water are offered daily.

This breeding female sloth bear was denned up using this protocol in the fall of 1999 when breeding behavior was seen between the pair and she was speculated to possibly be pregnant. She was denned in a holding area adjacent to the exhibit and was free to move about into an adjacent small outside holding cage away from the exhibit and the male. Food and water were offered daily. No cubs were produced, and she was noted to remain active throughout the denning period.

Events Surrounding the Incident

The female sloth bear had again bred in the summer of 2000. Plans to discuss denning her up were placed on the Veterinary Department/ Large Mammal Department monthly rounds agenda by the senior veterinarian prior to his departure for field work from 13 November to 21 November 2000.

On 20 November, the associate veterinarian received a distressed call from the sloth bear keeper informing her for the first time that the animal had already been denned up as of 17 November 2000, and that plans were to withhold food and water as well to minimize any keeper contact as directed by the mammal curator.

These plans were discussed at Large Mammal/ Veterinary Rounds on 21 November by the mammal curator, mammal keepers and the associate veterinarian. The mammal curator stated that he did not plan to give any more food or water other than what was given on 17 November 2000, and that the keepers should only be checking the female once per week to minimize disturbance. He was then questioned by the veterinarian and keepers as to the safety of this change in denning protocol, especially in regard to withholding water and reduced frequency of monitoring. He stated that he had discussed this protocol with the previous holding institution and that this was how they had done it, resulting in successful cubbing. He also alluded to other facilities that used this protocol. There was further discussion of placing a remote monitoring device in the den for better monitoring, which the curator said he would address.

The associate veterinarian informed the senior veterinarian upon his return on 22 November 2000. The conversation included a discussion of whether a warm climate bear would undergo a state of dormancy. Since both veterinarians' experiences included only hibernating species, the associate veterinarian called the previous holding institution's veterinarian to double check on their protocol. A message was left with one of their veterinarians. Miscommunication between the Toledo veterinarians was later discovered in that the senior veterinarian did not understand from the conversations that the bear was actually denned up at that time, but thought that discussions were in reference to future denning protocol.

The reply from the previous holding facility was not received by the associate veterinarian until 29 November 2000 due to her absence during the holiday interim and family illness. The message received stated that the institution previously holding the bear did not withhold food or water when they denned this or any other bear.

This message was passed on to the mammal curator and the senior veterinarian asking for further clarification or justification to proceed with the protocol the mammal curator had mandated. The assumption was that the bear would be checked, and water offered as a minimum until further information could be verified. The bear had been denned up for 13 days at this point.

On 4 December 2000 the sloth bear was discovered dead in her den by one of the mammal keepers and the curator who had come to check on the animal and feed and water her. It had been 18 days since she had been denned up. Gross necropsy findings indicated a nonpregnant animal whose cause of death was dehydration. The mammal curator did confirm that he had received the message from the veterinarian, and took full responsibility for the death of the animal. The director and deputy director, and the mammal curator's immediate supervisors, reportedly had not been informed of any of the protocols for the sloth bear denning before its death was reported to them.

Aftermath

There was extensive media coverage of the incident, followed by internal (Toledo Zoological Board), USDA, and local humane society investigations. A full USDA inspection of the zoo was also conducted as a result. Several violations of the Animal Welfare Act were cited, three in relation to the sloth bear death. These violations cited by the USDA Animal and Plant Health Inspection Service (APHIS) include noncompliance in the following areas:

9 CFR 3.129 (a)&(b)	FEEDING
9 CFR 3.130	WATERING
9 CFR 2.40 (a) (2)	VETERINARY CARE

The veterinarian lacked proper authority to ensure the provision of adequate veterinary care and oversee the adequacy of other aspects of animal care and use. The mammal curator resigned his position. The zoo sustained a fine from the USDA.

The zoo took steps to prevent problems of this nature in the future by evaluating and adopting several new procedural protocols under the direction of the senior veterinarian and deputy director.

These include:

1) Set policy that all animal care staff, including veterinarians, curators, and zookeepers, understand that they are to inform the deputy director of any situation that could negatively impact the welfare of any zoo animal. These situations might include housing, feeding, transport, psychologic stress, environmental conditions, medical conditions, and animal safety issues.

2) Written policy that each animal will be observed daily unless exempted by prior written approval of the curator, the veterinarian, the deputy director and the executive director or for circumstances specified as appropriate in an approved species management plan, such as nesting birds, groups of birds, reptiles, amphibians, and fish in natural enclosures, where daily observation is not possible, and reptiles and amphibians in hibernation and aestivation.

3) The protocol for any isolation of an animal must include plans for regular visual inspection, either directly or through video monitors, noting that practices specified in an approved management plan as routine and/or appropriate do not require prior approval.

4) Enforced requirement of a completed “Diet Change Request” form submitted to the veterinary department for approval before any radical diet changes are implemented for husbandry or medical reasons

5) Set policy that the withholding of food and water from any animal in the zoo is prohibited without prior written approval of the curator, veterinarian, the deputy director, and the executive director except for the following:

a) When deemed medically necessary by the veterinarian, such as fasting for newborns or in preparation for anesthesia or for treatment of gastrointestinal problems

b) Under circumstances specified as appropriate in an approved species management plan, such as part of an animal's normal husbandry regime or shot term fasting of food for training purposes. Requests for approval must be accompanied by a diet change request.

c) Species Fact Sheets are to be filled out for each species in the collection. These are to be made available to the veterinary, animal care and management staff for reference at any time. These will include basic natural history, behavioral, and husbandry information or cite references in that regard, and management plans for the species in the collection

d) Periodic internal or "self" inspections of different animal areas (including non-mammal and veterinary areas) by a committee made up of the deputy director, veterinarian, curatorial and maintenance staff. A checklist based on USDA inspection guidelines was drawn up and is to be used as a guide. These inspections will be done semiannually once all the areas have been done.

Discussion

The loss of an animal under one's care is often difficult to accept, especially when a well-intentioned, knowledgeable, caring staff is involved. The fact that the sloth bear's death was a preventable loss made it even more difficult. It was devastating to many staff members and came on the heels of another near tragic elephant keeper injury incident.

Although the incident (in conjunction with the elephant keeper injury which occurred prior to this) served to bring to light the problems of a dysfunctional department with internal morale problems and communication breakdown, and illustrated just how serious the consequences of these problems can become without proper management guidance and communication, some good has come from it.

The roles, rights, authority, and responsibility of the attending veterinarians, management, and animal care staff have become better defined. Communication lines are opening between management and animal care staff, and animal care staff and the veterinary department. The attending veterinarians have been empowered to take action when potential animal welfare issues are found and seek assistance from the USDA if their recommendations in that regard are being ignored. It has also stimulated healthy dialogue between the veterinarians, management and animal care staff to better define what is meant by "adequate veterinary care" as stated in the Animal Welfare Act.

U.S. DEPARTMENT OF AGRICULTURE UPDATE: ANIMAL WELFARE ACT COMPLIANCE

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Abstract

Beginning with the passage of the Laboratory Animal Act in 1966, responsibility for assuring the public that exhibitors provide humane care, handling, and treatment for the animals they exhibit has been given the United States Department of Agriculture (USDA). Public concern in animal welfare has grown considerably, and the USDA has been given the challenge to address these concerns, while simultaneously maintaining an environment in which animals can be maintained in captivity, exhibited in their natural habitats, the public can have, where appropriate, direct contact with animals, and facilities can conduct studies to improve and maintain their collection of animals.

The Inspection Process

Congress mandated that each registered research facility be inspected at least once every year. Congress did not mandate an inspection interval for licensed facilities, including exhibitors. Using a risk-based inspection system (RBIS), the Animal and Plant Health Inspection Service (APHIS) determines the minimal inspection interval for each licensed exhibitor. RBIS is based on the facility's compliance history, type of animals housed, and methods by which animals are exhibited.

Although each inspector can develop their own inspection process, there are several basic guidelines all inspectors must follow. The inspections will be unannounced and conducted at an interval that assures compliance with the Animal Welfare Act (AWA). Inspections will be conducted with a representative from the facility who is at least 18 yr of age. Areas of noncompliance will be noted and an inspection report completed. At the end of each inspection, the facility will receive a copy of the USDA inspection report along with an opportunity to discuss (exit interview) with the inspector each area of noncompliance noted.

Disagreeing with an Inspection Report

Each facility will sign or validate that they have received the inspection report. A signature on the report does not mean concurrence with the report. Each facility, at the time of the exit-interview, should discuss each item on the report to assure they understand each deficiency and what needs to be done to correct them. If the facility disagrees with a documented item, they should bring this to the attention of the inspector before he/she leaves the premises and state the facility's reasons for disagreeing. In most cases, issues are resolved at this time and the report, if appropriate, amended. If not resolved at the exit-interview, the facility may follow the procedures outlined in Animal

Care's Tech Note "Understanding Compliance Inspections" to have the inspection reviewed by the inspector's supervisor.

Common Violations Found at Exhibitor Facilities

In fiscal year 2000, there were 4,804 violations documented at licensed exhibitor facilities involving 60,863 animals. Six hundred eighty-eight of these violations involved the attending veterinarian and/or inadequate veterinary care.

Responsibility and Authority of the Attending Veterinarian

Each licensed exhibitor is required to have under formal arrangement a full or part-time attending veterinarian who shall provide adequate veterinary care to the facility's animals. In the case of a part-time attending veterinarian, the formal arrangement shall include a written program of veterinary care and regularly scheduled visits to the facility's premises. Each licensed exhibitor shall assure that their attending veterinarian has the appropriate authority to assure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use. Adequate veterinary care includes (1) availability of appropriate facilities, personnel, equipment, and services; (2) use of appropriate methods to prevent, control, diagnose and treat diseases and injuries, and the availability of emergency, weekend and holiday care; (3) daily observations of all animals to assess their health and well-being; (4) adequate guidance to personnel involved in the care and use of animals regarding handling, immobilization, anesthesia, analgesia, tranquilization and euthanasia; and (5) adequate pre-procedural and post-procedural care in accordance with established medical and nursing procedures.

Compliance Through Education Rather Than Enforcement

Animal Care focuses on education efforts through the inspection process, industry meetings, Animal Care website, and other mechanisms. Animal Care inspectors use each inspection of a facility as a means to educate an exhibitor on maintaining compliance with the AWA. Animal Care personnel have attended and participated in national meetings of the Association of Aquatic Animals, American Zoo and Aquarium Association, Society of Marine Mammalogy, and Association of Aquatic Life Support Systems Operators. In FY 1999, Animal Care personnel attended more than 297 industry meeting and training sessions and presented papers or informal talks at more than 97 of them. In February 1999, Animal Care launched its new and improved website (www.aphis.usda.gove/ac). The new site contains the same information as the old site but organizes it in a more logical and user-friendly way for both the general public and program stakeholders. In 1997, Animal Care began publishing a periodic report on animal welfare issues and now has over 2000 subscribers.

Innovative Penalties that Benefit the Animals

Animal Care uses a two-pronged enforcement strategy. For licensed exhibitors who show an interest

in improving the conditions for their animals, Animal Care actively pursues innovative penalties that allow the facility to invest part or all of their monetary sanctions in facility improvements, employee training, research on animal health and welfare issues, or other initiatives to improve animal well-being. In doing so, USDA enables the facility to immediately improve the conditions for their animals while sending a clear message that future violations of the AWA will not be tolerated. Prior to 1997, most fines were either suspended or paid directly to the U.S. Treasury, but neither of these methods directly improved the plight of the violator's animals. On the other hand, for licensed exhibitors who do not improve conditions for their animals, Animal Care moves swiftly and pursues stringent enforcement actions. Such action typically includes significant monetary penalties and/or license suspensions or revocations. It may also include confiscation of their animals and relocation to another facility if the animals are found suffering.

NUTRITIONAL CONSIDERATIONS OF THE CAPTIVE MANED WOLF (*Chrysocyon brachyurus*) WITH EMPHASIS ON MANAGEMENT OF CYSTINURIA

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Abstract

An important and widely recognized condition of the maned wolf is cystinuria, or excess levels of cystine in the urine. Cystinuria has been identified in the majority of wolves tested, both in captivity and in the wild. Cystinuria also occurs in humans and domestic dogs, and has been demonstrated in these species to have a genetic basis. In all species afflicted with this condition clinical disease may occur secondary to urolith formation. The solubility of cystine in urine is pH-dependent and the unusually low urine pH of many captive maned wolves may predispose them to morbidity and mortality related to cystine urolithiasis. Dietary modification is a significant component of cystinuria management in both human and canine patients. Based on present knowledge, we hypothesize that through dietary modifications we can reduce urinary cystine concentrations as well as produce alterations in urinary pH which increase cystine solubility and decrease cystine urolith formation in captive maned wolves.

Introduction

The maned wolf is a large South American canid which inhabits the grasslands, savannahs, swamplands, and scrub forests of central and eastern Brazil as well as regions of Argentina, Paraguay, Bolivia, and Peru. Due to declining numbers in the wild primarily from habitat destruction and fragmentation, the maned wolf is classified as endangered in its natural habitat by the US Fish and Wildlife Service, as "lower risk, near-threatened" on the 2000 IUCN Red List of Threatened Species, and is listed under Appendix II of the Convention on International Trade in Endangered Species (CITES). A relatively small captive population of maned wolves is maintained in zoologic parks across the United States under the guidance of a Species Survival Plan. For much of its history in captivity, the maned wolf has been known to be generally unthrifty and have marginal reproductive success. Relatively poor body condition, extremely rapid passage of digesta, chronically soft stools, and gingivitis are common conditions that are being or have been remedied primarily with husbandry changes.[5,7] One serious and widely recognized medical condition afflicting captive maned wolves with an alarming prevalence is cystinuria, or excess levels of the nonessential amino acid cystine in the urine.[3,4,6,17]

Cystinuria, a disorder which affects both humans and domestic dogs, is an autosomal recessive metabolic defect characterized by defective transport of cystine and the dibasic amino acids lysine, ornithine, and arginine through the epithelial cells of both the renal tubular and intestinal brush

borders.[13,22,24] The disorder is common in humans, with an overall prevalence of approximately one in 7,000-15,000, although in certain ethnic groups, such as those of Libyan Jewish descent, the prevalence is as high as one in 2500.[12,25] The prevalence in dogs varies with geographic region and breed, being much higher in most European countries than the United States, and being most frequently reported in Dachshunds, English Bulldogs, Mastiffs, Basset Hounds, and Newfoundlands.[8,19] Although few studies have been performed to clearly characterize the prevalence of cystinuria in both captive and wild maned wolves, one report described abnormally elevated cystine and dibasic amino acid excretion in approximately 80% of 42 wolves tested.[4] Of the eight wild wolves tested, six were found to be positive for cystinuria. Although it was concluded based on this finding that the prevalence of cystinuria in wild maned wolves is comparable to that in captive wolves, the extremely low sample size in addition to the testing of animals from the same geographic region may have significantly biased the conclusion of a high prevalence in all wild populations. Maned wolves in captivity have historically been plagued by complications related to cystinuria which cause significant morbidity and mortality. To date, no research has been done to characterize the incidence of clinical disease related to cystinuria in wild maned wolves.

Clinical manifestations of cystinuria only develop secondary to the formation of cystine uroliths, which, depending on their location, can predispose the patient to complications such as urinary tract infections and renal insufficiency, as well as cause life-threatening upper or lower urinary tract obstruction.[14,15,23] Although cystine and all three of the dibasic amino acids (lysine, ornithine, and arginine) are excreted in excess in cystinuria, only cystine is involved in crystal and urolith formation. Cystine, the least soluble of the amino acids, has a solubility which is highly dependent on pH, with an increase in pH over 7.0 resulting in marked increases in cystine solubility.[1,10,18]

Amino acids pass freely through the glomerular membrane and their concentration within the glomerular filtrate is equal to that in plasma. Approximately 98% of these amino acids are reabsorbed in the proximal renal tubular epithelial cells and returned to the plasma. Cystine and the dibasic amino acids utilize an identical active tertiary transport mechanism. The high affinity heterodimeric apically-located transporter is composed of -heavy (rBAT) and light ($b^{o,+}AT$) protein subunits. The gene coding for the rBAT protein, SLC3A1, was localized to human chromosome 2.[9] More recently, the gene for the $b^{o,+}AT$ protein subunit was identified on human chromosome 19, and designated SLC7A9.[12,20] Although cystinuria was once considered to be a genetically homologous disorder with multiple phenotypic expressions, these findings confirm genetic heterogeneity, with each gene being responsible for one of only two phenotypic categories.[20]

Identification of flat, hexagonal cystine crystals in a urine sediment or of cystine calculi by urolith analysis are both pathognomonic for cystinuria, although neither may be present in a cystinuric patient. Elevated urinary cystine levels or a positive cyanide-nitroprusside test may support a diagnosis, but suspicions should ideally be confirmed by direct urine quantification of cystine by HPLC or other methods over a 24-hr period.[23]

Therapy of human and canine cystinuria focuses on increasing the urinary solubility of cystine as well as reducing urinary cystine concentration. These objectives can be accomplished by urinary

alkalinization, dietary modification, and administration of thiol-containing drugs.[1,14,18] Pharmacologic agents, such as D-penicillamine and mercaptopropionylglycine, which act by a thiol-disulfide exchange reaction, have had limited success in the long-term management of cystinuria primarily due to a high incidence of undesirable side effects. These and other agents are typically reserved for patients responding suboptimally to other forms of therapy.

Dietary manipulation includes three main factors. Hyperdiuresis decreases urine cystine concentration, decreasing the likelihood of cystine crystallization. Secondly, the amount of cystine excretion has been demonstrated to be correlated with dietary sodium levels, and thus a low sodium diet is advocated to reduce cysteine excretion.[21] Finally, a reduction in dietary protein, especially that derived from animal sources which are inherently higher in sulfur containing amino acids, is proposed to decrease urinary cystine excretion as well as increase urinary pH. Alkalinization of the urine to increase cystine solubility can also be accomplished through pharmacologic means. Although sodium bicarbonate has been used, potassium citrate has been advocated due to the effects of sodium on cystine excretion.

Although classified in the order Carnivora, the maned wolf's natural diet is hardly that of a carnivore. The normal diet of the maned wolf in the wild consists of approximately 51% plant matter and 49% animal matter.[11,16] The animal portion consists primarily of small rodents and birds, while the plant portion is largely accounted for by the tomato-like fruit, *Solanum lycocarpum*. In captivity, the maned wolf has traditionally been fed like a carnivore, with dietary protein sources being almost entirely meat based. This was most likely a major contributor to the acidic urine pH (5-6) which is typical of captive maned wolves in the United States, predisposing them to precipitation of cystine and the formation of cystine uroliths (M. Allen, personal communication).

Recent research in this area produced a commercially manufactured maned wolf diet which is currently being fed to all captive maned wolves in the United States.[2] This diet, as compared to a canine maintenance formula, has higher levels of fat, fiber, and sodium, as well as moderate levels of protein. Most importantly, it contains reduced cysteine and methionine levels, and was demonstrated to result in significantly lower urinary cystine concentrations and improved stool quality in the maned wolves studied.

Research Goals

The current research project was designed in an effort to improve the overall nutritional status of the captive maned wolf as well as reduce the risk of cystinuria-related complications without the use of pharmacologic agents. We hypothesize that maned wolves in captivity are predisposed to cystinuria-related complications due primarily to an improper diet which promotes excess cystine excretion and decreased cystine solubility secondary to a low urine pH. By decreasing dietary sodium content and increasing the percentage of dietary protein derived from plant sources we hope not only to better mimic the nutrient composition of the wild maned wolf diet but also to decrease urinary cystine concentration and increase cystine solubility through an increase in urine pH. Therefore, dietary manipulations will feature modifications of the current low cysteine diet including

a change in the sodium:potassium ratio as well as an increase in the proportion of protein derived from plant sources. Effects of these changes on urine pH, urinary amino acid excretion, and overall digestibility will be monitored. There are three main objectives of this project:

(1) To develop a new diet for captive maned wolves which results in both a decrease in cystine excretion as well as an increase in urine cystine solubility.
   a. Decreasing dietary sodium content is proposed to decrease urinary cystine excretion.
   b. Increasing the proportion of plant-derived protein in the diet is proposed to decrease urinary cystine excretion as well as increase average urine pH.
   c. By increasing urinary pH, the incidence of cystine crystallization and subsequent urolith formation will be decreased.

(2) To gather baseline digestibility information on the diet on which captive maned wolves in the United States are currently maintained as well as determine effects on digestibility of changes in this diet.

(3) To lay the groundwork for further studies on the nutrition and physiology of the maned wolf and its successful maintenance in captivity.

Conclusion

It is assumed by most that the molecular basis and pathogenesis of cystinuria in the maned wolf parallels that found in humans and dogs, and although this has not yet been scientifically proven, the probability that cystinuria in maned wolves has a genetic origin is high. The presence of a renal tubular defect in cystine reabsorption that is genetically based and present in the majority of both captive and wild maned wolves could be disastrous to the future of this species. Successful management of such an obstacle would require determination of the prevalence of the disorder in wild populations, identification and characterization of the molecular and genetic basis of the disorder, the development of a diagnostic test to determine its presence, the identification of carrier animals, and the development of a successful breeding and reintroduction program that does not include affected animals. Until such investigations are performed, our efforts will focus on addressing management of cystinuria in captive populations through nutritional modification.

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NUTRITIONAL STATUS IN CAPTIVE BOTTLENOSE DOLPHINS (*Tursiops truncatus*)

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Abstract

Nutritional status of captive dolphins (*Tursiops truncatus*) was examined using biochemical analysis. Voluntary blood samples, obtained from four healthy captive bottlenose dolphins at Brookfield Zoo, were analyzed for vitamin D metabolites [25(OH)D and 1,25$(OH)_2$D], lipids (total cholesterol, triacylglycerides, HDL-cholesterol, and LDL-cholesterol), and fatty acids. Fish fed to these dolphins were analyzed for dry matter, crude protein, fat, energy, vitamin D, cholesterol, and fatty acids. Plasma values for 25(OH)D were higher than those published for a variety of cetacean species, but similar to published bottlenose dolphin data. Total cholesterol was lower than that reported for captive beluga, but similar to wild beluga. Palmitic, oleic, eicosapentaenoic and docosahexaenoic acids were high in both dolphin plasma and the fish sampled. Vitamin D in herring was similar to that found in other species of fish and was reflected in the high circulating levels of 25(OH)D. Capelin was found to be considerably lower in vitamin D than other species of fish. Total cholesterol in fish samples was higher than that of fillets of other fish species.

Introduction

Determinations of circulating levels of vitamins, lipids and fatty acids can provide a base for examining nutritional status of captive cetaceans.[9] Limited information exists regarding circulating concentrations of these nutrients in cetaceans. This study examines vitamin D metabolites, lipids and fatty acids in captive bottlenose dolphins and relates those values to the fish they consumed.

Methods

Blood

Voluntary blood samples were collected from two male (age: 17 and 19 yr) and two female (age: 18 and 7 yr) bottlenose dolphins at Brookfield Zoo. Both females were gestating at the time blood samples were obtained. Animals were housed in their usual exhibit and fed their usual fish diet with supplemental thiamin, vitamin E and a multivitamin. Venous blood was drawn by animal management staff into tubes containing either heparin or EDTA and chemically analyzed. Plasma was separated by centrifugation and frozen for less than 2 mo at-80ºC until thawed for analysis. Samples from all four animals were analyzed for fatty acids. Samples for vitamin D metabolites and lipids were not obtained from the 7 yr old female.

Samples were analyzed for the vitamin D metabolites 25(OH)D and 1,25$(OH)_2$D at Boston University School of Medicine (Boston, MA). 25(OH)D was extracted from 0.05 ml plasma with 100% ethanol, followed by a protein-binding assay using rat serum vitamin D- binding protein which has a high affinity for 25(OH)D and using a high specific activity [^{3}H]25$(OH)D_3$ as a tracer.[6] To determine 1,25$(OH)_2$D, 1.0 ml of plasma was extracted with acetonitrile. The extracts were then applied to C-18-OH reversed-phase cartridge to separate 1,25$(OH)_2$D from 25(OH)D and tri-hydroxylated vitamin D metabolites. The quantitation of 1,25$(OH)_2$D was accomplished by a radio-receptor binding assay using calf thymus receptor and high specific activity [^{3}H]1,25$(OH)_2$D.[3,7]

Total cholesterol[1,4] triacylglycerides, high density lipoprotein-cholesterol (HDL-cholesterol) and low density lipoprotein-cholesterol (LDL-cholesterol)4 concentrations were measured at Loyola University Medical Center (Maywood, IL) using the Synchron Delta CX7 Analyzer (Beckman-Coulter, Inc., Brea, CA).

Fatty acid analyses were conducted at the Kennedy Krieger Institute (Baltimore, MD). Fatty acids were determined by capillary gas chromatography of fatty acid methyl esters as described by Moser et al.[14]

Diet

Proximate analysis of whole capelin (*Mallotus villosus*) and herring (*Clupea* sp.) was conducted at the Brookfield Zoo Nutrition Laboratory. Fish were pureed with liquid nitrogen until they formed a fine powder, then dried at 60C using a forced draft oven. Crude protein was determined using the Kjeldahl method × 6.25.[2] Crude fat was determined by Soxhlet ether extraction and energy by bomb calorimetry (Parr Instruments Moline, IL). Vitamin D, cholesterol and fatty acid analyses of fish were performed by Covance Laboratories (Madison, WI). Vitamin D was determined by HPLC using AOAC method 45.1.22, modified 2. Total cholesterol was analyzed according to AOAC method 994.102. Fatty acids were determined by gas chromatography according to the American Oil Chemists Society.[3]

Results and Discussion

Blood

Vitamin D metabolite, lipid and fatty acid concentrations in bottlenose dolphin samples are listed in Table 1. There was little variation among dolphins in the concentrations of vitamin D metabolites. Plasma concentrations of 25(OH)D in Brookfield Zoo dolphins were similar to those published for wild bottlenose dolphins.[11] Values were higher than published values for wild beluga whales (*Delphinapterus leucas*), wild pilot whales (*Globicephala malaena*), and wild white-sided dolphins (*Lagenorhynchus acutus*).[11] Concentrations of 1,25$(OH)_2$D have not been reported in cetaceans. Total cholesterol and HDL cholesterol showed less individual variation than did triacylglycerides and LDL cholesterol. Total cholesterol was lower than previously noted for captive beluga[8] but within the ranges obtained for wild beluga.[8] No data published for other cetaceans were found.

Monounsaturated and polyunsaturated fatty acid percentages in dolphin plasma were similar at 36% and 38%, respectively, with saturated fatty acids accounting for roughly 20% of the total. Fatty acid composition of dolphin plasma was similar to that previously published for captive bottlenose dolphins[15] with oleic, eicosapentaenoic and palmitic as the three highest fatty acids for both groups. Omega 3 and omega 9 fatty acids made up more than 50% of the fatty acids measured.

Diet

Composition of fish is known to vary with species, gender, age, season and the location where they are caught.[5] Nutrient composition of the capelin and herring fed to Brookfield Zoo dolphins on a dry matter basis (DMB) is presented in Table 2. Protein content was similar between the fish types, however fat content was higher in herring. Vitamin D content of the herring was higher than that for capelin. Fish liver oils are known to be a good source of vitamin D, yet few studies have examined vitamin D content of whole fish. Published values for Baltic herring fillets with skin had higher levels of vitamin D than the herring sampled in this report (3828 IU/100 g and 1257 IU/100 g, respectively DMB). Fresh water perch fillets without skin had lower levels of vitamin D (326-1527 IU/100 g DMB) than the Baltic herring with an upper range similar to the sampled herring.[13]

Total cholesterol in capelin was higher than the herring. Lie et al.[12] examined the fatty acid and cholesterol content of a variety of fish species, but analyzed fillets, not whole fish. Cholesterol of the sampled fish was twice that of the fish fillets examined by Lie, which was to be expected.

Myristic, palmitic, palmitoleic, oleic, eicosenoic, eicosapentaenoic and docosahexaenoic acids were the highest in both herring and capelin. Eicosanoic was highest in capelin while palmitic was highest in herring. Overall, herring had higher levels of both saturated and polyunsaturated fatty acids. Palmitic and oleic acids were high in many fish species examined by Lie et al.[12], as it was in the sampled fish. However, herring and capelin were considerably higher in eicosenoic acid compared to most species reported.

Plasma concentrations of vitamin D metabolites, lipids and fatty acids in captive bottlenose dolphins appear to be reflective of the fish diet consumed. The supplemental multivitamins contributed to the vitamin D concentrations in plasma, but were consumed at such low levels that the impact should not have been substantial. Diet was noted as the primary factor for difference found in the fatty acid composition of the liver of freshwater and marine ringed seals.[10]

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Table 1. Circulating concentrations of vitamin D metabolites, lipids and selected fatty acids[a] in the plasma total lipids of captive bottlenose dolphins.

Vitamin D metabolites and lipids *n* = 3		Mass percentage of fatty acids *n* = 4	
Vitamin D metabolites		Saturated fatty acids	%
25(OH)D, ng/ml	210 ± 12.9	Myristic 14:0	1.9 ± 0.09
1,25 $(OH)_2$ D, pg/ml	135 ± 13.1	Palmitic 16:0	11.3 ± 0.36
		Stearic 18:0	6.0 ± 0.34
Lipids		Arachidic 20:0	1.2 ± 0.07
Total cholesterol, mg/dL	136 ± 6.3	Monounsaturated fatty acids	
Triacylglycerides, mg/dL	61 ± 13.4	Oleic 18:1ω9	20.9 ± 0.60
HDL-cholesterol, mg/dL	101 ± 2.9	Gondoic 20:1ω9	2.1 ± 0.11
LDL-cholesterol, mg/dL	25 ± 5.4	22:1ω11	1.9 ± 0.07
		Nervonic 24:1ω9	2.9 ± 0.19
		Palmitoleic 16:1ω7	5.9 ± 0.39
		Vaccenic 18:1ω7	2.6 ± 0.17
Fatty acid totals, %		Polyunsaturated fatty acids	
Total saturated fat	21.63 ± 0.78	Linoleic 18:2ω6	1.5 ± 0.03
Total ω9	28.7 ± 0.28	Arachidonic 20:4ω6	3.6 ± 0.16
Total ω7	9.4 ± 0.58	Alpha linolenic 18:3ω3	2.2 ± 0.010
Total ω6	6.2 ± 0.09	Eicosapentaenoic 20:5ω3	15.3 ± 0.62
Total ω3	32.0 ± 0.57	22:5ω3	3.4 ± 0.31
Total fatty acids (μg/ml)	1419 ± 363.6	Docosahexaenoic 22:6ω3	10.9 ± 0.45
		Palmitelaidic 16:1T	1.1 ± 0.05

[a]Fatty acids that exceeded 1% were listed.

Table2. Selected[a] nutrient composition of fish fed to Brookfield Zoo dolphins on a dry matter basis.

Nutrient	Capelin	Herring
Dry matter, %	19.14	25.79
Crude protein, %	69.93	62.79
Crude fat, %	12.12	22.21
Energy, kcal/g	5.46	6.88
Vitamin D, IU/100 g	99	1257
Cholesterol, mg/100 g	893	477
Saturated fatty acids, g/100 g		
Capric 10:0	0.104	0.04
Myristic 14:0	1.25	1.75
Pentadecanoic 15:0	<.05	0.08
Palmitic 16:0	1.88	2.76
Stearic 18:0	0.26	0.31
Monounsaturated fatty acids, g/100 g		
Palmitoleic 16:1	1.78	1.13
Oleic 18:1	1.62	2.37
Eicosenoic 20:1	3.29	2.68
Polyunsaturated fatty acids, g/100 g		
Linoleic 18:2	0.26	0.27
Linolenic 18:3	< 0.05	0.16
Octadecatetraenoic 18:4	0.10	< 0.04
Eicosapentaenoic 20:5	0.94	1.20
Docosapentaenoic 22:5	0.157	0.16
Docosahexaenoic 22:6	1.20	1.75
Saturated fat, g/100 g	3.24	4.85
Polyunsaturated fat, g/100 g	2.45	3.76

[a]Fatty acids that exceeded 0.05 g/100 g were listed.

AN OVERVIEW OF MEDICAL PRODUCTS USED IN SMALL EXOTIC MAMMALS

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Abstract

Small mammals pose a challenge for veterinarians. In the treatment of these animals, new, innovative methods need to be employed in order to obtain the same quality work-up as with larger, relatively more manageable animals. This review will offer several different methods in the handling and medical management of these creatures.

Introduction

Small exotic mammals are inherently challenging to work with because of the difficulty in safe handling and the low demand for tools designed to work with little species. The techniques and tools presented in this paper are intended to facilitate their medical care. Suppliers and manufacturers of products mentioned in this review are presented in Table 1.

Restraint

Gas anesthesia is often the best method for safe handling of small mammals. Induction chambers can be fashioned from large dog masks, acrylic boxes or plastic bags. Several commercial chambers are available (Braintree, Stoelting) as well as small face masks (AM Bickford, MDS Matrx). Preparing face masks is possible by modifying syringe cases and small plastic bottles to fit at the end of anesthetic tubing; latex gloves can be placed across the face end as a seal. Endotracheal tubes (ET) tubes as small as 1 mm can be purchased (VSP); alternately, you can use red rubber catheters and i.v. catheters that can be attached to anesthetic non-rebreathing tubing. A penlite laryngoscope (Dr. G's Unique Products) increases visibility in small oral cavities.

Manual restraint may be facilitated by laboratory animal products such as semicylindrical plastic restrainers for rodents (Braintree) which allow for observation and safe needle injections (i.p, i.v.-tail). Plastic cake decorating bags can also be used for restraint, with the animal's head placed at the tip.

Monitoring Equipment

1) ECG monitors are available that read 10-999 BPM (Bioanalytical Systems). Needles can be used in lieu of clips, or gauze can be placed between clips to reduce tissue trauma. Doppler machine probes can be taped in place over the heart or vessels for audible heart beats.

2) Pulse oximetry machines have various sized attachments that can wrap around a tail or foot; some

small (1 × 1 × 0.3 cm) probes can be placed on the underside of a tail or other vascular surface (Nonin).

3) A feline respiratory tidal volume monitor is available that attaches to an ET tube (Medical Engineering and Development, Inc.). A ventilator is available for small, intubated animals up to 10 kg (Vetronics by Bioanalytical Systems).

Physical Examination

Ocular head gear (MDS, Inc., Opticaid by Edroy, Orascoptic Research) increases magnification and provides appropriate light for good visualization of small anatomic features.

1) Ocular exam: a handheld slit lamp (Eidolon) can be employed to visualize adnexa, a 26- mm lens (Volk) and transilluminator allow full fundic exams in the dilated patient. Ultrasound 10 MHz probes can be used on animals as small as 60 g.

2) Aural: limited by the diameter of otoscope specula, 2 mm is the smallest available (Welch Allyn), or you can use a rigid endoscope (1.8 mm, Storz).

3) Oral: items that assist opening the mouth include metal specula (Petiatric Supply Co.), mini rubber spatulas, radiograph film, guitar picks, rigid ID cards, pediatric/infant nasal specula (Spectrum), and non-slip cylindrical specula (Max Associates). Cotton-tipped applicators act as a great extension of your fingers; use an otoscope or rigid endoscope to increase visibility.

Diagnostic Sampling

1) Blood: Venipuncture sites include jugular, cephalic, saphenous, femoral, tail, ear, and orbital sinus (some rodents). Collection method is as follows: a) use a heparinized 0.5-ml insulin syringe (28-ga needle) or b) insert a hubbed needle (25-28 ga) into the vessel and when a flash is seen, use a capillary tube to obtain the sample. There is a small centrifuge available that spins 10 µl HCT tubes (StatSpin, Inc.). A whole blood volume of 20 µl is enough for a CBC; 250 µl can give you about 20 serum chemistry tests (dependent on PCV) on most standard machines. A smear and a PCV tube get you a lot of valuable information. For immediate results on small volumes of blood, the i-Stat system (Heska) will run whole blood; three drops (~60 µl) will provide a mini-chemistry panel. Blood can be collected in 750 µl microtainers (Becton Dickinson).

2) Tissue: Fine needle aspiration using a 25-ga needle can achieve a good diagnostic sample. Punch biopsy tools are made as small as 2 mm (Fray). Endoscopic biopsy instruments as small as 3 Fr (1.8-mm trochar) are available (Storz). Touch preparations can be performed on any lesion on any size animal. After placement of an i.o. catheter, a bone marrow sample can often be obtained.

3) Other: For a tracheal wash or rectal wash, use a 24-27 ga. intravenous catheter. For gastric washes, use a red rubber tube as small as 5 Fr microculturettes (Becton Dickinson) are good for

bacterial sampling of dental socket, rectums, etc. Nasolacrimal duct cannulas for nasal flushes are available in 21 and 23 ga sizes (Jorgenson Labs).

Diagnostic Imaging

1) Radiographs: Radiographs without manual restraint can be accomplished using plastic boxes, bowls, plastic bags and small cloth bags. If the patient is several inches away from the plate, magnification will occur. Mammography units provide excellent detail, but detail plates and dental radiographs are sufficient. Standing lateral films can be accomplished by placing an animal on a plastic box while supporting a film directly behind the animal. To shoot through a plastic kennel/crate, increase the kVP. In the anesthetized patient, use masking tape for positioning or boards fashioned with Velcro tape. For positioning, a 40 × 60 cm vacuum positioner is available (Hug-U-Vac).

2) Ultrasound: 7.5 MHz probes can be used on many animals, although 10 MHz and even 16 MHz vascular probes are available (Neurosoft, Inc.). Stand-off pads (Aquaflex from Parker; ATS) facilitate the use of larger probes in small animals. Alternately, a thick gel layer and light pressure or a plastic bag filled with gel can be used as a makeshift stand-off pad.

Treatment Administration

1) Oral: Tube feed with or without a speculum using an intravenous catheter, a steel gavage tube (smallest size is 24 ga) (Petiatric Supply, ACES, Braintree), a red rubber tube (5-10 Fr), or a tomcat catheter. Food items that can pass through most of these feeding tubes include A/D diet (Hill's Science Diet), Herbivore Critical Care diet (OxBow Pet Products), baby food, Nutrical (ESCVO), ground insectivore diet (Reliable Protein Products), and CliniCare (Abbott). A coffee grinder, food processor, or blender can be used to grind dry pellets of any kind, for later mixture with water.

Oral medications: suspensions of antibiotics can be obtained from many compounding pharmacies or can be made by mixing crushed tablets or capsules with a 1:9 mixture of KY jelly: water. Flavor additives (extracts and sugar-free drink mixes) may be added to increase palatability. Children's formulations work well in many cases for palatability. Medicated gelatin may be made by following package instructions and adding oral medications (calculated by mg/ml gelatin); pureeing food items into gelatin can increase palatability.

2) Topical: a) Orabase (ConvaTec) can be mixed with ophthalmic ointments, which is great for mucosal surfaces; b) ophthalmic solutions can be dropped onto wounds or lesions; and c) Bag Balm (Dairy Association) and Preparation H can be used on non-ulcerated inflammation of soft tissue. Tuf-foot (Bonaseptic) has shown some efficacy in treating pododermatitis in rodents.

3) Intravascular/Intraosseus: Treatment can be facilitated using syringe pumps that can administer volumes as low as 0.01 ml/min (Braintree, Yale).

4) Intralesional: Antibiotic impregnated methylmethacrylate beads can be made in any size.[3]

Surgery

1) Instruments/materials: a) A surgical tray is available for animals 300-2000 g with plastic bands for maintaining body position (Lone Star Medical Products, Inc). An operating board (Deltaphase by Braintree) maintains body temperature via special pads and has an 8 × 8 inch (20 cm × 20 cm) operating surface with instrument holding areas. b) Surgical packs should include a microblade and handle, Stevens tenotomy scissors, Castroviejo or Derf needle holders, wire speculum, chalazion forceps, Bishop Harmon forceps (Spectrum, Braintree), cotton tip applicators, and small gauze. c) Dental equipment (Jorgenson Laboratories, Spectrum) is available for medium sized rodents.[1] Two mm elevators (feline or microelevators) are available separately (Peerless International Inc.; Spectrum). Small feline mouth gags or a 1-ml or 0.5-ml syringe can be cut and used as a mouth gag by placing the canines in either end. d) Rigid endoscopes are available now as small as 1.9-mm telescope diameter with biopsy tools as small as 3 Fr (Storz). e) Surgical preparation: use moustache clippers to shave a small surgical area. Clear drapes allow full visualization of your patient during surgery (Veterinary Specialty Products) or use store bought oven bags.[5] f) Closure: suture material 5-0 to 9-0 (Ethicon), tissue glue, and stainless steel suture.

2) Hemostasis: Cotton swabs will hold off a bleeding vessel until you can permanently control the bleeding. Mechanical cautery can be accomplished using small 4-mm hemoclips (Weck) or standard ligation. Chemical cautery can include silver nitrate sticks or Monsel's salts (ferrous sulfate powder). Clotting enhancers include Gelfoam (Pharmacia and Upjohn) and Clotisol (Benepet). Electric cautery using a handheld cautery pen can be handy (Petiatric Supply Co.). Radiosurgery units producing 3.8 MHz have less lateral heat and can be used on animals as small as rodents (Ellman).

3) Hypothermia: A 250-ml fluid bag microwaved for 30-45 sec and wrapped in a towel serves as a warm waterbed. Heated exam tables are helpful. Heating pads should be made with heated water coils to prevent burns. Pediatric quilts are available which blow warm air on the patient (Bair Hugger by Gaymar/Augustine).[4] Isothermal reusable pads are available (Deltaphase by Braintree) which maintain 39º C for 6 hr via a chemical solution which is solid at room temperature and becomes fluid when heated.

4) Fracture repair: Intramedullary pins can be made using cerclage wire, stylets from spinal needles or catheters, and needles. Splints can be made from sturdy bandage tape, toothpicks, paper clips, Vet-O-Plast (Orfit), syringes, or straws[2] with methylmethacrylate or epoxy in aquarium tubing or red rubber catheters.

5) Wound treatment: a) sealants: tissue glue, Nu-Skin (available at pharmacies), surgical drape, and No-Sting Barrier film (3M Animal Care Products). b) granulation enhancers: BioSyst (Cook), IAMIN-vet (Procyte), Carravet (Veterinary Product Labs), Silvadene; or Granulex (Bertek) c) Bandage material: VetRap pre-cut into 0.6-cm pieces, Tegaderm, bandaids.

Critical Care

Incubators/critical care chambers are available in many sizes with a variety of accessories (Petiatric Supply, Thermocare, HotSpot for Birds). Most critical care systems have nebulizer attachments available from the company. A 10-gallon aquarium can be modified with an 'Aquatop' which has a 17.5 × 20 cm access door and controls temperature and humidity (Petiatric Supply).

Summary

Small mammals can be successfully managed medically by stocking a few specialty items and modifying existing supplies into workable equipment for patients < 500 g. The product manufacturers listed can provide further ideas for small patient management.

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Table 1. Product suppliers and manufacturers.

Abbott Laboratories, Abbott Park, Illinois, www.abbott.com/animal_health/animal_health.html

ACES (Animal Care Equipment and Services, Inc); P.O. Box 3275; Crestline, CA 92325; 1-800-338-ACES (2237); www.animal-care.com/010.htm

A.M. Bickford 12318 Big Tree Rd, Wales Center, NY 14169; 1-800-795-3062; www.ambickford.com/

ATS Laboratories, Inc.; 404 Knowlton St., Bridgeport, CT, 06608; 1-203-579-2700; www.atslabs.com/Contact%20Us%20ATS%20Laboratories.htm

Augustine Medical, Inc. 10393 West 70th St. Eden Prairie, MN 55344; 1-800-800-4346; www.bairhugger.com

Becton Dickinson; 1 Becton Drive; Franklin Lakes, NJ 07417; 1-201-847-6800; www.bd.com

Benepet Pet Care Products; P.O. Box 8111, St. Joseph, MO 64508; 1-816-279-3449; 1-800-825-0341

Bertek Pharmaceuticals; 3711 Collins Ferry Road, Morgantown, WV 26505; 304-285-6420; www.bertek.com

Bioanalytical Systems, Inc.; 2701 Kent Avenue, West Lafayette, IN 47906; 1-765-463-4527; 1-800-845- 4246; www.bioanalytical.com/vetronics/cardio.htm#test

Biopure; 11 Hurley St., Cambridge, MA 02141; 1-617-234-6822; 1-888-337-0929; www.biopure.com

Bonaseptic Company; The Roberts Building-Suite 107, 2996 Grandview Avenue NE, Atlanta, GA 30305; 1-888-TUF-FOOT; www.tuffoot.com/about.htm

Braintree Scientific, Inc.; 1-781-843-2202; www.braintreesci.com

CeramOptec Industries Inc.; Biolitec Inc.; 515 A Shaker Road, East Longmeadow, MA 01028 1-413-525-0600; www.ceramoptec.de/

ConvaTec, Professional Services; P.O.Box 5254, Princeton, NJ 08543-5254; 1-800-422-8811; www.convatec.com/en_US/contact/index.html

Cook Veterinary Products; 501 N. Rogers Street, Bloomington, IN 47404; 1-800-826-2380; www.cookgroup.com

Dairy Association Co, Inc.; P.O. Box 145, Lyndonville, VT 05851; 1-800-232-3610; www.bagbalm.com/page5.htm

Dr. G's Unique Products; Avian Medical Center, 43112 John Mosby Highway, Chantilly, VA 20152; 1-800-478-0355; www.avianmedicalcenter.com/products

Edroy Products Co, Inc; 245 North Midland Ave, P.O. Box 998, Nyack, NY 10960-0998; 1-914-358-6600

Eidolon Corp, 3 Erie Dr, Natrick, MA 01760; 1-800-651-0015; www.slitlamp.com

Ethicon, 1-800-862-2422; www.ethiconinc.com

EVSCO Pharmaceuticals; P.O.Box 685, Harding Hwy, Buena, NJ 08310; 1-800-225-0270, 1-856-691 2411 ext 265; www.evscopharm.com/pages/nutrtion.html

Fray Products Corp.; 2495 Main Street, Buffalo, NY 14214; 1-800-288-6580; 1-716-837-2461; www.frayproducts.com/email.html

Gaymar industries, Inc.; 10 Centre Dr. Orchard Park, NY 14127; 1-800-828-7341; www.gaymar.com

Heska Corporation; 1-800-464-3752; www.heska.com/heskateam/product_catalog.asp

Table 1. Product suppliers and manufacturers (continued).

HotSpot for Birds; 27 Essex Drive, Northridge, CA 91324; 1-323-851-1878; 1-888-246-8776; hotspot@multiscope.com

Hug-U-Vac (Sports Doc Emergency Response Systems); 3871 Concomly Dr.; Salem, OR 97306; 1-503- 364-4340; huguvac@aol.com

Jorgenson Laboratories; 1450 N. Van Buren Ave.; Loveland, CO 80538; 1-800-525-5614; www.jorvet.com; info@jorvet.com

Lone Star Medical Products, Inc.; 8733 Knight Rd., Houston, TX 77054; 1-713-796-0505, 1-800-331-7427; www.lsmp.com/html/veterinary.html

Max Associates; 1-301-776-8824; maxassociates@erols.com

MDS (Medical Diagnostics Services, Inc); P.O. Box 1441; Brandon, FL 33509;1-813-653-1180

MDS Matrx; 1-800-847-1000, 1-716-716-662-6650; www.matrxmedical.com

Medical Engineering and Development Inc., 3334 Vrooman Rd., Jackson, MI 49201; 1-517-789-9060, 1-800-784-9060; www.med-engrg.com

Neurosoft, Inc.; 45150 Business Court, Suite 100, Sterling, VA 20166, USA; 1-703-904 9600; www.neuro.com/medsys/DWLtech2.htm

Nonin Medical Inc.; 2605 Fernbrook Lane North; Plymouth, MN 55447; 1-800-356 8874; www.nonin.com

Orascoptic Research; 1-800-369-3698; 1-608-831-2555; www.orascoptic.com/

Orfit Industries n. v., Vosveld 9a, B-2110 Wijnegem-Belgium (+32).3.326.20.26; www.orfit.be/

Oxbow Pet Products, 29012 Mill Rd., Murdock, NE 68407, 1-800-249-0366; www.oxbowhay.com

Parker Laboratories, Inc.; 286 Eldridge Road, Fairfield, NJ 07004; 1-973-276-9500, 1-800-631-8888; www.parkerlabs.com/us4.htm

Petiatric Supply Co., 3030 Mascot, Witchita, KS 67204; 1-316-831-9500; www.petiatric.com

Pharmacia and Upjohn, 100 Route 206 North, Peapack, NJ 07977; 1-908-901-8000, 1-888-768-5501; www.pharmacia.com

ProCyte Corporation; 8511 154th Ave. NE, Bldg. A., Redmond, WA 98052; 1-425-869-1239; www.procyte.com

Reliable Protein Products; 44-489 Town Center Way, Suite D, PMB 505; Palm Dessert, CA 92260-2723; 1-760-321-7533; www.zoofood.com/insectivore.shtm

Spectrum; 4575 Hudson Dr. Stow, OH 44224-1773; 1-800-444-5644; custserv@spectrumsurgical.com; www.spectrumsurgical.com

StatSpin, Inc; 85 Morse Street, Norwood, MA 02062-4327; 1-781-551-0100; 1-800-STATSPIN; www.statspin.com/index.html

Stoelting Co.; 620 Wheat Lane Wood Dale, IL 60191; 1-630-860-9700

Storz (Karl Storz Veterinary Endoscopy); 175 Cremona Dr., Goleta, CA 93117-3085; 1-800-955-7832; www.karlstorzvet.com

ThermoCare Inc; P.O. Box 60669, Incline Village, NV 89450; 1-800-262-4020

Volk; 1-800-345-8655; www.volk.com

Veterinary Products Laboratories; P.O. Box 34820, Phoenix, AZ 85067-4820; 1-888-241-9545

VSP (Veterinary Specialty Products); P.O. Box 812005 Boca Raton, FL 33481; 1-561-362-7340; 1-800-362 8138; www.vet-products.com

Table 1. Product suppliers and manufacturers (continued).

Welch Allyn; 1-800-535-6663; www.welchallyn.com/misc/search/country.asp
Weck Closure Systems, 2917 Weck Drive, P.O. Box 12600, Research Triangle Park, NC 27709, 1-800-234-9325; www.weckclosure.com/products/7_1_5.html
Yale Apparatus; 1-516-221-0059; www.syringepump.com

DIABETES AND CATARACT IN CAPTIVE PLAINS VISCACHAS (*Lagostomus maximus*) AND LONG-TERM EFFECTS OF A DIET CHANGE

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Abstract

Sixteen captive plains viscachas (*Lagostomus maximus*), including nine animals with bilateral cataracts, were diagnosed with type II diabetes mellitus. Diagnosis was based on the presence of obesity, hyperglycemia, hypercholesteremia, elevated serum fructosamine levels, and glucosuria during a clinical examination of animals under general anesthesia. A complete diet change with marked reduction of energy was initiated, and six individuals received additional monitoring at 3, 6, and 9 mo. Mean body weight decreased by 15% over this period. Glucose and fructosamine were within reference ranges at 3 mo, and cholesterol normalized by 6 mo. After diet change, the captive population of plains viscachas had increased vitality and activity.

Introduction

The plains viscacha (*Lagostomus maximus*) is a large, colonial rodent that inhabits the pampas grasslands of Paraguay, Bolivia, and Argentina. These nocturnal herbivores live in communal burrow systems called "viscacheras" in social groups of 10-30 individuals.[3] Detailed data on their current distribution are not available and they are still hunted commercially for meat and fur. As a result, this species has disappeared from most prime grasslands and agricultural areas in South America. The Zurich Zoo has bred plains viscachas regularly since 1964.[8] For many years, bilateral cataract formation has been observed in neonates, which continue to worsen with age. A clinical study was initiated to ascertain whether hereditary, traumatic, biochemical, or infectious diseases were underlying factors in the development of bilateral cataracts in this species.

Methods

The plains viscachas are housed in an indoor enclosure together with a pair of burrowing owls (*Steotyto cunicularia*). The ground is covered with rocks, sand, and earth which provide digging activity to the animals, but additional artificial burrows of concrete tubes are also present. The diet consisted of apples, carrots, salad, bread, a commercial mineral/vitamin supplement, and rye grass hay (protein 8.9%, fat 1.7%, fiber 29.4%) with branches offered ad libitum. Sixteen animals (nine males, seven females) were anesthetized by mask induction and maintenance with isoflurane in oxygen. All animals received a complete physical and ophthalmologic examination, and the morphology of the cataract was defined in affected individuals. Blood samples were obtained from the medial branch of the saphenous vein and complete hematology counts and serum chemistry profiles were performed. Urine was collected for urinalysis from filled urinary bladders through gentle transabdominal manual pressure or cystocentesis. Results of hematology and serum chemistry

were entered into a computerized database and analyzed statistically. T-tests for independent samples were used to compare means and to evaluate significant differences between viscachas with and without cataract, and for both groups between reference values which were obtained by the author from 44 anesthetized free-ranging plains viscachas from Argentina.[11]

Results

All animals were obese and nine animals showed ophthalmoscopic evidence of lens fiber and/or lens capsule degeneration, varying from focal lens opacities to severe mature cataracts. Intraocular pressure ranged from 4 to 8 mm Hg. Ocular alterations were inconclusive as to the etiology of the cataract. Glucosuria was present in four of eight animals tested. Abnormal hematologic and biochemical values ($P < 0.01$) are listed in Table 1.

Serum glucose values were higher in cataract-affected animals than in non-affected animals. Cholesterol, calcium, fructosamine levels, lymphocyte and monocyte counts were lower in cataract-affected animals. However, these differences were not statistically significant between the two groups of the zoo population.

Due to significant elevations in glucose, cholesterol and fructosamine levels with glucosuria, a presumptive clinical diagnosis of diabetes mellitus with consequent bilateral cataract formation was made. Because previous necropsies of plains viscachas with cataracts revealed immunohistochemically normal insulin production of pancreatic islet cells, the condition was classified as non-insulin dependent or type II diabetes mellitus (T2DM).

Therapy and Follow-Up

Because cataract formation was irreversible in affected animals and surgical intervention was not a feasible option, further efforts focused on the therapy of T2DM to improve the biochemical situation of the animals and prevent further cataract formation. In humans and cats with T2DM, correction of obesity by dietary measures is essential for diabetic control.[2] For the captive plains viscachas, a complete diet change with marked reduction of energy and protein with an increase of fiber was instituted. Carrots, bread, apples and salad were no longer offered. The basis of the new diet was a 1:1 rye grass hay and straw mixture which was enriched with a vitamin/mineral supplement and 15 mm herbivore pellets (Pre Alpin Lepo, Agrops GmbH, 82541 Degerndorf, Germany, protein 10%, fat 3.5%, fiber 22.7 %). During the summer, fresh cut grass was offered. The new diet was well accepted by the animals and showed a welcome behavioral enrichment in the form of greater activity.

A total of six animals, three cataract-affected and three non-affected animals, were selected for further monitoring. Anesthesia and sampling was performed as described above at 3, 6, and 9 mo after the diet change. Mean alteration of body weight was -10.7 % for the 0-3 mo period, -5.5 % for the 3-6 mo period, and +1.2 % for the 6-9 mo period. Mean body weight decreased over the whole observation period by 15.0 %. Reductions in serum glucose, fructosamine, and cholesterol is

illustrated in Figs. 1-3. After the diet change, glucosuria was absent. No further cataract formation occurred in previously non-affected animals and overall, the animals showed increased vitality and activity.

Discussion

Hyperglycemia, hypercholesteremia, and glucosuria were consistent findings for cataract-affected as well as non-affected animals, indicating a diabetic condition in the plains viscachas. However, these values can show large variability between animals, and may be influenced by food intake and stress. A glucose tolerance test after fasting was not feasible. Therefore, fructosamine levels, which have been used for diagnosis of diabetic dogs and cats and reflect glycemic control over the previous 2 to 3 wk,[7] were important to verify the diagnosis in the plains viscachas. Moderate hypercalcemia in the zoo animals may reflect the different mineral intake between the mineral-supplemented captive animals and the free-ranging reference animals. However, disturbances in calcium homeostasis are a common metabolic abnormality in diabetic humans and contribute to cataract formation.[6] Lymphocytosis and monocytosis occur in chronic or acute stress situations and were related to capture and handling as well as to the diabetic condition of the animals.

Although inbreeding in the viscacha group at the zoo occurred over a long period and a genetic basis of the disease cannot be ruled out, inappropriate diet was the main factor causing T2DM and secondary cataract formation in these animals. T2DM is known to occur in several species of wild rodents in captivity if they are fed a high energy diet.[1,4,5,9,10] Most of these reported species originate in harsh, arid climates similar to the pampas. Further studies will be necessary to characterize the pathogenesis of the disease in the plains viscacha more completely, which may be a useful model for human T2DM.

ACKNOWLEDGMENTS

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Table 1. Abnormal hematologic and biochemical values of viscachas ($P < 0.01$).

Variable	Reference values from free-ranging viscachas ($n = 44$) Mean ± SD[a]	Zoo viscachas with cataract ($n = 9$) Mean ± SD[a]	Zoo viscachas without cataract ($n = 7$) Mean ± SD[a]
Glucose (mmol/L)	7.58 ± 2.26	17.24 ± 4.40	14.27 ± 3.20
Cholesterol (mmol/L)	1.30 ± 0.47	2.94 ± 0.78	3.27 ± 0.52
Calcium (mmol/L)	2.24 ± 0.18	2.72 ± 0.17	2.68 ± 0.09
Fructosamine (µmol/L)	255.68 ± 40.08	345.50 ± 44.94	364.17 ± 35.58
Lymphocytes (/µl)	2025 ± 1144	5372 ± 1945	6053 ± 2735
Monocytes (/µl)	501 ± 220	1232 ± 420	1312 ± 305

[a]Standard deviation.

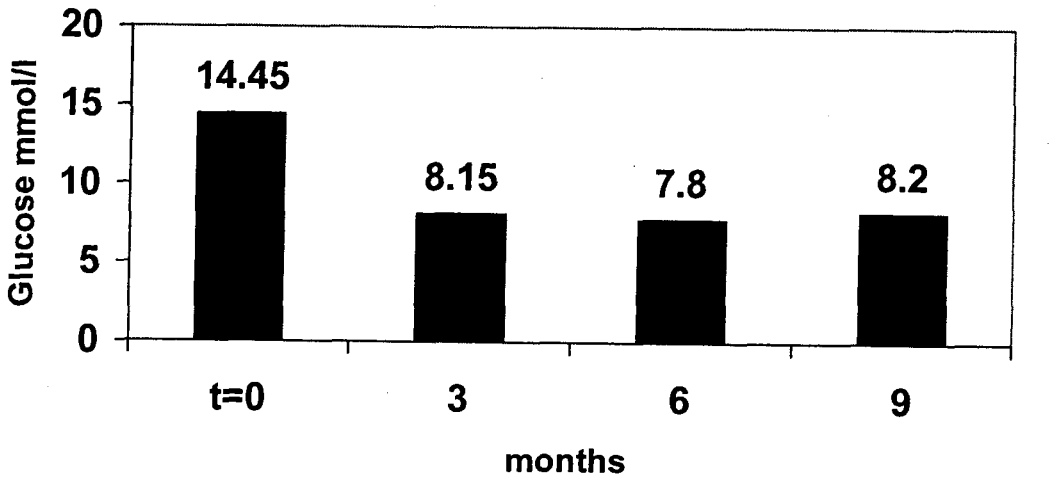

Figure 1. Mean long-term glucose values after change of diet in six captive plains viscachas.

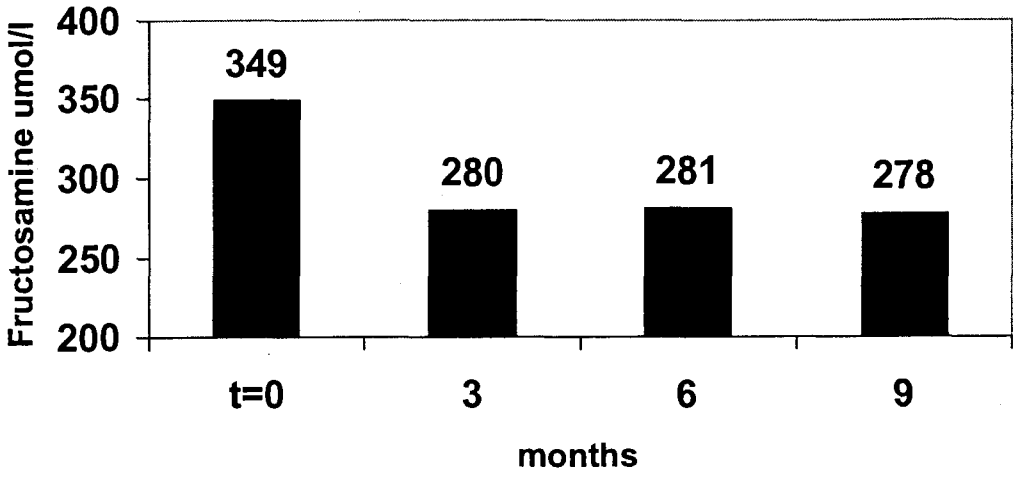

Figure 2. Mean long-term fructosamine values after change of diet in six captive plains viscachas.

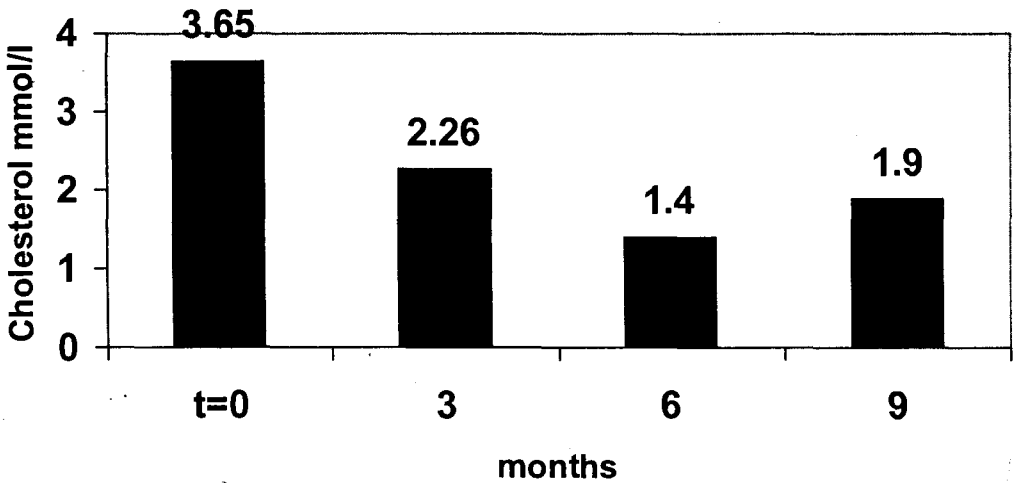

Figure 3. Mean long-term cholesterol values after change of diet in six captive plains viscachas.

COMMON MEDICAL PROBLEMS OF MEGACHIROPTERANS IN CAPTIVITY

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Abstract

Many species of Megachiroptera are being kept in increasing numbers in zoos throughout North America and Europe. Megachiroptera have a number of medical problems associated with trauma and nutritional deficits. Traumatic injuries are usually seen during transportation or conditions of overcrowding, as well as inappropriate social groupings (i.e., male groups). Dietary imbalances have resulted in dilated cardiomyopathy, fluorosis and metabolic bone disease. Dental diseases mimic those seen in small animal medicine. Infectious diseases are uncommon and sporadic, but recent reports of zoonotic viral infections in Astral-Asian bats warrant careful quarantine of wild caught bats.

Introduction

After rodents, bats comprise the second largest group of mammals and are found across the entire globe with the exception of Antarctica and north of the Arctic circle.[8] The morphology of bats has been questioned in recent years, and it is estimated that there are some 900 species of living bats. All bats belong to the order Chiroptera, which consists of two suborders, the Megachiroptera and Microchiroptera. Megachiroptera are the Old World fruit bats or flying foxes, which are found in subtropical regions of Africa, Asia and Indo-Australia. These bats are all in one family, the Pteropodidae, and consume fruits, flowers and or flower products.[6] The remaining bats are in the suborder Microchiroptera, which is composed of 17 families of bats. The Microchiroptera have greater diversity and consume insects, flesh, blood, nectar, flowers and pollen.[6] With bat persecution and environmental loss continuing worldwide, bat populations are becoming more endangered. Bats are now identified as major pollinators and distributors of seeds in tropical forests.[9] Bat welfare plays an important role in the conservation of tropical forests. Recently bat conservation has taken a step forward, with work being completed in both the field and in captive situations.

Trauma

Abscesses and Swellings

Bite wounds are very common and are not usually noticed until a swelling or a draining wound develops. Bite wounds to the head may result in abscess formation on the snout and head; lacerations of the eyelid, and third eyelid have also been documented. *Streptococcus, Staphylococcus, Pasteurella* and various anaerobes have been cultured from these bite wounds.[5] Treatment involves lancing the abscess and flushing with 2% chlorhexidine diacetate (Nolvasan®,

Fort Dodge, Fort Dodge, IA 50501 USA) under anesthesia; oral antibiotics may decrease recurrence. Antibiotic treatments, such as trimethoprim-sulfamethoxazole (Septra® Glaxo Wellcome, Research Triangle Park, NC 27709 USA; 20 mg/kg p.o. every 12 hr for 7 days) or amoxicillin-clavulanate acid (Clavamox® Beecham, Philadelphia, PA 19101 USA; 20 mg/kg p.o. every 12 hr for 7 days), have been effective. The abscess may resolve in about 1 wk, however, multiple debridements are often warranted. Damage to the eye may include lacerations of the eyelid, and possibly cornea. Fluoroscein staining followed by treatment with topical ophthalmic ointments is recommended.

Patagial Trauma

Trauma to the wing patagium may result in small holes or large lacerations. These wounds rarely become infected and will heal through constriction over a few weeks. Poor husbandry, such as malnutrition and chronic injury, may result in excess scar tissue formation along the healing edge, which may result in a permanent hole in the patagium. Palpable thickening of the wound edge may be noted, along with loss of pigmentation. Once husbandry conditions have been improved, excess scar tissue may be removed using electrocautery; suturing is not necessary. Trauma to greater than 75% of the wing patagium can result in constriction of the wing tissue and permanent loss of flight. This is rare and the wing should be given a number of weeks to heal before any drastic measures, such as amputation, are taken.

Iatrogenic thermal burns caused by artificial heating devices such as warm water bottles and heat lamps occur very easily. Wing tissue is very thin, and even warm water bottles that do not feel hot to the touch may result in large areas of necrosis within a few days after exposure. Circulating water blankets should be covered by a towel during prolonged anesthetic procedures. Wet bats can be dried using hair dryers set on low. Bats recovering from anesthesia are best placed in a avian or infant incubator to increase body temperatures

Nail Avulsion

Besides the feet, the thumb (digit number 1 on each wing) is the main grasping claw and is necessary for ambulation. These nails are easily damaged, especially while moving around in wire caging. The trauma is similar to that of a dog or cat who has torn off a nail and may be painful for a few days. Thumbnail avulsion can be treated by application of Soft Nails ® (SmartPractice, Inc., Phoenix, AZ 85008 USA), a plastic cap designed to glue over cat nails as an alternative to declawing. In this procedure, a small amount of surgical glue is applied to the nail bed and the appropriate sized cap is applied. This cap will protect the nail bed and will fall off once nail starts growing. In most cases, the cap will stay on for several weeks, but reapplication may be necessary if the cap falls off prematurely. These caps have worked very well, and speed recovery and the use of the thumb.

Frost Bite

Megachiropterans housed in outdoor enclosures are susceptible to frostbite. Injuries seen at the

Lubee Foundation have been limited to the nail beds of the toes and the wing tips. This injury presents similarly to the thumbnail avulsion, with nail bed necrosis and subsequent sloughing of the nail. The toe injuries are problematic, as the bats cannot roost in the normal hanging position. This problem can be solved by placing the bats in a smaller indoor enclosure equipped with plastic hammocks for the bats to lie in. Hammocks made of plastic fencing material are easy to install and clean. The hammocks were placed about 60 cm from the top of the cage, allowing the bats to lie down without using their feet. Soft Claws® can be used to cover the nail beds until the toenails regrow. Antibiotic treatment may be warranted for the first few weeks. As the nails heal, the bats perch by placing most of their foot through the wire cage and grip with the toes. Regrown nails may be straight, not curved, which does not allow for normal roosting behavior. Repeated grooming of the new nails over several months with a Dremel tool to place a curve on the plantar surface of the nail may facilitate return to normal roosting.

Fractures

Fractures of long bones such as the femur and radius, may be repaired using techniques similar to those used in avian medicine.[1] Metacarpal fractures of the finger bones are very common and may result from fighting or trauma. If the fracture is closed and is not in the distal 1/3 of the metacarpal bone it may heal without external fixation. Confine the bat for 1 mo to stop flying and facilitate healing. Fractures near the distal end of the metacarpals may result in open fractures or malunions. Due to the small size of these bones, external fixation or coaptation are difficult to apply successfully. Alternatively, the chronic non-healing fracture or exposed bone fragment may be removed using electrocautery to dissect out the bone and loose wing skin. Fortunately, these fractures usually occur in the second phalanx (Megachiroptera do not have a third phalanx) and do not affect flight. Once the loose fragment is removed, shorten the exposed bone with a pair of Mayo scissors after pulling the skin proximally, allowing the skin to cover the end of the bone. A drop of tissue glue on the end will facilitate healing. The wound is subject to being damaged and reopened. Fortunately, progressive osteomyelitic lesions that ascend up the bone appear to be rare. Treatment with oral antibiotic may be warranted.

Nutrition

Dilated Cardiomyopathy

Dilated cardiomyopathy has been documented in wild caught *Pteropus* sp.[3,4,9] These bats may show cranial edema, lethargy, muscle wasting and blue mucous membranes. Enlarged hearts can be visualized on thoracic radiographs, while ultrasonography may reveal ventricular and atrial dilation, myocardial wall thinning, and poor contractility. All bats reported with this condition had undetectable blood levels of vitamin E (< 56 IU/kg). Supplementation of vitamin E in the diet to 240 IU/kg (dry matter basis) has almost eliminated this problem in megachiropterans. Treatment of affected animals has been successful with medical management.[4]

Fluorosis

Excess fluoride ingestion causes multicentric hyperostosis of bone surfaces with resultant proliferation of bone in the carpus. Lesions seen at the Lubee foundation appeared as enlarged thumb joints in lactating *Pteropus vampyrus*. These lesions have also been described in *Pteropus giganteus, Pteropus poliocephalus* and *Rousettus aegyptiacus* at the Toronto and Calgary Zoos.[2] These proliferations result in large firm raised areas which cause the overlying skin to become taut and subject to abrasion, laceration and secondary infection. Fluoride levels of affected bats measured at necropsy were 2200-4700 ppm; unaffected bats at another collection had levels of 290-320 ppm.[2] Dicalcium phosphate from shrimp meal which contained ~1900 ppm of fluoride was the main source of toxicosis. A change to monocalcium phosphate has resolved the problem.

Hypocalcemia and Metabolic Bone Disease

Swollen joints, curvature of long bones, and flattened faces were found on visual exam of *Pteropus vampyrus* pups. Serum calcium levels were low at 2.5 mg/dl (9.5 mg/dl ± 0.7mg/dl), ISIS Physiological Reference Ranges, 1999). Discontinuation of the dicalcium phosphate supplement for a period of 6 mo during the fluorosis problem resulted in metabolic bone disease in these rapidly growing pups of the largest megachiropteran. Affected pups were supplemented with oral calcium (calcionate syrup, Rugby, Norcross, GA 30071 USA; 23 mg/ml) 2 ml p.o. every 12 hr as a supplement to the diet. After 2 wk of treatment, the serum calcium levels had normalized and the deformities in the long bones began correcting themselves.

Infectious Disease

Respiratory Disease

Respiratory disease is uncommon in the Megachiroptera. Sneezing and clear nasal discharge are common in healthy fruit bats. However, Wahlberg's epauletted fruit bats (*Epomorphus wahlbergi*) with pneumonia frequently demonstrate anorexia and seclusion from the colony.[5] Respiratory cultures have isolated a *Pasturella*-like bacteria as the pathologic agent.[7] Treatment with enrofloxacin (Baytril, Bayer Corp., Shawnee Mission, KS 66201 USA;10 mg/kg i.m. or p.o. every 12 hr) may be indicated. Unfortunately, this species of bat is easily stressed and may die during treatment.

Gastrointestinal Disease

Acute death in three Rodrigues fruit bats (*Pteropus rodricencis)* was diagnosed as severe necrotizing enteritis caused by *Yersinia enterocolitica*. The primary enteric infection resulted in embolic spread of bacteria with hemorrhage, necrosis, and inflammation present within multiple organs. Fecal culture of 17 additional bats in the same enclosure was negative for *Yersinia enterocolitica*. Fecal culture for *Yersinia* is recommended for any "down" bats.

Dental Disease

Dental problems such as fractured teeth, dental abscesses and staining are common in most species of Megachiroptera. Staining in wild-caught bats appears as dark brown to black areas of discoloration and may be related to enamel defects.[5] The staining does not cause problems and should be considered an incidental finding. Tooth root or apical abscesses appear as swellings on the ventral mandible or dorsal maxilla. Treatment involves lancing the abscess and removing the tooth under general anesthesia. In smaller bats < 500 g, an 18-ga needle can be used as an elevator to loosen the dental ligament allowing removal of the tooth. Postoperative analgesia such as butorphanol (Torbugesic®, Fort Dodge, Fort Dodge, IA 50501 USA; 0.2 mg/kg i.m.) should be used in association with a course of antibiotics. These wounds heal quickly, and usually resolve within 2 wk. Chronic abscesses involve bone and may appear as small hard lumps under the ventral mandible. These lesions should be evaluated radiographically, and extended courses of bone-penetrating antibiotics such as amoxicillin-clavulanate acid at 20 mg/kg p.o. every 12 hr may be indicated.

Viral Diseases

A number of viral diseases of megachiropterans have been recently identified in the Australian/Southeast Asian region, including Hendra virus, Bat lyssavirus (bat-like rabies), and Nipha virus. These viruses are zoonotic and have caused fatalities in humans, horses and pigs. Newly imported bats should be quarantined appropriately, including serial titers for these diseases. For additional information, see the CDC Conference on Emerging Infectious Diseases at www.cdc.gov.

Conclusion

Nutritional deficiencies and trauma are the main causes of disease in captive megachiropterans. Infectious disease and neoplasia also occur. The use of volatile gas anesthesia (Isoflurane®, Abbott Labs, Chicago, IL 60004 USA) has made physical examination and treatment of common problems safe and easy. Megachiropterans respond well to treatment, and learn to take medications without restraint using behavioral conditioning.

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ORAL AND RECTAL MICROBIAL FLORA OF THE GREAT INDIAN FRUIT BAT *(Pteropus giganteus)*

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Abstract

Although bats are popular exhibit animals at zoologic institutions, little is known about alimentary tract microflora of these animals. Few studies characterizing bat rectal microbial flora have been performed and oral cavity microflora has not previously been investigated.[1,6,7,9,10] Hence, antimicrobial therapy of bat bites to handlers or other bats is usually empiric and determination of disease status of the alimentary tract may be difficult.

Eight (five male, three female) apparently healthy great Indian fruit bats (*Pteropus giganteus)* were manually captured and restrained for induction and maintenance of anesthesia with isoflurane in oxygen administered via face mask. After physical examination, bats were sampled to identify oral and rectal microbes. Oral samples were obtained by swabbing the buccal and lingual gingiva, tongue, and hard palate. These samples were cultured for aerobic and anaerobic bacteria and fungi. Rectal samples were collected by insertion of the culturette into the rectum, and were cultured for aerobic bacteria. The diet was also sampled by inserting a culturette into the diet prior to its removal from the bats' enclosure on 3 consecutive days. The diet was composed of oranges, grapes, apples, raisins, spinach, bananas and monkey chow. Diet samples were cultured for aerobic bacteria and fungi. All samples were transported at ambient temperature to the Louisiana Veterinary Medical Diagnostic Lab.

Aerobic, anaerobic, and fungal isolates were cultured using standard culture techniques and observed for colony and gram stain or lactophenol blue mount morphology for identification to the generic level. Catalase testing, hemolysis and lactose fermentation were used to determine whether api STAPH, api STREP, api 20E, or api 20NE biochemical tests (API Analytab Products, Biomerieux, Hazlewood, MO 63042-2320 USA) were used for further characterization of aerobic microbial isolates. Yeast were characterized with api 20CAUX biochemical test kit (Yeast Strip, Biomerieux, Hazlewood, MO 63042-2320 USA).

The eight *P. giganteus* tested had a mixed oral microbial flora, which was predominantly Gram positive with anaerobic and fungal components. Aerobic oral and rectal microbial isolates found in more than one bat are listed in Table 1. Common anaerobic oral isolates were *Fusobacterium* spp. (n = 4) and *Peptostreptococcus* spp. (n = 5). Common fungal isolates of the oral cavity were *Cladosporium* spp. (n = 5), *Candida albicans* (n = 4) and *Trichosporon* spp. (n = 2). Many of the

organisms isolated from the oral cavity of *P. giganteus* have also been identified in bite wounds inflicted by dogs and cats.[2,4] Wound infections from animal bites are often polymicrobial and *P. giganteus* appears to be no exception. All bats sampled had greater than one aerobe, at least one anaerobe and most had fungal isolates in their oral cavity. *Aeromonas* spp., *Citrobacter freundii,* and *Clostridium* spp. were identified as potential pathogens not previously reported in bite wounds.[3,5,8]

Rectal aerobic isolates from the eight *P. giganteus* tested were predominantly Gram negative bacteria (Table 1), similar to those previously reported for Pteropodidae.[6,9] The aerobic bacteria most commonly isolated from the diet was *Pantoea* spp., and the most common dietary fungal isolate was *Candida parapsilosis*, with each isolate found on 2 separate days of dietary sampling. *Salmonellae* spp. were not isolated from oral, rectal or dietary samples. Dietary isolates and oral flora were dissimilar, having only Gram positive streptococcal and staphylococcal organisms in common. However, most bacteria isolated from the diet were also found in the rectal flora.

For empiric bacterial treatment of *P. giganteus* bite wounds, the clinician should consider therapy targeted toward Gram positive microbes and anaerobic bacteria while culture and susceptibility results are pending. In wounds unresponsive to appropriate antibacterial therapy, fungal culture and antifungal therapy should be considered. Rectal bacterial isolates should be interpreted carefully as they may be influenced by diet.

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Table 1. Common oral and rectal aerobic bacterial isolates of the giant Indian fruit bat *(Pteropus giganteus) n* = 8.

Isolate	Oral (*n*)	Rectal (*n*)
Citrobacter freundii	2	-
Enterobacter cloacae	-	2
Pantoea spp.	-	2
Pasteurella spp.	-	2
Proteus mirabilis	-	4
Bacillus spp.[a]	2	-
Gemella morbillorum [a]	2	-
Staphylococcus sciuri[a]	2	-
Staphylococcus spp. (coagulase negative)[a]	3	4
Staphylococcus simulans [a]	2	-
Streptococcus spp. (alpha hemolytic)[a]	6	2

[a]Most oral isolates are gram-positive.

RETROSPECTIVE STUDY OF MORBIDITY AND MORTALITY IN THE TWO-TOED SLOTH (*Choloepus hoffmanni* AND *Choloepus didactylus)* IN NORTH AMERICA

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Abstract

A retrospective study of morbidity and mortality of two-toed sloths (*Choloepus didactylus* and *Choloepus hoffmanni*) was conducted to elucidate medical problems diagnosed in captivity in North America. Eighteen out of 39 institutions responded to a request for medical records with a total of 101 animals represented. Findings were tabulated by organ system and divided into age groups. Major causes of morbidity in all groups included parasitemia, dental disease, and trauma. End-stage renal disease accounted for the majority of deaths. Phlebotomy sites included the cephalic, superficial antecubital, jugular, and femoral veins. Multiple anesthetic protocols were described with varying degrees of success.

Introduction

Sloths belong to the order Edentata, suborder Xenarthra, family Bradypodidae. This family contains two genera: *Choloepus* (the two-toed sloth) and *Bradypus* (the three-toed sloth). Species differ in anatomy, physiology, behavior and habits. The term "sloth" applies to *Choloepus* in this paper.. *Choloepus didactylus* is found in Guyana, northern Brazil, eastern Colombia and eastern Venezuela. *Choloepus hoffmanni* is found from Nicaragua, through Central America into Panama and then southward into Columbia, Venezuela, Guiana, Ecuador, Peru, Brazil and Bolivia. Both species are found at the tops of tall trees in tropical rain forests, mixed deciduous forests, and secondary growth forests. They thrive in the high humidity found in the vicinity of rivers. Sloths are almost strictly arboreal, but are good swimmers. They do not ambulate well on land.

C. didactylus usually has seven (range: six to eight) vertebrae, while *C. hoffmanni* usually has six (range: five to six). Sloths have limbs vascularized by rete mirable and an elongated thorax (21-25 pairs of ribs). They can withstand 10-40 min of apnea, but do not possess a specific "diving reflex." Heart rate ranges from 70-130 beats/min, with a respiratory rate of 13-14 breaths/min. Mean body temperature is 34.4º C (94º F). Sloths are heterothermic and unable to shiver, but pant and do have sweat glands.[5] Their thermoneutral zone is above 25º C (77º F), but critical ambient temperatures are from 12º C (53º F) to 30ºC (86º F); sunshine can increase body temperature up to a lethal level.[10] Sloths do well in enclosures that range from 24-29.5º C (75-85º F) with humidity at 60-70% (minimum 40%).[3,4] Sloths weigh from 2.7-8.1 kg, but the gastrointestinal contents and urine can account for up to 20-30% of their body weight. Sloths generally defecate and urinate at the same time with a frequency of once every 3-8 days. The bladder of an adult sloth can hold up to 1.5 L of urine. Sloths have a low metabolic rate, which is attributed to low thyroid function not hypothyroidism.[5] In captivity they rest or sleep approximately 20 hr/day. Free-ranging sloths

remain active through most of the night resulting in approximately 11 hr of activity/day.[8] Sloths have one set of teeth their entire life; five upper and four lower. The teeth consist of dentine and vasodentine and are surrounded by cement. There is no enamel. Sloths have a ruminant-like stomach with three compartments. In the wild they eat a variety of plant material including buds, leaves, flowers, fruit, twig tips and young stems. Animal material may also be consumed when available,[8] although no documentation was found of this in wild sloths. Meat based products are added to captive diets because sloths consume it readily when offered.[5] They eat better when their food is diced as opposed to chunked or sliced.[8] Vitamin K deficiency has been noted in other xenarthrans and has been added prophylactically to some diets. The sex of sloths can be difficult to determine, as the penis cannot always be expressed and the testis are intraabdominal. The opening at the genital protuberance appears more slit-like in the female than the male. Age in captivity can reach over 20 yr and has gone beyond 30 yr in a few instances.

Materials and Methods

Institutions housing two-toed sloths in North America were contacted and medical records requested for all deceased, transferred and living animals at their institutions. Eighteen institutions responded with 101 animals represented. Some medical records contained very limited medical information, but were included for completeness. Findings were tabulated by organ system and divided into age groups. Divisions include: neonates < 4 wk; 4 wk to 4 yr; 4 to 10 yr; 10 to 15 yr; 15+ yr; and age unspecified. Disease processes were not separated by species because correct speciation of all animals was not known.

Morbidity

Neonates < 4 wk (five events)
Integument (20%): Tropical rat mite.
Trauma (20%): Laceration.
Gastrointestinal (20%): Constipation.
Misc (40%): Two were hand-reared (poor suckling; maternal neglect).

4 wk-4 yr (39 events)
Respiratory system (15%): Nasal discharge, either mucoid or mucopurulent.
Gastrointestinal ailments (38%): Stomatitis; Intestinal parasites; Constipation; Enteritis.
Musculoskeletal (20%): Dislocation of the carpus/metacarpus; Osteosclerosis; Bite or fight wounds; Traumatic compression at L2- L3.
Urinary tract (2.5%): Urethritis.
Integument (20%): Burns; Alopecia; Ulcerative dermatitis; Superficial abrasions; Decubital sores; Cutaneous abscess; Pruritis with seborrhea sicca.
Ocular (2.5%): Bilateral conjunctivitis.

4-10 yr-old (56 events)
Respiratory (5.4%): Nasal discharge.

Gastrointestinal (18%): Intestinal parasites; Abdominal and umbilical hernia; Dental tartar; Dead tooth; Enteritis.
Musculoskeletal (47%): Bite or fight wounds; Porcupine quills in the mouth; Torn/broken nails.
Urinary (2%): Cystitis.
Reproductive (10%): Serosanguinous vaginal discharge; Aborted fetus.
Integument (10%): Abrasions; Cutaneous abscesses; Dermatosis; Decubital sores; Ulcerative dermatitis.
Other (7.3%) Heat stroke/stress.

10-15 yr-old (58 events)
Respiratory (3%): Nasal discharge.
Gastrointestinal (39%): Dental problems; Lingual ulcerations; Mycotic glossitis.
Musculoskeletal (22%): Fight or bite wounds; Fall; Broken nails; Puncture wound.
Urinary (5%): Renal calculi; Pyelonephritis; Urinary tract infection.
Ocular (3%): Corneal scratch; Corneal scar.
Reproductive (9%): Mastitis; Abortion in second trimester; Mid-term abortion.
Cardiovascular (3%): Mineralized descending aorta; First degree AV block.
Integument (16%): Ectoparasites; Abrasions; Moist dermatitis; Infected puncture wounds; Burns; Periocular alopecia; Generalized alopecia and pruritis.

15+ yr-old (57 events)
Respiratory (10%): Nasal discharge; Thoracic mass.
Gastrointestinal (30%): Dental problems; Intestinal parasites; Oral Candidiasis; Protozoal enteritis.
Musculoskeletal (12%): Nails ripped off; Deformed nail; Fight or bite wounds.
Urinary (14%): Cystitis; Renal calculi; Cystic calculi.
Ocular (12%): Cataracts; Mucopurulent discharge; Foreign body; Corneal ulcer; Mucoid discharge.
Reproductive (9%): Minimal multifocal fibrinopurulent placentitis; Early term abortion; Penile discharge.
Cardiovascular (2%): Possible calcification of aorta.
Integument (11%): Abrasions; External parasites; Interdigital dermatitis; Aural hyperkeratosis; Bowenoid carcinoma in situ; Mucocutaneous ulcers; Ulcerative epidermitis; Lymphoplasmacytic superficial dermatitis.

Age Unspecified (24 events)
Respiratory (17%): Nasal discharge.
Gastrointestinal (29%): Intestinal parasites; Mycotic stomatitis.
Musculoskeletal (12.5%): Broken nails accounted for all of these incidences.
Ocular (8%): Conjunctivitis; Mucopurulent discharge.
Integument (33%): Cutaneous cysts; Burns; Moist dermatitis; Periorbital dermatitis; Laceration.

Mortality

Neonates (nine sloths)
Aborted fetus' (22%): Early term and second trimester.
Stillborn (22%): Amniotic aspiration; Acute Fetal Distress Syndrome.
Respiratory (11%): Drowning.
Musculoskeletal (11%): Perinatal trauma
Unknown (33%): No necropsy performed; Passive congestion of all organs with no specific disease process identified; possible abortion.

4 wk-4 yr (eight sloths)
Respiratory (25%): Drowning; Aspergillosis.
Cardiovascular (12.5%): Systemic septicemia.
Unknown (62.5%): No necropsy performed.

4-10 yr-old (two sloths): Systemic septicemia; No necropsy performed.

10-15 yr-old (four sloths)
Gastrointestinal (25%): Gastric impaction.
Cardiovascular (25%): Terminal septicemia.
Urinary (50%): End stage renal disease; Tubulointerstitial nephritis.

15+ yr-old (four sloths)
Gastrointestinal (25%): Perforating ulcer in fourth chamber of stomach.
Unknown (25%): Euthanatized due to old age; "many age related problems" at necropsy.
Urinary (25%): Renal disease.
Mixed (25%): End stage kidneys (cystic) with severe diffuse histiocytic to purulent necrotizing bronchopneumonia.

Age Unspecified (nine sloths)
Gastrointestinal (11%): Gastric ulceration.
Cardiovascular (11%): Cardiac valvular disease.
Unknown (33%): No necropsies performed.
Urinary (45%): End stage kidney disease; Carcinoma of bladder. Transitional cell carcinoma of the bladder.

Discussion

Parturition was noted in all age groups over 4 yr of age. Overgrown nails were seen in all age groups except neonates. Abnormal nail growth was not uncommon, and associated with osteosclerosis of the distal interphalangeal joints in one case. Nasal discharge was not always associated with a specific etiology, and many of the organisms cultured were considered commensals. Increased humidity improved or resolved clinical signs in many cases. In the sloths

that died of pneumonia, nasal discharge was only occasionally seen premortem.[2] Fungal organisms were seen on tracheal lavage of one sloth with a thoracic mass. Madurella and Penicillum were cultured and the animal responded to anti-fungal therapy.

Candida was present in several cases of glossitis/stomatitis. The diagnosis was based on cytologic exam of oral lesions. *E. coli*, *Aeromonas*, *Enterobacter* and *Salmonella* have been cultured from feces of animals with enteritis, but their contribution to the disease process is unknown. *Salmonella* was also cultured from the gastrointestinal tract of animals without clinical disease, and has been reported in asymptomatic free-ranging animals.[6] Intestinal parasites included strongyles, hookworms, flukes, ciliates, flagellates, tapeworms, strongyloides, nematodes, coccidia and trematodes. Dental diseases included tartar, dead teeth, points on cheek teeth, overgrown canines, mild gingivitis, fractured teeth, periodontal disease, gingival hyperplasia, malocclusion, excess wear and abscessed teeth. More dental disease was reported in the older animals, especially points that were causing oral ulcerations/lacerations.

Bite or fight wounds occurred frequently, and slow introduction of sloths in enclosures large enough to provide a territory for each animal is recommended. Fighting was alleviated in some cases when more food pans were added to the enclosure, with hanging branches placed close enough to allow animals to eat comfortably. Over half of the bite wounds reported became infected. Burns from hot water pipes in the enclosure were common. Torn or broken nails were suspected to occur from entrapment of nails in portions of the exhibit, especially air or heating vents.

Urinary tract infections were reported frequently. While the cause the infections was not always known, *E. coli* was cultured in a few instances, and *Streptococcus* Group D was isolated from an animal with pyelonephritis. Pyuria did not always accompany bacteruria. Flagellates and fungi were identified in urine sediment collected by cystocentesis. These conditions were not treated and their clinical significance was not determined. Renal calculi were diagnosed radiographically multiple times. Cystic calculi were associated with rectal prolapse in one animal.[9] Calculi were composed of calcium, magnesium, ammonium and phosphorus in one sloth, while another animal had crystals containing magnesium phosphate hydrate, carbonate apatite and protein.[9] Previously urinalysis values reported for sloths differ from those recorded in the medical records reviewed.[7] Isosthenuria was noted in clinically normal animals. Water deprivation tests are not recommended in this species, as death occurred in some of the sloths when attempted.[5]

Kidney disease was a frequent cause of death. Blood values obtained prior to death in animals which died from renal failure did not consistently show azotemia. The death from gastric impaction was secondary to interstitial nephritis and dystrophic calcification of the stomach. Gastric mucosal mineralization was identified in another animal that died with end stage renal disease. Mineralization of the descending aorta was visualized radiographically in more than one animal.

Phlebotomy sites included the superficial antebrachial or the cephalic vein, the jugular vein using ultrasonic location in some cases, the femoral vein using doppler detection,[1] and a quicked nail. Cardiocentesis and the dorsal vertebral vein have been reported as phlebotomy sites.[3,4] The dorsal

vertebral vein is a 10 mm diameter vein that lies extradurally to the right of the spinal cord in the dorsal region, with nerve roots passing dorsal to it.[5] Heparin and EDTA were utilized as anticoagulants, but hematologic profile studies use EDTA.[1,11,12]

Various anesthetic protocols have been utilized including isoflurane for induction and maintenance; ketamine 2-6 mg/kg for sedation; ketamine plus halothane, nitrous oxide and oxygen, and telazol 2-6 mg/kg. During translocation of 202 wild sloths, four anesthetic protocols were evaluated,[10] including acepromazine 0.1 mg/kg + ketamine 10 mg/kg; xylazine 1mg/kg + ketamine 10 mg/kg; tiletamine + zolazepam 10 mg/kg combine; and medetomidine 0.04 mg/kg + ketamine 3 mg/kg. The two protocols recommended for 30-40 min procedures including minor surgeries were medetomidine 0.04 mg/kg + ketamine 3 mg/kg or xylazine 1 mg/kg + ketamine 10 mg/kg.[10] Atipamezole at five times the medetomidine dose is recommended for reversal. Intubation can be very difficult in sloths.

Although early reports recommended reduction in dosage and frequency of medication due to the large stomach and low metabolic rate,[3] subsequent articles suggest this is not necessary. The injectable route may be preferable due to the unknown effect of oral medications on the forestomach and vice versa. Selection of oral medications should be guided by ruminant protocols, and the use of doxycycline or certain sulfa drugs is recommended.[4]

The clinical presentation of a sick sloth was similar in many cases and included weakness, dehydration, anorexia and weight loss. Routine physical examination of sloths was not common. Animals were generally evaluated only when problems arose, which may have resulted in disease processes being missed if the clinical signs did not warrant evaluation of the animal under anesthesia. Routine health assessments of sloths under anesthesia is encouraged and should include whole body radiographs, blood collection for complete blood counts and serum chemistry evaluation, and cystocentesis for urinalysis.

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CASE CLUSTER OF PANCREATIC ISLET FIBROSIS IN ROCK HYRAX (*Procavia capensis*)

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Abstract

From 1990 to 2000, 10 cases of pancreatic islet fibrosis were documented in the Dallas Zoo collection of 64 rock hyrax (*Procavia capensis*). Eight (one male, seven females) of these animals entered the collection as adults and were presumed to be unrelated to each other. Four of the females arrived in 1993 pregnant by wild, unknown males, and produced nine (two males, four females, three unsexed) offspring collectively. In 1994 and 1995, the single captive adult male sired 47 (17 males, 26 females, 4 unsexed) offspring from these wild-caught females and one of their 1993 offspring. During the late 1990s, three rock hyrax presented with clinical diabetes mellitus (Cases 4, 6, and 10), ultimately related to pancreatic islet fibrosis. Additional cases of pancreatic islet fibrosis were documented in four unrelated (Cases 1, 2, 3, and 5) and five related animals (Cases 6-10). This clustering of disease supported a review of the medical history, family tree, and pathology for this species in the collection (Fig. 1, Table 1).

Both the Dallas Zoo and five other institutions maintained offspring from this line. Review of the medical history provided minimal insight to the disease etiology. Clinical signs were vague and insidious in onset: rough hair coat, soft but not unformed feces, and weight loss. Minor trends in clinical pathology were identified in those animals with islet fibrosis. All but one animal (Case 6) presented with persistent hyperglycemia. All animals presented with persistent elevation in creatinine phosphokinase and amylase. Lipase concentrations were steady (9-39 U/L) until shortly before presentation of clinical signs. Although ISIS data were consulted for amylase and lipase, it is likely that animals in this line were providing data to these physiologic reference ranges.

Histologically, lesions were similar in all affected animals. The pancreas had severe islet fibrosis with extension of the fibrous tissue into the surrounding interstitium, and associated disruption of the acinar structures. Density of islet cells in the areas of fibrosis was variable, with some islets having few or no islets cells but others having approximately normal cellular density. Cases with mild fibrosis had islet cell hyperplasia. Occasionally, ducts were mildly dilated and contained inspissated secretory material. A battery of special stains was performed on the pancreas from Case 7. Iron and copper stains were negative. Sclerotic sections of the pancreas were negative for amyloid with Congo red. A trichrome stain confirmed that the islet deposition was collagen and accentuated that distribution was earliest and most severe around and in the islets. A Cherukian-Schenk stain for endocrine granules accentuated the presence of islet cells in areas of early interstitial fibrosis, further supporting the islet as the initial site of fibrosis. Electron microscopy

performed on case 7 supported the histologic findings and revealed extensive collagen deposition in the interstitium between clusters of sequestered islet and acinar cells. Frequent fibroblasts were associated with the collagen deposition. Islet cells were partially degranulated, and some had vacuolar changes in the nucleus. No viral agents or inflammatory processes were detected. The cause for this condition could not be determined morphologically. Additional significant lesions in many of these animals included hemosiderosis, glomerulonephritis or glomerulosclerosis, opportunistic infections, and degenerative cardiac disease, all of which are common complications of diabetes in mammals.

Differential diagnoses for this novel pancreatic lesion in adult rock hyrax include nutritional toxicity, infectious source, and genetic predisposition that manifests with age. Diet review, clinical pathology, and evaluation of serum for autoantibodies to the pancreatic islets will be pursued in this species.

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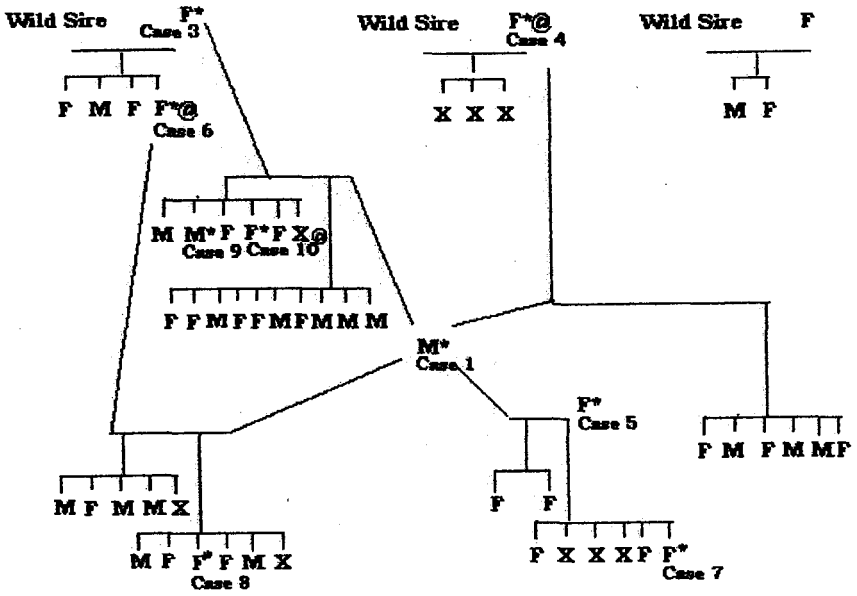

Figure 1. Pedigree of rock hyrax (*Procavia capensis*) at the Dallas Zoo (1990-2000) with presentation of pancreatic islet fibrosis. M = male, F = female, U = stillborn; *Pancreatic islet fibrosis (post-mortem); @ = clinical diabetes mellitus (ante-mortem). Note: Case 2 is not related to the family tree presented. Case 5 is a wild-caught female from 1993 that was not pregnant by a wild sire.

Table 1. Signalment, medical history, and pathology of rock hyrax (*Procavia capensis*) at the Dallas Zoo affected with pancreatic islet fibrosis (1990-2000).

Case number	Age	Gender	Clinical signs	Islet fibrosis	Other pathology
1	6 yr	M	Wasted body condition Dental disease Azotemia	3+	Nephrosclerosis Gastric ulceration Cystitis
2	2 yr	F	Comatose Pneumonia	4+	Purulent pneumonia
3	~4 yr	F	Comatose Alopecia Wasted body condition	4+	Cardiomyopathy Gastritis Hemosiderosis Nephritis
4	~6 yr	F	Euthanasia Weakness Azotemia Diabetes mellitus	3+	Hemosiderosis Glomerulosclerosis
5	~3 yr	F	Found dead	3+	Myeloproliferative disease Hemosiderosis Myocardial hypertrophy
6	7 yr	F	Euthanasia Diabetes mellitus	4+	Hemosiderosis Glomerulosclerosis Lingual candidiasis
7	1 yr	F	Found dead	3+	Lymphoma
8	1 yr	F	Comatose	3+	Nephritis Sepsis
9	4 yr	M	Chronic diarrhea Found dead	4+	Hemosiderosis Glomerulosclerosis
10	4 yr	F	Euthanasia Diabetes mellitus	4+	Hemosiderosis Cardiomyopathy Glomerulosclerosis

SQUAMOUS CELL CARCINOMA IN TWELVE KOWARI (*Dasyuroides byrnei*)

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Abstract

A relatively high incidence of neoplasia has been historically noted in marsupials, and in dasyurid marsupials in particular.[2-5,8] Neoplasms noted in kowari (*Dasyuroides byrnei*) have included a schwannoma[6], a thoracic fibrosarcoma,[2] a cerebellar medulloblastoma,[2] a glossal squamous cell carcinoma (SCC) with pulmonary metastases,[1] and SCC of the face (one case) and pinna (one case).[3] Squamous cell carcinoma has also been noted in other dasyurids, including the Tasmanian devil (*Sarcophilus harrisii*),[3-5,7] the tiger quoll (*Dasyurus maculatus*),[3,9] the eastern quoll (*Dasyurus viverrinus*),[3,8] the northern quoll (*Dasyurus hallucatus*),[3] the common planigale (*Planigale maculata*),[3] the fat-tailed dunnart (*Sminthopsis crassicaudata*),[3] and the brown antechinus (*Antechinus stuartii*).[3] This report describes several additional cases of SCC in kowari, usually originating from oral gingiva.

Between October 1997 and July 2000, twelve kowari from three zoologic institutions were diagnosed with SCC out of approximately 24 case submissions (50%). Ten kowari were euthanatized, and of these, eight were euthanatized primarily because of the neoplasm. Four had antemortem diagnosis by biopsy, which was later confirmed on necropsy. Eleven carcinomas were within the oral cavity: five arose from the maxillary gingiva, five from the mandibular gingiva, and one from the mucocutaneous junction at the oral commisure. A single lesion arose from a ceruminous gland at the base of the ear. Oral neoplasms usually presented as rapidly growing mandibular or maxillary masses, usually surrounded the molars, and were multilobulated, tan, and firm. Metastasis was not observed in any case, however, the lesions were locally aggressive, with consistent invasion of surrounding osseous and soft tissues, and occasional invasion of the nasal cavity, nasolacrimal gland, tongue, or teeth. Loss of teeth and dental calculus/gingivitis of remaining ipsilateral teeth and the apposing arcade was frequently observed. Concurrent gingival hyperplasia was noted in three cases, and was papillomatous in one of these cases. Of the affected kowari with known lineages, most had at least one affected parent.

Factors leading to this common neoplasm in this species may include genetic/familial disposition, viral infection such papillomavirus, or chronic gingivitis leading to neoplastic transformation. The observed gingival hyperplasia may represent a preneoplastic lesion in this species.

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SPONTANEOUS TUMORS IN HEDGEHOGS: A RETROSPECTIVE STUDY OF FIFTY CASES

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Abstract

There is only limited published information on diseases of hedgehogs, including neoplastic disease.[2,4,6,8,9,10,11] Most of these publications are individual case reports with a few involving multiple animals. Certain types of sarcomas in hedgehogs have been associated with retroviral infection.[3,5] In two previous retrospective studies concerning necropsy findings in hedgehogs, neoplastic disease was noted in approximately 30% of hedgehog necropsies.[1,7] The present study summarizes a series of tumors diagnosed in captive African hedgehogs and includes several tumors that have never been previously reported in hedgehogs.

Specimens were obtained from a total of 97 hedgehog submissions recorded at Northwest ZooPath (NZP) from 1994 to 2000. Sixty of the 97 hedgehogs were from zoologic parks and 37 were privately owned. Thirty-nine of the 97 hedgehogs were male, 46 female, and 12 of unknown gender. All were adult (>1 yr old). The tumors were classified according to histomorphologic features and the presence or absence of metastases. For evaluating possible gender predisposition, χ^2 test was performed and a *P*-value ≤ 0.05 was considered significant.

Neoplastic disease was diagnosed in 50 (51.5%) of the 97 hedgehogs, and four of these 50 animals had more than one type of tumor. Twenty-two of the hedgehogs with tumors were female, 16 were male, and 12 were of unknown gender. The occurrence of tumors was unrelated to gender ($P \geq 0.25$). The median age at time of tumor diagnosis was 3.5 yr with a range of 2-5.5 yr. The average life expectance for hedgehogs in captivity is approximately 3-5 yr. Seventeen of the hedgehogs with tumors were of unreported age and were classified as adult (>1 yr old). Thirty-one hedgehogs were from zoologic parks and 19 were privately owned. Of a total of 56 tumors diagnosed, 11 were classified as mesenchymal, 29 as epithelial, and 16 as discrete round cell neoplasms.

Fifty (89%) of 56 tumors were classified as malignant and six (11%) as benign. The diagnosis of a malignant tumor usually warranted a guarded to poor prognosis. The body systems in which the tumors were found (in order of frequency of occurrence) were as follows: integumentary (18 tumors), hemolymphatic (12 tumors), digestive (nine tumors), endocrine (eight tumors), genital (six tumors), musculoskeletal (two tumors) and nervous (one tumor).

The most commonly diagnosed tumors were: mammary gland tumor (nine), lymphosarcoma (eight), and oral squamous cell carcinoma (seven). Mammary tumors were usually malignant and presented as large, subcutaneous swelling along the ventral thorax and abdomen. In three cases, the mammary tumor reoccurred within 6 mo of surgical removal. Five of the eight cases of lymphosarcoma were

of the multicentric form and the other three were of the alimentary form. Diarrhea and weight loss were common clinical signs in hedgehogs with gastrointestinal lymphosarcoma. Cases of oral squamous cell carcinoma (SCC) usually presented as swelling of the maxillary or mandibular gingiva. Hedgehogs with oral SCC presented with loose teeth, tooth loss, swollen gingiva, and/or gingivitis. In all cases, the tumor was locally infiltrative but there was no evidence of metastasis.

In conclusion, neoplastic disease is very common in adult African hedgehogs and occurs without gender preference. In the majority of cases, the neoplasms were malignant and usually carried a poor prognosis. The integumentary, hemolymphatic, digestive, and endocrine systems were the most common sites for tumors. Mammary gland carcinoma, lymphosarcoma, and oral SCC were the most common types of tumors.

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INTRAABDOMINAL RADIOTRANSMITTER PLACEMENT IN NORTH AMERICAN RIVER OTTERS (*Lontra canadensis*)

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Abstract

North American river otters (*Lontra canadensis*) from northern and eastern New York were captured as part of a translocation project during the months of September to November in 1998, 1999 and 2000. Radiotransmitters were surgically implanted into the abdominal cavity of the otters to allow monitoring of the animals after relocation. The otters were maintained, assessed and preconditioned for release at Cornell's College of Veterinary Medicine as previously described.[1] Otters which had not sustained severe trap-related injuries, were in good body condition and health and weighed more than 4 kg were selected for radiotansmitter implant surgery (n = 24).

The otters were anesthetized with intramuscular ketamine (Ketaset, Ft Dodge Laboratories, Fort Dodge, IA 50501 USA; 15 mg/kg) and midazolam (Versed, Roche Laboratories, NJ 07110 USA; 0.4 mg/kg). After induction, the animals were intubated and maintained on isoflurane in oxygen throughout the surgical procedure. In contrast to previous reports of intraabdominal implant surgeries in otters, our technique involved an approach through the paralumbar fossa.[2-8] The otters were placed in right lateral recumbency and the hindlimbs were tied back. A 12-cm × 12-cm square was shaved immediately cranial to the cranial border of the hindlimb. The surgical incision was centered in the middle of this square. The skin, the subcutaneous layer, the cutaneous trunci muscle, and a thick layer of fat immediately deep to this muscle were sharply transected. The external abdominal oblique, the internal abdominal oblique and the transverse abdominal oblique were dissected bluntly. The transverse fascia and parietal peritoneum were transected for entry into the abdominal cavity. The radiotransmitter implants (Telonics Inc., Mesa, AZ 85202 USA) weighed approximately 28 g and were introduced into the abdominal cavity where they were allowed to free float. The surgical incisions were closed by suturing the abdominal muscle layer with 3-0 polydioxanone (Maxon, Davis & Geck, Manati, Puerto Rico USA) in a simple continuous pattern. The subcutaneous tissues were sutured with 3-0 polyglactin 910 (Vicryl, Ethicon, Johnson & Johnson Co. Somerville, NJ 08876 USA) in a simple continuous pattern. The skin was opposed with an intradermal pattern made with 3-0 polyglactin. No deaths occurred as a direct result of the surgical technique, but two complications were encountered. Approximately 10 ml of whole blood were lost as a result of hemorrhage during one procedure when a branch of the circumflex iliac vein was accidentally transected. The circumflex iliac artery runs on the lateral abdominal wall supplying the abdominal musculature, and can be transected if the surgical incision is extended too far ventrally. The otter was treated with intravenous fluids for blood loss and recovered uneventfully.

Infection of the surgical incision occurred in another case, in which *Pasteurella multocida, Staphylococcus aureus, Clostridum* spp. and *Fusobacterium* spp. were cultured from the surgical incision. The otter was treated with metronidazole and enrofloxacin, the wound was cleaned daily with diluted chlorhexidine (Nolvasan, Fort Dodge Laboratories, Fort Dodge, IA 50501 USA), and the otter recovered uneventfully.

Following recovery from radiotransmitter implant surgery, otters were released in the western part of New York State. Monitoring via radiotelemetry for 12-24 mo post-release revealed that four otters died. Three died when struck by vehicles. The fourth otter carcass was never found. The radiotransmitter was found, perhaps removed from the cadaver by a predator or scavenger. The remaining otters, including the two otters in which surgical complications occurred, became established in their new ranges. The intraabdominal implants did not appear to affect their survival or reproductive potential.

The standard approach to the abdominal cavity of mammals is a ventral midline incision, which allows transection of the linea alba, a relatively avascular tissue plane. However, river otters rub their ventral abdomen as part of their grooming behavior, and this behavior can disrupt the surgical incision and promote surgical incision infection. The paralumbar surgical approach is recommended for the implantation of radiotransmitters in North American river otters, since it carries a lower incidence of surgical incision infection and dehiscence.

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MOLECULAR TOOLS FOR POPULATION MANAGEMENT: SEARCHING FOR THE CAUSE OF ALBINISM IN A GROUP OF QUEENSLAND KOALAS (*Phascolarctos cinereus adustus*)

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Abstract

Two albino Queensland koalas (*Phascolarctos cinereus adustus*) have been born at the San Diego Zoo, one in 1985 and the other in 1997. Both appear to be instances of complete oculocutaneous albinism. In other animals, this defect is most often due to a mutation in the gene for tyrosinase enzyme, which catalyzes the first steps of the melanin-synthesis pathway in melanocytes of the skin, hair follicles, and retina.[1-4] Consequently, along with unpigmented skin and fur, albinism is associated with altered development and anatomy of the retina and optic nerve leading to reduction in visual acuity. The albino koalas at the San Diego Zoo showed signs of similar visual deficits. An autosomal-recessive pattern of inheritance is observed for such mutations. Pedigree analysis has unambiguously identified an original carrier in addition to five other carriers in three succeeding generations, all of which were phenotypically normal (A. Kumamoto, unpublished data). The original carrier, imported from Lone Pine Koala Sanctuary (near Brisbane, Queensland, Australia), gave birth to the first albino individual as a result of a mother/son mating. The second albino was generated by a pairing of two less closely related animals which were nevertheless both descendants of the original carrier. By use of the molecularly based techniques of polymerase chain reaction assay (PCR), cloning, and sequencing, we have obtained genetic sequence information from the entire exon 1 region of the koala tyrosinase gene for the purpose of detecting individuals harboring the mutant gene before they are bred.

Koalas are now listed by the U.S. Fish and Wildlife Service as a threatened species (50 CFR, Part 17.11). The Australian Koala Foundation reports that this change of status will not likely slow the koala population decline in the wild, as natural-habitat areas are essentially unprotected and are rapidly being degraded by logging, fire, and development. Although captive propagation is not the solution to koala conservation needs, it will be of increasing importance in monitoring and maintaining genetic diversity as fragmentation of habitat leads to reduction of gene flow in disjunct wild populations. Besides maintaining diversity of alleles, however, the identification of specific mutations in phenotypically abnormal animals allows the development of diagnostic tests for the detection of carriers of heritable genetic problems. As the first zoo-based, full-time laboratory dedicated to such work for genetic and infectious diseases, the Molecular Diagnostics Laboratory at the San Diego Zoo is uniquely positioned for this task.

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EOSINOPHILIC GRANULOMA IN THE BLACK RHINOCEROS (*Diceros bicornis*)

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Abstract

Mucosal and cutaneous ulcerative syndrome, recently described as superficial necrolytic dermatopathy (SND),[3] is a common cause of morbidity in captive black rhinoceros. As part of the clinicopathologic investigation of SND, a subset of animals were identified that had distinct eosinophilic inflammatory lesions concurrently manifested with characteristic histologic lesions of SND. These oral, nasal and cutaneous inflammatory lesions had several features that closely resembled eosinophilic granuloma (EG) as described in domestic cats, dogs and horses and were reviewed in greater detail. In all, eight rhinoceros with EG were identified and all had oral lesions. Oral EG occurred most often behind the prehensile lip and consisted of large discrete fungating masses with areas of ulceration that usually presented clinically with a history of oral bleeding. Nasal lesions occurred in the external nares and presented with epistaxis. Simultaneous nasal and oral lesions occurred in three animals and simultaneous cutaneous lesions occurred in a single animal. Three animals had recurrent lesions over periods of several years and no apparent seasonality was identified. EG lesions in some animals spontaneously resolved, whereas others were apparently responsive to corticosteroid treatment or cryotherapy.[2] Importantly, two animals treated with corticosteroids died due to secondary systemic fungal infections. Histologically, EG lesions were characterized by marked submucosal or dermal neovascularization (granulation tissue) with prominent infiltrates of eosinophils and scattered foci of collagen degeneration. Mucosal or epidermal hyperplasia was also present and in many cases was associated with epithelial degenerative changes consistent with SND. The EG lesions were interpreted as having a distinct pathogenesis from the SND lesions. The cause of the EG could not be determined, but factors associated with EG or EG-like lesions in domestic species include hypersensitivity,[4-6] hereditary factors,[4] trauma[6] and, in cats, cutaneous herpesvirus infection.[1] Clinical differentials for EG lesions include neoplasia and SND without EG; histologic examination of biopsy specimens is required for definitive diagnosis.

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INSULAR AMYLOIDOSIS IN CAPTIVE FELIDS: TWELVE CASES

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Abstract

Introduction

Amyloidosis consists of heterogeneous disease processes due to the extracellular deposition of an homogeneous glycoproteic material called amyloid in one or more tissues. Amyloid differs from other hyaline materials in that it stains positively with Congo red, and induces green birefringence under polarized light. The distribution of amyloid in tissues and its association with specific diseases aid in the diagnosis.

Insular amyloidosis (IA) is associated with type II diabetes in humans and animals. IA has been described mostly in humans, domestic cats, degus (*Octodon degus*) and nonhuman primates.[1-4,7,12,14-16,18-20] In nondomestic felids, IA has only been documented in cougars (*Felis concolor*).[8] The amyloid precursor deposited in the islets of Langerhans of IA patients is called amylin or insular amyloid polypeptide (IAPP), and is a normal secretion product of β-cells in the islets as insulin. In addition, IAPP is produced by δ-cells in the islets and endocrine cells in the gastrointestinal tract.[5,9,14,16] Although IAPP is involved in IA and amyloidosis associated with some neoplastic diseases (e.g., insulinoma), there is evidence that IAPP is an active islet hormone involved in the control of glucose metabolism together with insulin and glucagon. Specifically, IAPP inhibits gastric emptying, glycogen synthesis in skeletal muscle, as well as insulin and glucagon secretion, and stimulates hepatic gluconeogenesis.[9] IAPP is a 37-aminoacid molecule with a critical amyloidogenic sequence highly preserved in the different species studied. This sequence is located between residues 20 to 29 and is identical in humans, macaques, dogs, cats and cougars except for the aminoacid at position 25 (alanine in humans, threonine in the other species).[14]

There is controversy regarding the role of IA in type II diabetes. Although most humans, cats and macaques with type II diabetes have concurrent IA, IAPP deposits are also found in nondiabetic humans and animals, however, the number of islets affected and the severity of IA are considerably reduced as compared to age-matched diabetic patients.[1,14,18] Furthermore, insulin resistance, a key factor in the development of type II diabetes, is accompanied by an overproduction of IAPP[6,10] but it is not sufficient to induce IA and thus some other additional mechanisms may be involved in the

deposition of IAPP in the islets of Langerhans.[17,20] Interestingly, IAPP amyloid fibrils are toxic to βcells and, therefore, IA can contribute to the progression of type II diabetes.[11] In conclusion, although IA and type II diabetes are highly correlated, the exact pathogenesis of IAPP deposition and its relationship with insulin resistance and type II diabetes are not fully understood.

The importance of IA in the domestic cat and the diagnosis of IA cases in nondomestic felids at the authors' institutions led us to conduct a retrospective study of IA in these species and to document, whenever possible, the glycemic control of the IA cases identified in this survey at or close to the time of diagnosis.

Materials and Methods

A retrospective review of the medical and pathology records of nondomestic felids at the institutions involved in this study (Africam Safari, Puebla, México; Smithsonian National Zoological Park, Washington, D.C.; Northwest ZooPath, Snohomish, WA; Department of Veterinary Diagnostic Medicine, University of Minnesota, St. Paul, MN) was performed. All clinical histories, clinical pathology data (with special reference to serum glucose and urinalysis), and paraffin blocks or histologic sections containing pancreas from all IA cases identified were obtained and reviewed. The pancreas of all felids previously unprocessed for histology was routinely embedded in paraffin and stained with hematoxylin and eosin for microscopic examination. Pancreas from all IA cases was processed for IAPP and insulin detection by immunohistochemistry as previously described.[13]

Results and Discussion

Twelve cases of IA were identified in this preliminary survey. Clouded leopards (*Neofelis nebulosa*) accounted for eight of these cases; the other animals were two tigers (*Panthera tigris*), a Mexican bobcat (*Felis rufus escuinapae*), and a snow leopard (*Panthera uncia*). All these felids were above 10 yr-old; six were males (all clouded leopards) and five were females. Nine were euthanatized mostly due to osteoarticular or neoplastic diseases. None of these felids was reported to exhibit clinical signs of diabetes, however, three clouded leopards and one of the tigers were hyperglycemic at or close to the time of death. The snow leopard had slightly elevated glucose levels. All animals with urinalysis available were negative for glucosuria. Three cases had hypercholesterolemia or hypertriglyceridemia in the absence of hyperglycemia. Serum insulin-like immunoreactivity as well as IAPP and insulin immunohistochemical studies are in progress at the time of writing of this manuscript.

The results of this retrospective study suggest that IA may be a common pathologic finding in clouded leopards. In this species, IA may be frequently associated with hyperglycemia. Captive clouded leopards should be monitored for their endocrine pancreatic function on a regular basis.

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STUDIES ON ENCEPHALOMYOCARDITIS VIRUS (EMCV) IN A ZOOLOGIC CONTEXT

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Abstract

Encephalomyocarditis virus (EMCV) is a ubiquitous picornavirus with a worldwide distribution, capable of establishing infection in a vast range of species. Episodes of EMCV-associated fatality have been reported in zoos and national parks around the world, including sporadic cases at Taronga Zoo, Sydney, N.S.W.

An inactivated EMCV vaccine was evaluated by inoculating Barbary sheep (*Ammotragus lervia*), Indian antelope (*Antilope cervicapra*), Eastern wallaroos (*Macropus robustus*) and chimpanzees (*Pan troglodytes*). A proportion of the vaccinated ungulates were administered a booster vaccination 4 wk following the initial dose. Neutralizing antibody titres were monitored over a period of 6 mo. All vaccinated groups developed significant antibody titres by 1 mo that persisted at least 6 mo post-vaccination. Boosted animals had significantly higher titres at 3 and 6 mo following the initial vaccination compared with animals receiving a single vaccine dose. We recommend this vaccine preparation for the protection of non-domestic species against EMCV.

An immunoperoxidase staining technique to identify EMCV antigen in formalin-fixed specimens was successfully applied to zoo animal tissues. Archival specimens from cases of acute myocarditis were retrieved and subjected to immunohistochemistry. EMCV antigen was identified in the myocardium of two of these retrospective cases. This is the first time immunohistochemistry for EMCV has been described in a diagnostic context for zoo animals, and the first report of EMCV infection in a quokka (*Setonix brachyurus*). This technique offers an additional method of EMCV diagnosis, especially where tissues suitable for virus isolation are not available.

SURGICAL APPROACH TO ARTIFICIAL INSEMINATION IN ELEPHANTS

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Abstract

Artificial insemination is a recent development for assisted reproduction in elephants. Non-surgical insemination requires a cooperative elephant, well-habituated to the various procedures. In addition, a well-trained and equipped insemination team is needed to successfully complete the procedure. A surgical approach for artificial insemination in elephants reduces both the technology needed for success and the level of cooperation needed from the elephant to be inseminated. The first successful, surgical, artificial insemination was accomplished by making a 3-cm incision into the urogenital canal just below the anus. This vestibulotomy incision was guided by placement of an 8-cm PVC tube with a 3-cm opening at the upper end, up through the vulva to a level just below the anus. The opening in the PVC tube was used as a guide by palpating the opening through the skin. The incision was made following injection of a local anesthetic above the proposed incision site. The PVC guide prevents incision into the opposite wall of the urogenital tract. After the incision is complete a sterile disposable vaginal speculum is introduced into the urogenital canal. The intact hymen or cervix can be visualized directly with a flashlight or, for documentation of the procedure, a short endoscope can be utilized. Placement of semen into the vagina can be accomplished with little difficulty using sterile disposable horse insemination pipettes. Multiple inseminations are possible through the incision for the 2 to 3 days of estrus. Following the last insemination, a local anesthetic is administered and the edges of the incision are freshened and four to six simple interrupted sutures are placed to close the incision. Healing of the incision requires 4 to 6 wk with good aftercare.

COMPARATIVE INVESTIGATIONS ON REPRODUCTIVE BIOLOGY IN CAPTIVE GIANT PANDA AND URSIDS

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Abstract

Until captive and free-ranging populations of giant pandas *(Ailuropoda melanoleuca)* are self-sustaining through natural breeding, reliance on assisted reproduction will continue to grow. Although artificial insemination is frequently used in captive pandas, little is known about the reproductive physiology in this species.

In a 5-yr study, a pair of giant pandas was reproductively monitored and three artificial inseminations were performed from 1997 to 1999. After the arrival of the wild-caught, 10-yr-old female (SB #378) at the Berlin Zoo in 1995, non-invasive urinary hormone analysis was performed weekly. Estrogen and progesterone concentrations as well as the behavior of the female indicated no sexual activity during her first 2 yr in Berlin. In February 1997, she was evaluated by transrectal ultrasonography which revealed a normally developed genital tract but both ovaries appeared inactive. Estrus induction protocols were developed using PMSG with or without FSH priming. After treatment the animal showed clear signs of estrus based on urinary estrogen and progesterone levels, but she did not exhibit sexual behavior. The female was inseminated with frozen (first artificial insemination) and fresh (second and third artificial insemination) semen from the male (SB #208) 5 days after PMSG administration. The disposable artificial insemination catheter (2 mm in external diameter, Cook Inc., Queensland 4113, Australia) was first inserted into the external cervical os by endoscopic visualization and then further guided by ultrasound into the uterine body. One milliliter of diluted semen was deposited in the uterus. We achieved no full-term pregnancy in the female, however there was an indication of an embryonic absorption after the first artificial insemination. We suspect a pituitary malfunction may be responsible for the female's acyclicity and the embryonic resorption.

Semen was collected from the male by electrostimulation after sonographic evaluation of the accessory sex glands. Between February and May, seven very good quality ejaculates were obtained with 2.75-4.0 ml volume, 85-95% motility, and 63-85% membrane integrity. Semen was used immediately for artificial insemination of the female or was cryopreserved. Post-thaw motility of the best ejaculate fractions was 35-40%. In September 1999, only an aspermatic ejaculate could be collected, indicating seasonal changes of spermatogenesis in the giant panda. More experimental research is needed but is limited by the small number of individuals and the panda's endangered

status. It is imperative that carefully designed studies be carried out first on a more numerous, non-endangered species to avoid inadvertent mistreatment of pandas.

To improve the knowledge on the reproductive physiology of bears, we extended our study and performed comparative ultrasonographic and endocrinologic investigations[1] in various ursid species (15 *Ursus arctos arctos*, three *U. maritimus*, two *U. americanus*, five *Tremarctos ornatus*, one *Melursus ursinus*). The reproductive physiology of ursids is characterized by seasonality and a period of delayed implantation between mating in April/May and implantation in November. There is convincing evidence that progesterone is produced during this period and that progesterone concentrations increase towards the end of diapause.[2,4] Giant pandas are also believed to utilize delayed implantation.[3] However, monitoring of embryonic and fetal development has not been described to date.

Present results suggest that the reproductive biology of the giant panda finds its closest counterpart in ursids in the spectacled bear. However, due to the endangered status and limited access to this species, the American black bear and the European brown bear are being considered as a model for reproductive studies in giant panda.

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MYASTHENIA GRAVIS SECONDARY TO THYMOMA IN A POLAR BEAR (*Ursus maritimus*)

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Abstract

A 15-yr-old, 290-kg, female polar bear presented with weakness of the hind legs in June of 2000. The animal was able to bear weight on the hind limbs, but could not climb out of the pool in which she had been found. As the day progressed, the animal's hind legs became weaker. The animal was no longer able to bear weight on the hind limbs by early afternoon.

The following day the bear was found in sternal recumbency and was exhibiting forelimb weakness. It animal was anesthetized using 5 mg/kg Telazol i.m. (Telazol, Fort Dodge, Fort Dodge, IA 50501 USA) via pole syringe. Physical examination did not reveal the cause of the problem. A blood sample was obtained for complete blood count, serum chemistry, and serum banking. No abnormalities were detected. Lateral radiographs of the thoracolumbar spine were obtained using a portable x-ray unit. The films were of poor quality and not diagnostic. The animal was treated using 11 IU/kg Vitamin E (Vital E–300, Schering-Plough Animal Health, Union, NJ 07083 USA) i.m., 1.1 mg/kg flunixin meglamine (Banamine, Schering-Plough Animal Health, Union, NH 07083 USA) i.m., 0.7 mg/kg dexamethasone (Dexamethasone Solution, Phoenix Pharmaceutical, Inc., St. Joseph, MO 64506 USA) i.m., 80,000 IU/kg procaine penicillin G s.c. (Pen-G, Phoenix Pharmaceutical, Inc., St Joseph, MO 64506 USA) s.c., and 1.7 mg/kg calcium gluconate (Calcium Gluconate 23% Solution, Phoenix Pharmaceutical, Inc., St. Joseph, MO 64506 USA). One half of the amount of calcium gluconate was given i.v., the remainder was given s.c. The bear's condition was unchanged after recovery from anesthesia.

The animal's condition remained unchanged the following day. A visual exam was performed by a veterinary neurologist later that day. A spinal lesion was suspected, but could not be confirmed by a complete neurologic exam. Plans were made to perform spinal radiographs, spinal tap and myelogram the following day.

The bear was anesthetized using 5 mg/kg Telazol i.m. via pole syringe. The animal was intubated and anesthesia maintained using isoflurane (IsoFlo, Abbot Laboratories, North Chicago, IL 60064 USA). Fluids were administered throughout the procedure. A physical exam was repeated, again revealing no pertinent abnormalities. A blood sample was obtained for complete blood count, serum biochemistry and serum banking. Lateral radiographs of the thoracolumbar spine were obtained. No spinal lesions were noted on these films. Numerous unsuccessful attempts were made to perform a spinal tap in the lumbar region. A successful spinal tap was performed at the cisterna magna. Twelve ml of cerebrospinal fluid were collected and utilized for cytology, total protein and bacterial culture. No abnormalities were detected. A myelogram was then performed by a veterinary

radiologist. Radiographs were taken of the thoracic and lumbar spine. Although the films were not high quality, no spinal lesions were evident. At this time, the decision was made to euthanatize the animal based on a poor prognosis and the difficulty associated with providing the nursing care for a polar bear.

A necropsy was then performed by a consulting pathologist. A mass 6.0 × 4.0 × 6.0 cm was located in the cranial mediastinum. The mass was para-aortic in location and consisted of friable, apparently necrotic material. A degenerative intervertebral disc was identified at L7/S1. The spinal cord in this area appeared edematous. No signs of traumatic injury could be identified.

Histopathologic examination of the mass led to a presumptive diagnosis of thymoma, spindle cell variant. Immunohistochemical stains confirmed the presence of a thymoma. Serum collected after the onset of clinical signs was submitted to be analyzed for acetylcholine receptor antibodies (Comparative Neuromuscular Laboratory, University of California, San Diego, La Jolla, CA 92093 USA). In addition a serum sample from the affected bear collected prior to the onset of signs and serum samples from two clinically normal female bears were also submitted. The sample collected from the affected polar bear after the onset of clinical signs exhibited a titer of 0.86 nmol/L. Utilizing canine or feline reference values this sample would be compatible with a diagnosis of myasthenia gravis. All other samples submitted would be considered to be within normal limits, using either reference range.

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ULTRASONOGRAPHIC MONITORING OF ELECTROEJACULATION IN THREE SPECIES OF RHINOCEROS (*Ceratotherium simum, Diceros bicornis, Rhinoceros unicornis)*

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Abstract

Electroejaculation has been generally unsuccessful for the rhinoceros. Two black rhinoceros (*Diceros bicornis*), two white (*Ceratotherium simum simum*) and one greater one-horn rhinoceroses (*Rhinoceros unicornis*) were monitored by rectal ultrasonography during electroejaculation. The electroejaculation procedure was modified as described previously.[1] The ultrasound probe was incorporated into a custom engineered electric probe, designed by the authors. Seminal emission into the posterior urethra was ultrasonographically observed for each animal except the greater one horn rhinoceros. Seminal fluid was successfully collected in 12 attempts to electroejaculate these five animals. Two later collections from the greater one-horn rhinoceros produced semen of low to zero sperm concentration. Electric thresholds for the first ejaculate occurred between 200-500 milliamperes, and 5-9 volts within 25-90 stimulations. Semen accumulated in the pelvic urethra during pauses between series of electric stimulations above these thresholds. This accumulation was then cleared by either further electric stimulation or massage of the penis. Overall semen quality measurements included semen volume (15-168 ml), sperm concentration (0 to 493×10^6 ml), sperm motility (10-85%), and pH levels (7.5-8.5). Seminal fluid was produced in all procurement attempts on all animals. Of these attempts, 11 out of 12 contained motile sperm. Recovered semen exhibited parameters superior to previous reports. Ultrasonographic monitoring of the electroejaculation procedure improved sperm collection in the rhinoceros.

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SEMEN COLLECTION, ANALYSIS, AND CRYOPRESERVATION IN JACKSON'S (*Alcelaphus buselaphus jacksoni*), AND CAPE HARTEBEEST (*Alcelaphus buselaphus caama*)

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Abstract

The various subspecies of hartebeest (*Alcelaphus busalaphus*) formerly ranged throughout all Africa. Encroachment of farming and livestock has resulted in a critical loss of habitat and food and water resources for the hartebeest. These factors, along with hunting for meat and sport, have taken their toll on hartebeest populations in the wild.[3] The subspecies *A. b. tora* and *A. b. swaynei* occurred formerly from Southern Egypt to Somalia, are classified as endangered by the IUCN/SSC.[3] Much of the remaining range of *A. b. tora*, in southern Sudan and northwestern Ethiopia was devastated by drought in the early 1980s, and only a few animals are thought to survive. *Alcelaphus busalaphus* has also disappeared from most of South Africa but is still locally common in parts of Botswana, Nambia, Tanzania, and Kenya.[4]

Successful captive propagation of hartebeest in zoologic institutions is an important step in the conservation of this species. This need will become more critical, as habitat becomes increasingly threatened and hartebeest numbers subsequently decline. The captive management of hartebeest in zoologic institutions has proven challenging. At present, three subspecies exist in six North American zoologic institutions. The Wildlife Conservation Society's St. Catherines Island Wildlife Survival Center (WSC) houses two of these subspecies: the Jackson's hartebeest (*A. b. jacksoni*) and the Cape hartebeest (*A. b. caama*). The WSC has been working with the Jackson's hartebeest since 1978, and has been very successful at maintaining breeding herds. The WSC now holds all the remaining Cape hartebeest in North America.

The purpose of this study was to perfect semen collection, evaluation, and long term storage techniques for these two subspecies of hartebeest. We are currently evaluating whether there are any seasonal differences in the sperm quality and quantity in these animals. The long term objective will be to develop assisted reproductive techniques (e.g., artificial insemination) in captive hartebeest. This technology will allow the capability of importing semen into this country from captive and wild hartebeest in Africa and Europe to increase the genetic diversity of current captive populations.

Adult male hartebeest were immobilized in individual holding stalls. Carfentanil (Wildnil, Wildlife Laboratories, Inc, Fort Collins, CO) at 2.1-2.4 mg per animal was the primary immobilizing agent used in this study. Once the animals were sternal they were given 10 mg xylazine (X-ject SA, Burns Veterinary Supply, Inc., Rockville Centre, NY) intravenously incrementally as needed to provide

muscle relaxation and analgesia. An intravenous catheter was placed in the lateral saphenous vein for fluid and drug administration throughout the procedure. Anesthesia was monitored via pulse oximetry, respiration, and digital pulse rate.

Once anesthetized, semen was collected from hartebeest by electroejaculation, using a 38 mm diameter probe with longitudinal electrodes. A standardized electroejaculatory regimen consisting of administering electric stimuli in sets of 10 at 2, 3, and 4 volts (series 1), 3, 4, and 5 volts (series 2) and 5 and 6 volts (series 3) were used to collect semen.[2]

Laboratory calipers were used to measure the width (W) and length (L) of each testicle, recorded in cm. Combined testes volume is calculated by using the following formula ($W^2 \times L) \times 0.524$ for each testis.[1] Representative ejaculate and sperm traits are shown in Table 1.

The semen was evaluated grossly and noted to have a distinct pink color. Examination of the ejaculate showed that no red blood cells were present and the preliminary diagnosis is that the semen contains porphyrins. Preliminary results indicate that the presence of porphyrins is not detrimental to short term sperm storage. Semen pH, volume and sperm concentration were measured. Percent sperm motility and progressive motility (forward progressive movement on a graded scale: 0 = no movement to 5 = rapid, steady forward progression) were assessed by examining 5 µl of raw ejaculate at 37° C. A 20-µl aliquot of the raw ejaculate was fixed in 0.3% glutaraldehyde and sperm morphology was assessed by phase contrast microscopy (×100). Sperm morphology assessments were conducted by classifying 100 spermatozoa as normal or abnormal. In the Jackson's hartebeest, one of the most striking findings is significant abnormal sperm morphology. The abnormal classes of sperm identified are: cytoplasmic droplet, bent midpiece with droplet, abnormal midpiece, coiled flagellum, bent flagellum with a cytoplasmic droplet, bent flagellum without a droplet, bent midpiece without a droplet, biflagellate and other (includes spermatids; Table 1). In contrast, Cape hartebeest have high proportions of morphologically normal spermatozoa (Table 1). Table 1 presents the results of the fresh semen evaluation for Cape and Jackson's hartebeest.

The semen was divided into three aliquots and extended in either TEST (0.4% glucose, 4.83% tes-n-tris, and 1.15% tris), BF5F (1.6% glucose, 1.6% fructose, 1.2% tes-n-tris, 0.2% tris and 0.5% sodium triethanolamine lauryl sulphate) or Tris diluent containing 5% glycerol to a concentration of ~200 $\times 10^6$ /ml.[1] Extended semen was assessed for motility and status and cooled to 4° C over 2 hr. Cooled extended semen was loaded into 0.25-ml straws and frozen over liquid nitrogen (LN2) for 8 min before plunging into LN2. Straws were stored in LN2 for a minimum of 24 hr before thawing. Straws were thawed by immersion in a water bath at 37° C for 15 sec. Straw contents were released into sterile microcentrifuge tubes and assessed for motility, status and viability at 37° C. Thawed sperm was diluted 1:3 with Talp medium supplemented with pyruvate and 5% fetal calf serum. Talp was added in increments to reduce the osmotic shock of removing glycerol. After 30 min, diluted sperm was re-assessed for motility, status and viability. Assessments were repeated hourly. Table 2 presents the preliminary results of cryoprotectant addition, cooling and thawing.

The results of this study demonstrate that hartebeest spermatozoa do not respond well to the cryopreservation process and low numbers of motile sperm are recovered. The higher pre-freeze sperm motility in the Cape hartebeest resulted in higher post-thaw values. However, preliminary results indicate that proportionally, similar numbers of both Jackson's and Cape hartebeest sperm survived the freeze-thaw process. BF5F results in higher pre-freeze and post-thaw motilities, but insufficient data is available to compare this statistically. Further studies will investigate whether the high numbers of abnormal sperm are related to possible seasonality of the species or to a lack of genetic diversity in the captive population. Also, the effects of increasing glycerol concentration will be investigated to attempt to increase the numbers of post-thaw motile spermatozoa.

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Table 1. Ejaculate and sperm traits of Jackson's and Cape hartebeest.

	Jackson's hartebeest (n = 4)	Cape hartebeest (n = 3)
Ejaculate volume (ml)	3.9	6.7
pH	7.9	7.8
Sperm concentration ($\times 10^6$)	1320	3494
Sperm motility (%)	43.8	63.6
Progressive motility	4.1	4
Structurally normal sperm (%)	39.8	87.3
Structurally abnormal sperm (%)		
Cytoplasmic droplet	2.25	1
Bent midpiece with droplet	4.5	0
Coiled flagellum	34.5	13
Bent flagellum with droplet	0.5	0
Bent flagellum without droplet	7	0
Bent midpiece without droplet	3.8	3.3
Abnormal acrosome	0.25	0
Biflagellate	0.5	0
Abnormal midpiece	0.25	0
Other	0.25	1.3

Table 2. Cape and Jackson's hartebeest sperm characteristics after cooling, freezing and thawing in different extenders.

Treatment	Cape hartebeest		Jackson's hartebeest	
	Motility (%)	Status	Motility (%)	Status
Raw semen				
Extender				
TEST	42 ± 11.7	3.5 ± 0.0	25.0 ± 7.4	3.5 ± 0.4
BF5F	70 ± 5.8	4.0 ± 0.0	46.3 ± 9.4	4.0 ± 0.2
TRIS	20 ± 10.4	2.8 ± 0.33	15.0 ± 8.4	3.3 ± 0.6
After cooling to 4º C				
TEST	44.3 ± 12.6	2.5 ± 0.6	12.5 ± 4.3	2.6 ± 0.4
BF5F	70 ± 5.7	3.5 ± 0.3	12.5 ± 4.3	2.6 ± 0.4
TRIS	26 ± 11.4	1.0 ± 0.0	11.3 ± 1.6	1.6 ± 0.7
After thawing				
TEST	29	1	14.7 ± 4.9	1.7 ± 0.7
BF5F	34 ± 14	3.8 ± 0.3	15.7 ± 8.7	2.3 ± 1.2
TRIS	29	1.5	9.0 ± 4.0	1.0 ± 0.0

GUIDELINES FOR VETERINARY ASSISTANCE DURING THE REPRODUCTIVE PROCESS IN FEMALE ELEPHANTS

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Abstract

In February 2000, a group of European zoo veterinarians met at Tierpark Hagenbeck, Hamburg to evaluate a questionnaire about 31 parturitions in Asian elephants. The results were presented at the 40th International Symposium on Diseases of Zoo and Wild Animals.[1] These results were combined with the experiences of some North-American zoo veterinarians, which resulted in the protocol presented in this paper. This protocol may serve as a guideline for institutions that wish to breed elephants. The proper application of the recommendations given in these guidelines should increase the reproductive success in elephants. It is the moral obligation of everyone who is responsible for the management and breeding of elephants to consider utilizing the guidelines as they apply to their situation and to collect data that may help increase our knowledge.

The breeding process in elephants requires monitoring of several parameters in both males and females. The most crucial parameters are the determination of the estrous cycle through progesterone and, perhaps, LH assay, evaluation of the genital tract in both sexes,[2,3] determination of the number of fetuses and finally, parturition. The first part of this paper will mention briefly the tools that can be used in female elephants to achieve these goals. The second part describes a protocol for veterinary intervention in elephant parturition.

Monitoring and Assisting Female Reproduction in Elephants

Determination of Estrous Cycle

Monitor blood (progesterone)[4] or urine (pregnanetriol)[5] at least biweekly during the luteal phase; if more accuracy for estrous prediction (e.g., for breeding) is required, monitor every week. Monitor every week during the follicular phase. When using the urine test, be aware that the pregnanetriol concentration in the urine is compared with creatinine. If the creatinine level is too low, a new sample should be submitted. Even when monitoring the cycle by urine-pregnanetriol, much effort should be made to train all elephants to allow blood sampling.

Breeding

The occurrence of mating is not an indicator of impending ovulation; some animals mate even during advanced pregnancy or outside the estrous period. Though not yet documented, we suppose that fertilization can only result from mating during an estrous preceded by the pre-ovulatory (= second) LH-peak. The fertile period is restricted to the time around the rise in progesterone. For the prediction of the next ovulation, follicular phase length has to be determined and therefore it is important to know the moment of the preceding fall of progesterone/pregnanetriol. For better accuracy, weekly samples throughout the cycles have optimal predictive value. LH monitoring daily during the non-luteal phase is necessary for artificial insemination programs. Determination of the post-luteal-phase (= first) LH-peak can help predict the first opportunity for (natural) breeding, since the interval between both LH-peaks is rather constant.[6]

Pregnancy Confirmation

Immediate increase of progesterone/pregnanetriol around the time of mating is suggestive of the correct timing for breeding. Preliminary experiences with the use of an LH-test in Asian elephants have shown that many Asian elephants have a 1-day drop in progesterone and then a rapid rise the day following the LH surge[7].Continuation of high progesterone/ pregnanetriol level continuing for at least 16 wk after mating is highly suggestive of pregnancy. Ultrasonographic examination at 8-20 wk after mating allows easy visualization. Between 10 and 20 wk, the larger mature animals may need to lay down on its side for reliable ultrasonographic examination of the uterus. Uterine vascularization can be visualized to determine viability of the fetus, to exclude embryo absorption and mummification. At 6 mo post breeding pregnancy can be confirmed by elevated serum prolactin concentration.[7]

Monitoring at the End of Gestation

Progesterone assays are recommended every other day from week 89 to 91. From week 91 (637 days) daily progesterone assays are recommended and as soon as progesterone starts to decrease, sample twice daily. Daily monitoring of progesterone is only possible if you have a nearby facility that runs these assays on a daily basis. Many labs do not have sensitivity for levels of progesterone (P4) in elephants 1-3 days prior to delivery. Loss of mucous plug (not seen in many facilities) is another indication of impending delivery.

Behavioral changes may be observed in the elephant that is due for calving (short periods of separation from the group, beating the vulva with the tail, frequent production of small-sized feces and small quantities of urine[8]. Group members may react differently (vocalizations, restlessness) 24-hr observation including the use of a (time-lapse) video recorder starting in week 85 may add to information. The most relevant information for later decisions is the moment the amniotic sac ruptures. It is interesting to investigate the implications if only the allantois membrane ruptures. In most cases (75%) birth is seen within 2 hr of rupture of fetal membranes.[1] Development of

mammary gland and production of milk shortly before birth is often seen. Pre-and post-parturition ventral edema may be noticed.

Preparations for Calving

If possible, store some colostrum (freezer) or store plasma obtained from the dam in weeks prior to parturition. Have artificial milk available (hand rearing has been done at Emmen Zoo and Berlin Zoo). Check restraint chains and fixation points. Check the stable and place bars where a calf could possibly escape. Take out all obstacles. Make sure there is a good stock of bran or saw dust to be used on a concrete floor as soon as the calf is born. This will absorb much of the amniotic fluids and prevent the animals from slipping on the wet floor.

Supplies to have on hand include two or three pairs of keeper-gloves to get a better grip on the wet, slippery calf when needed, plastic hose pipe (with pump, if necessary) for rectal cleaning with lukewarm water, three birth-chains with proper handles (two for the legs, one for trunk or tail), find a way to avoid back sliding when manual extraction (vaginal vestibulotomy is required). Drugs to be kept in store include Betadine iodine, oxytocin, Ca-borogluconate, estrogen, lidocaine, xylazine, azaperone, atipamezole, doxapram and oxygen. With the limited knowledge that is available now, we suppose that normal calving should take place within 2 hr after rupture of membranes (release of fetal fluids).

The moment when veterinary intervention should start is still to be determined. At this time we have to rely on the data known from 20 cases.[1] Fifteen cows delivered within 2 hr: 12 live and three dead calves. One cow delivered a healthy calf after 15 hr. Four cows delivered after > 25 hr: one hypocalcemia (alive), three dead calves (2x episiotomy). Normal calving should take place within 48 hr after decrease of progesterone to below 0.1 ng/ml.

Not much validation has been done regarding the interval between the moment of complete absence of detectable blood progesterone level and the birth of a live calf. Efforts should be made to collect more data about this very important moment. In a few cows progesterone levels in the last 8 wk of pregnancy may be near baseline and determination of this event is difficult. However, most cows have a 50% drop in a 12-24-hr period and then drop to baseline levels in the next 12-24-hr period.[9] Fig. 1 shows this correlation between progesterone drop and time of birth similar as to what was demonstrated by Brown.[9] There are other exceptions to this where a couple of cows have remained above baseline levels and parturition occurred.

Guidelines for Veterinary Assistance at Parturition

The normal birth process in elephants has been poorly documented. Data of most cases has been kept as medical records at different institutions. The recommendations made here are based on the personal experience of the authors, results of the European survey[1] and data from the literature.[8]

To determine the moment when calving starts, two parameters are essential, the blood progesterone level and the relaxation of the cervix, monitored by ultrasonographic examination. One of the authors has used a commercial kit (Mare Foaling Predictorkit, Animal Health Care Products, 2660 E. 37th St. Vernon, CA 90058 USA), that measures the calcium level in milk to predict the time of parturition. This requires daily milk samples at the end of gestation.

Progesterone: the sensitivity of the equipment and the time needed to run the assay are the bottleneck for using the progesterone concentration as a reliable tool. Today many human hospitals use advanced equipment with a minimal detection level of 0.1 ng/ml that can be determined in less than 2 hr.

Ultrasonography: to use this technique as a reliable tool, it is indispensable for the veterinarian to gain experience long before the elephant birth occurs. This will enable the veterinarian to distinguish the relaxation of the cervix from the normal cervix. A 3.5 or 5 MHz probe can be used transrectally to visualize these changes. During the last 2 wk of gestation, the mucous that is present in the vagina during gestation will be discharged gradually. This is a clear indication of impending parturition.

Once the birth process starts, two situations may be encountered: delivery has started with or without spontaneous rupturing of the fetal membranes. In the European study,[1] 13 out of 20 (65%) elephants delivered the calf within 5 min after rupture of the amniotic sac and another two animals within 2 hr. From the remaining five animals, one elephant suffered from hypocalcemia (Rotterdam Zoo, unpublished data) and three animals produced a dead calf (including two vaginal vestibulotomy cases). The different findings (closed cervix or open cervix) of transrectal ultrasound investigations illustrate that veterinary intervention may be required if parturition does not take place within 2 hr after rupture of the fetal membranes.

No Rupture of Fetal Membranes Noticed

Generally, the blood progesterone level drops from the maintenance level to baseline level (< 0.1 ng/ml) in the last 2-3 days of gestation. It is expected that generally the calf will be born within 48-72 hr after the blood progesterone concentration has dropped to baseline level. However, one of the authors found that most calves are born alive and active within 3-5 days after progesterone drops to baseline in over 15 parturitions he attended and another 10 on which he consulted. Some births took place 7-8 days following drop (as long as the cervix is not dilated and labor had not been initiated).

If the calf is not born naturally 24 hr after blood progesterone has dropped to baseline level, rectal palpation and ultrasonographic examination of the cervix is highly recommended. This will demonstrate the rate of relaxation of the cervix, the presence of the amniotic sac or parts of the fetus in the cervix or vagina and should be repeated daily according to the following schedule. Rectal palpation can be carried out in combination with ultrasonographic examination.

If there is no relaxation of the cervix at 72-96 hr, repeat rectal examination every 6-12 hr. If there is still no cervix relaxation at 120 hr, see section immediately below. If there is partial relaxation of the cervix at 24-120 hr, administer 50 IU oxytocin s.c. or i.m.

Ruptured Fetal Membranes or 120 hr After Progesterone Drop

The calf should be born within 2 hr after rupture of the fetal membranes. If not, veterinary intervention should take place according to the following schedule. Two hours after rupture of membranes or 120 hr after progesterone drop, collect a blood sample for calcium determination. Store an EDTA and heparin sample for *Herpes* virus diagnosis (both cells and plasma in freezer after separation). Perform a rectal palpation, transrectal ultrasonographic examination, transrectal massage of the pelvic area with two arms for at least 10 min (keep hands gripped together and press with the wrists or the palmar sides of the hands against the pelvic roof and the genital tract). This may stimulate labor activities. Care must be taken not to damage the rectal wall, which may get very edematous. Insert at least one catheter i.v. To stimulate further relaxation of the cervix, the parenteral administration of estrogens may be considered. However no data are available for elephants. Administer 50 IU of oxytocin i.m. or s.c. if there is (some) relaxation of the cervix.

Four hours after rupture of membranes or 122 hr after progesterone drop, treat for hypocalcemia if applicable. Perform a rectal palpation, transrectal ultrasonographic examination, and transrectal massage of the pelvic area 50 IU of oxytocin i.m. or s.c. If parts of the fetus are in the pelvic canal, the dose may be increased to 100 IU i.m.

Six hours after rupture of the membranes or 124 hr after progesterone drop, perform a rectal palpation, transrectal ultrasonographic examination, check blood Ca-level again, and perform transrectal massage of the pelvic area. If no progress was made, prepare everything to perform a vaginal vestibulotomy. Administer 100 IU of oxytocin in a 1-hr infusion.

Eight hours after rupture of the membranes or 126 hr after progesterone drop, perform a tectal palpation, transrectal ultrasonographic examination, and transrectal massage of the pelvic area. As long as progress continues, monitor and act as above. If no progress was made, perform a vaginal vestibulotomy.

Post-Partum Care

Fluegger et al.[1] report that in 20 out of 23 cases the afterbirth was expelled within 10 hr. In one unpublished case at Rotterdam zoo (2001) a piece of afterbirth was found 6 wk after a normal parturition. No illness of the cow was seen during this period. In Antwerp a 20-yr-old cow produced the first calf of a twin (first calving) after a 91-wk gestation period. The second calf was born 113 days after the first calf. Both calves were born dead. The second calf was hardly decomposed. The mother did not show significant symptoms of illness (Meurichy, personal communication).

Vaginal Vestibulotomy in Elephants

Vaginal vestibulotomy[10-15] is a surgical procedure, in which the vertical part of the urogenital tract (vestibulum vaginae) is exposed by a percutaneous approach. Vaginal vestibulotomy is indicated if there is no progress in calving despite treatment according to the guidelines for veterinary assistance around parturition. Vestibulotomy is contraindicated if there are no fetal parts in the birth canal, confirmed by ultrasonography and rectal palpation.

Protocol of the Procedure

Don't sedate the cow if not strictly necessary, as you will need the straining support during the extraction. If the animal is non-handleable and restraint in a chute is not an option, use a reversible sedation (xylazine/atipamezole) and antagonize the sedation as soon as the elephant is chained on four legs. Behavior may have changed during this phase of the calving, most likely in favor of the veterinary intervention (i.e., the animal is probably more interested in getting the calf out than in attacking the keepers around her). However, there is not sufficient information about this point. The situation has to be evaluated for each animal.

Use local anesthesia only. After cutting through the skin, place a flexible plastic tube (5-10 cm diameter or a rumen tube) retrograde into the vestibulum vaginae to locate the incision site. To facilitate orientation, a 10 × 2 cm window should be made in the tip of the tube, 3 cm from the end. This window can be palpated transcutaneously and facilitate a quick perforation incision.

Use normal calving chains. Don't use more than three people per leg. An extra ring in the floor between the hind legs should be present in any calving box for elephants. A pulley can be attached to it in order to provide optimal conditions for pulling the calf out (in a ventral direction). If not present, one should consider to use a steel bar fixed to the walls behind the cow. A pulley can be attached to this bar. When the calf is in posterior position and can't pass through the pelvic girdle, try to rotate the calf (90º longitudinal axis) during extraction. In cattle, active rotation is the normal way for a live calf to pass its pelvis through the pelvic canal of the mother. When the calf is dead, the absence of this phenomenon might be responsible for stagnation of the calving process. In the experience of one of the authors (Rotterdam Zoo, 1993) it was only possible to remove a dead calf in posterior position by vaginal vestibulotomy after 90° rotation of the calf.

Don't hesitate to push the calf in the direction of the uterus; it might return in a better position. During this action, both legs and the trunk or tail should be well connected to the chains. Refill the genital tract with at least 5 L of "artificial embryonic fluid" by using a pump (aquarium-type). Don't pull at both legs in one direction at the same time. You must cross the chains and pull alternatively on each leg. It is more likely that each leg will pass the pelvic canal bit by bit, rather than that the two legs can pass simultaneously. When the calf is out, flush the uterus with cold water until the placenta is expelled. Give 50 IU of oxytocin i.v. At this time (if needed) the cow can be given a xylazine sedation (if sedated and reversed previously, use azaperone).

Place a long balloon catheter in the urinary bladder. Cut it to the proper length (i.e., just below the wound). Use acrylic glue or suture it in place**.** Close the vaginal vestibulum wall in two layers with PDS or Vicryl.

Reverse the sedation, wash your hands, clean up the mess and have a beer. But don't close the skin. Forget everything you learned about surgery. Whatever fancy suture technique you may use, the skin wound will open again. Don't think that your special suture will work, because every possible suture technique has already failed. Even when closing the skin, feces will still contaminate the wound, because the skin property does not allow a watertight wound closure; suturing the skin has proven to result in a permanent fistula.[10,11] You may hope that by leaving the skin wound open, the vestibulum sutures may be exposed to a minimum of tension, giving the wound the chance to heal. By doing so, you might be the first veterinarian performing this surgery without leaving the animal with a fistula. Give antibiotics for at least 7 days.

If you were not able to remove the calf, do not try to perform a caesarean unless you are absolutely sure that the cow will not survive conservative treatment. All cesareans performed to date have resulted in the death of the mother. If you see yourself facing the critical situation that the calf can not be removed through the wound and fetotomy is either no option or it has failed, the first advice is to leave the wound of the vestibulotomy open completely and see what happens during the days to come. Flush the uterus frequently with large amounts of water with a disinfectant like Betadine iodine to prevent the occurrence of Bandl's rings that may cause necrosis of the uterus.[16] An antibiotic treatment is recommended.

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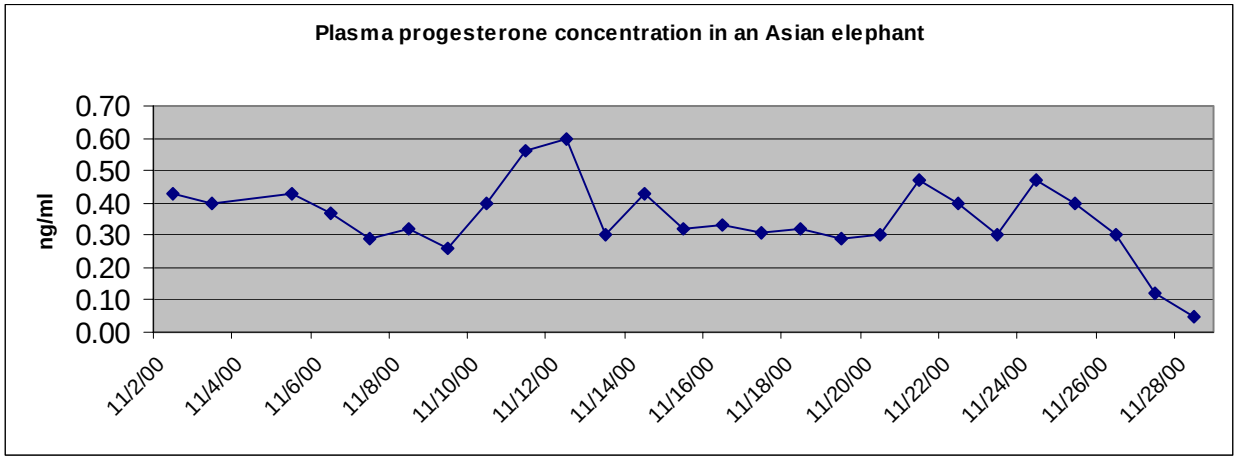

Figure 1. Plasma progesterone concentration in an Asian elephant. The calf was born at the end of the day when the progesterone level had dropped below the detection level of 0.1 ng/ml. (Rotterdam Zoo 2001.)

SYNOVIAL SARCOMA CASES IN PSITTACINES

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Abstract

Synovial sarcomas are relatively uncommon malignant tumors in animals arising from synovioblastic mesenchyme, usually in close proximity to synovial structures such as joints, tendons, or bursae. The neoplasm is characterized histologically by being composed of two intermingled cellular components, a synovioblastic or epitheliolid component and a fibroblastic component, where the proportions can vary considerably from tumor to tumor. The disease has been well described for humans, but less so in animals where cases have mainly been seen in dogs.[2] The clinical characteristics differ between human and canine cases, and probably also compared to avian cases. Previously only one avian case has been reported, in a Moluccan cockatoo.[1]

Case 1

A 4-yr-old female Senegal parrot (*Poicephalus senegalus*) was presented at a clinic with a mass on one wing, on the distal humerus surrounding the elbow joint. The skin was slightly ulcerated over the mass due to self-trauma. The mass had been observed for approximately 1 mo and was approximately 2 cm across and 1 cm in cross-section. It was firm in consistency and radiographs revealed an infiltration in underlying bone making it inoperable, as amputation was not an option. The bird was euthanatized and a portion of the mass was sent for histopathology.

Case 2

A >14-yr-old male sulphur-crested cockatoo (*Cacatua galerita*) was presented at a clinic with a mass on one wing, on the distal humerus, close to the elbow joint. The bird held the affected wing somewhat elevated. The mass, approximately 2 cm across, was soft and fluctuated on palpation. Traumatic hematoma was suggested. Three weeks later the mass was somewhat larger. Cytology showed pure blood, with some signs of infection but no suspicion of neoplasia. Enrofloxacin was given. Three weeks later the bird started biting the mass, puncturing it, causing hemorrhage and infection. Two months after initial presentation the bird was weak and dyspneic. The skin was ulcerated from self-trauma and the mass was now larger, involving most of the humerus and on the dorsal aspect firm in consistency. Pus and faintly yellow fluid oozed from the mass. The owner elected euthanasia and a portion of the mass was sent for histopathology.

Case 3

A 16-yr-old breeding female blue-eyed cockatoo (*Cacatua ophtalmicus*) living with a male was presented at the same clinic as Case 2, with a drooping left wing and a 2.5-cm swelling just distal to the elbow joint. Radiographs showed only soft tissue swelling without any bone involvement. The bird was sent home to rest, separated from the mate as trauma was suspected. The mass was initially only on the lateral side of the wing, but at the second visit 1 mo later the mass had expanded in size and was spread proximal and medial, including the elbow joint. The mass was firm and nonpainful on palpation, well attached to underlying bone. The bird was emaciated, weak, and very lethargic and had the previous day started biting the skin over the mass. The owner elected euthanasia, but did not allow necropsy or further histologic examination of the mass. Of course only a tentative diagnosis, but the similarities to Case 2 makes it very tempting to put synovial sarcoma as first on the list of differentials.

At histologic examination of the submitted neoplasms (Case 1 and 2) are surprisingly similar in appearance. Both consist of sheets of an abundant spindle cell component that stain faintly positive for collagen with van Gieson staining. A large number of reticulin fibers are seen in these cells with reticulin staining. In this fibroblastic tissue are irregular cavitary areas lined by the second component of the tumor, a mainly single layer of polyhedral epithelioid cells, sporadically growing into villous projections. In the cystic spaces a viscous fluid is found that stains positive with periodic acid-Schiff (PAS) stain, probably a synovial-like mucopolysaccharide secreted by the epithelioid cells as described.[2]

In humans these neoplasms rarely involve the underlying bone or the nearby joint but do metastasize readily, whereas in dogs the tumors tend to be more aggressive, infiltrative, and in time can destroy bone and destroy the adjacent joints or tendons. The clinical course is highly variable but the metastatic potential seems to be less in canine synovial sarcomas (25%) than that in humans. Metastasis occurs in dogs, but late in the clinical course, which can aid in differentiating this type of tumor from an osteosarcoma. Metastasis in humans occur to the lungs, regional lymph nodes and bones.[2]

The synovial sarcomas presented here as well as the previously presented avian case[1] seem to behave like canine cases, with soft tissue, bone, and joint involvement. Presumably metastasis may occur also in psittacines, but unfortunately in these avian cases not all tissues were examined histologically.

Bottom line: Make your pathologist and yourself happy – always necropsy and histopathology!

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BODY SCORING SYSTEM FOR CAPTIVE GIRAFFE (*Giraffa camelopardalis*)

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Abstract

Peracute mortality syndrome of captive giraffe as previously defined[1,2] may progress to a chronic wasting condition if certain conditions are met, the most notable condition being a lack of cold stress. At the present time there is no established method of evaluating muscling, fat, and overall body condition of giraffe (*Giraffa camelopardalis*). Such a scoring system in conjunction with serum chemistries, exocrine hormones, and body weights will allow for further investigation into peracute mortality syndrome/chronic wasting, a condition which we believe to be caused by an energy deficiency. The purpose of this project is to develop a standardized body scoring system that will assist zoologic staff in evaluating the condition of animals under their care. In some instances it may be the only feasible means for objectively evaluating these animals if physical limitations prohibit routine blood collection and body weights.

This method will use body scores of 1-8, with 4-6 being the optimal range, while higher scores indicate an animal that is overweight and lower scores indicate an animal that is underweight (Table 1). In keeping with methods currently used to evaluate cattle, horses, and other domestic livestock, muscling and subcutaneous fat are evaluated first in the hindquarters, taking note of the prominence of the sacral vertebrae and the hipbones (ilium). Prominence of the backbone is noted as an indicator of back fat. The neck and chest can be used as secondary indicators of condition, with well-fleshed animals displaying thickness in the neck, especially at the base, and a flat chest. Easy palpation and even visible prominence of cervical vertebrae begin to appear in animals that are below desired weight. Less fleshed animals often show a hollow in the center of the chest.

Due to differences in sex and conformation, some variation may occur between individual animals. Mature bulls tend to have thicker necks. Pregnancy in females will expand the abdomen, which may hide the prominence of the backbone and hipbones for a time. Examination of the hindquarters will provide a good indication of body condition during this period. Skin wrinkles do not appear to be a good indicator of condition, since wrinkles can often be found on prime and fleshy animals, as well as underweight animals.

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Table 1. Body condition score descriptors.

Score	Description	Notes/Comments
1	Emaciated. No fat can be palpated. Muscle wasting has occurred.	
2	Cervical vertebrae clearly visible. Protruding spine. Distinct hollows cranial to hipbones. Crest of illium is visible. Thin legs. Hips appear sunken and shoulders are slim.	Poor condition. Cause for concern.
3	Hipbones prominent. Definite outline of spine. Sacral vertebrae prominent. First two cervical vertebrae visible. Sunken chest.	Occasionally seen following a growth spurt in young adults.
4	Tailhead is noticeable and point of hipbone is visible. Outline of spine is visible. Hollow in center of chest.	Nicely muscled, but with little fat. Commonly seen in growing giraffe over 1.5 yr.
5	Back and hips rise smoothly to topline with no visible outline or denting along backbone. Some palpable fat around tailhead. Point of hipbone just visible. Slight hollow in chest.	Good condition.
6	Back is level and wide. Hipbone not visible, but easily palpated. Smooth chest. Visible thickening in lower neck.	Good condition. Ideal for calves under 1.5 yr.
7	Slight crease along backbone. Hipbones difficult to palpate. Smooth chest and thick neck.	Overweight.
8	Definite crease along backbone. Tailhead no longer clearly visible. Soft fat palpable along tailhead. Thick neck.	Obese.

CHARACTERIZATION OF THE BACTERIAL AND FUNGAL POPULATION PRESENT IN THE CLOACA AND CHOANA OF THE PATAGONIAN CONURE (*Cyanoliceus patagonus byroni*)

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Abstract

The Tricahue or Patagonian conure (*Cyanoliceus patagonus byroni*) is a native psittacine specie of Chile that is considered endangered and is listed in the red book of terrestrial vertebrates of Chile. There are just three colonies living in captivity in Chile, and only one of them, which lives in the National Zoo in Chile, is actively reproductive. Little is known about its biology, therefore populations in captivity must be studied in order to promote its conservation.

Cloacal and choanal swabs were collected from 23 healthy Patagonian conures living in captivity at the National Zoo in Chile for culture stain in two seasons of 2000, winter and spring. The samples were cultured and stained in order to determine the normal flora (bacteria and fungus) in the cloaca and choana of the birds. Of 192 strains identified, most were gram-positive bacteria (Table 1) from both the cloaca and choana. The most frequent bacteria cultured in both cloaca and choana was *Staphylococcus epidermidis* (Tables 2, 3). As other psittacine birds, Patagonian conures have mostly gram-positive bacteria in the choana and cloaca.

Twenty-four taxa of fungus were found: 13 filamentous and 11 yeast. In the choana, the most frequent fungus isolated were *Pichia* sp. and *Aspergillus* sp., whereas in cloaca *Aspergillus* sp. and *Penicillium* sp. were more commonly identified (Table 4).

Table 1. Total strains founded in cloaca and choana.

	Choana	Cloaca
Gram-positive bacteria	78 (89.66%)	82 (71.8%)
Gram-negative bacteria	9 (10.34%)	23 (21.9%)
Total	87 (100%)	105 (100%)

Table 2. Samples from the choana.

	Winter	Spring
Bacillus sp.	7 15.5%	3 7.1%
Corynebacterium non fermentative	13 28.8%	7 16.6%
Staphlococcus epidermidis	15 33.3%	22 52.3%
Bacillus firmus	5 11.1%	1 2.3%
Streptomyces sp.	3 6.6%	2 4.7%
Pseudomonas aeruginosa	2 4.4%	0 0.0%
Edwardsiella ictaluri	0 0.0%	2 4.7%
Serratia marcens	0 0.0%	2 4.7%
Enterobacter sp.	0 0.0%	1 2.3%
Other gram-negative	0 0.0%	2 4.7%
Total	45	42

Table 3. Samples from the cloaca.

	Winter	Spring
Bacillus sp.	15	15
	24.5%	34.0%
Corynebacterium non fermentative	14	6
	22.9%	13.6%
Staphlococcus epidermidis	19	6
	31.1%	13.6%
Bacillus firmus	3	0
	4.9%	0.0%
Streptomyces sp.	0	4
	0.0%	9.0%
Pseudomonas aeruginosa	2	2
	3.2%	4.5%
Edwardsiella ictaluri	0	3
	0.0%	6.8%
Serratia marcens	0	5
	0.0%	11.3%
Enterobacter sp.	0	1
	0.0%	2.2%
Other gram-negative	0	0
	0.0%	0.0%
Escherichia coli	0	1
	0.0%	2.2%
Enterobacter aerogenes	3	1
	4.9%	2.2%
Aeromona caviae	1	0
	1.6%	0.0%
Proteus vulgaris	2	0
	3.2%	0.0%
Klebsiella pneumoniae	1	0
	1.6%	0.0%
Enterobacter cloacae	1	0
	1.6%	0.0%
Total	61	44

Table 4. Frequency of fungus species present in the choana and cloaca.

	Choana	Cloaca
Aspergillus sp.	11.76%	37.50%
Cladosporium sp.	0.00%	4.17%
Paecilomyces sp.	5.88%	0.00%
Penicillium sp.	0.00%	12.50%
Scedosporium prolificans	5.88%	0.00%
Other fil.	29.41%	0.00%
Candida famata	5.88%	4.17%
Cryptococcus albidus	0.00%	4.17%
Geotrichum sp.	0.00%	4.17%
Pichia sp.	11.76%	4.17%
Rhpdptorula mucilaginosa	0.00%	8.33%
Other lev.	29.41%	20.83%
Total	100%	100%

A FOUR-YEAR STUDY ON REPRODUCTIVE STATUS AND LIFE HISTORY OF WILD EUROPEAN BROWN HARES (*Lepus europaeus*)

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Abstract

Since 1991 the European brown hare (EBH) (*Lepus europaeus*) has been classified as a CITES species in Germany within six states, at last listed as a vulnerable species in Nordrhein-Westfalen in May of 2000. Based on the critical situation of a nation-wide population decline, a basic research study on reproductive biology in free-ranging EBH was initiated in 1998. In general, population dynamics are mainly influenced in terms of successful pregnancies, birth rate, and postpartum survival rates. This joint project included the non-surgical evaluation of health status, reproductive fitness, and monitoring of life history of new-born. A total number of 302 EBH (142 females, 160 males) from 16 different areas were assessed by using endocrinology, ultrasonographic, and spermatologic parameters. Different ultrasound techniques (B-mode, color flow Doppler, and 3-D ultrasonography) were applied in both sexes to image the entire urogenital tract, including blood flow inside reproductive organs, heart activity of individual concepti, and to the precise pregnancy stage and integrity. Semen collections were performed with specially designed electroejaculation equipment adapted to the male hare anatomy. Additionally, blood samples were examined to detect several important viral and bacterial pathogens using different diagnostic methods (an ELISA to detect the agent of European brown hare syndrome, serology screening for *Brucella* sp., *Yersinia* sp., tularaemia, and *Toxoplasma* sp., and immunohistology to detect *Toxoplasma* sp.).

Of animals tested, 68.08% of the females were pregnant, 19.86% lactating, and in average 2.12 implantation sites per pregnancy were counted. Only 12.06% of females were non-pregnant and non-lactating, however, these individuals also showed ovarian activity. Mean serum concentrations of blood-plasma-progesterone were 97.9 ng in pregnant and 14.5 ng in non-pregnant females. Electroejaculation was practiced successfully in 134 cases. Each semen sample was assessed concerning volume, sperm concentration, motility, and morphology. In 13.75% of the males oligozoospermia was supposed, however, flow-cytometric-analysis did not show disturbance of spermatogenesis in this group. In addition, thermography technique (emerge vision, TM) was used to detect neonates in the natural environment. These new-born hares were monitored at night until they left the birth place naturally wise or they were hunted by foxes and birds of prey. These results did not indicate any kind of depression in reproduction or general health status monitoring of new born and juveniles results in a low abundance. Population development seems to be mainly determined by a high postnatal mortality and reduced survival rates.

FECAL HORMONE LEVELS IN THICK-BILLED PARROTS (*Rhynchopsitta pachyrhyncha*)

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Abstract

Characterizing endocrine profiles in birds can assist in non-invasive sex differentiation and in the facilitation of aviculture breeding programs. Often times, however, the stress and volume of blood involved in serum hormonal assays limits their use in avian species. By measuring these hormones in feces, levels can be measured frequently and easily without stress to the bird. Characterization of thick-billed parrot ovarian activity through noninvasive fecal monitoring would enable us to better facilitate the captive breeding of this endangered species.

To determine conjugated estradiol, progesterone, and testosterone levels, feces were collected from eight individually housed birds and one breeding pair, four times per week for 52 wk. The samples were divided, with one-half of each sample being frozen, and the other half being dried in a drying oven until a constant weight was achieved. The dried feces was weighed and then solubilized at a 1:10 (weight:volume) ratio in a solubilizer solution. Samples were vortexed briefly, then shaken for 24 hr. The samples were then centrifuged for 10 min at 3,000 rpm and the supernatant was removed, aliquoted, and stored frozen.

To establish a standard concentration range, samples were used to make serial dilutions in distilled water. This also provided the final dilution factor for the samples. Dilutions to the experimental samples were then made using these values. Each sample was run in duplicate at two different dilutions, using only one bird per plate to minimize interassay variation. Plates were read on a Spectramax 340 spectrophotometer at 405 nm and 650 nm. SoftMax Pro, SAS 612, and Microsoft Excel were utilized to analyze the resulting data.

The findings of this study led to preliminary conclusions about the characterization of the progesterone profiles of thick-billed parrots using enzyme immunoassay (EIA) techniques. Future characterization and analysis of fecal hormone levels for estradiol and testosterone conjugates will be performed. The characterization of the hormone profile in thick-billed parrots should greatly enhance opportunities to utilize reproductive technology in this species.

Aeromonas hydrophila SEPTICEMIA AND RESULTING ENCEPHALOPATHY IN A CAPTIVE JUVENILE WESTERN LOWLAND GORILLA *(Gorilla gorilla gorilla)*

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Abstract

A female 27-day-old mother-reared western lowland gorilla (*Gorilla gorilla gorilla*) that was raised in a naturalistic outdoor enclosure, in a group where social dynamics were unstable, became acutely septicemic with *Aeromonas hydrophila*. The infection resulted in an encephalopathy. Treatment extended over the course of 1 yr and involved numerous specialists, neurologic examinations, a computerized axial topography (CAT) scan, magnetic resonance imaging (MRI), and physical therapy. Despite the treatments, motor development was significantly delayed and paralleled human infant symptoms associated with cerebral palsy. At 1 yr of age the gorilla started to seizure.

The gorilla was euthanatized at 13 mo of age. Necropsy revealed the posterior bilateral cerebral convexities to be severely atrophic with focally corresponding diminished white matter and dilated posterior horns of the lateral ventricles and subarachnoid spaces. There was evidence of a 0.5 cm depressed area consistent with an old infarct in the superior aspect of the left cerebellar hemisphere. Histopathology showed neuron loss and gliosis with the most severe atrophy in the depths of the sulci in the posterior parietal occipital cortex. The affected areas had mineralized neurons and macrophages. The lesion in the left cerebellar hemisphere revealed a loss of cerebellar cortical neurons.

Introduction

Aeromonas hydrophila is a gram-negative bacillus that is commonly found in soil, sewage, and in fresh or brackish water in most of the United States.[14,21,22] It has been reported to infect amphibians, fish, turtles, birds, rabbits, grey seals, and nonhuman primates through ingestion of contaminated food or water.[1-5,10,11,13,18] *A. hydrophila* can infect any human and cause gastrointestinal signs. However young children, those with impaired immune systems, or people with malignant neoplasia may suffer from septicemia.[7,9,13,15] Septicemic conditions from this bacteria have also been reported in amphibians, a grey seal, turtles, a dog, rabbits, and a ground hornbill.[1-5,9,11,12,14,15-17]

In addition, myonecrosis due to *A. hydrophila* infection has been reported in human patients, particularly those who are immunocompromised or have neoplasia.[6,7,11,15,23] Ischemic insult to the cerebral cortex due to septic shock can lead to permanent damage and can result in motor developmental delays similar to cerebral palsy in human infants.[14,20] Infections of *A. hydrophila* have been reported in seven species of Old and New World Primates. In these species *A. hydrophila* showed both a septicemic form as well as peritonitis.[2] A septicemic condition and resulting encepahlopathy from this organism has not been reported in gorillas at this time.

Case Report

The female western lowland gorilla neonate had been raised for 3 wk in the summer, in a naturalistic outdoor enclosure, that occasionally had standing water. Its mother was primiparous, was part of a group with two young but mature males, and another first-time mother. The social dynamics of this young group led to group instability at times. There were numerous minor altercations between group members, and the neonate gorilla from this report sustained several scratches on its head as a result. These are speculated to be possible points of entry for the bacterial organism. When the neonate was 27 days old it became acutely weak and lethargic. The gorilla infant was pulled from the mother and upon examination and diagnostic work-up was found to have an *Aeromonas hydrophila* septicemia. This resulted in an ischemic encephalopathy that caused the neonate to have delayed motor responses, a hemiparesis of the left arm, and minimal use of the legs. The muscular weakness was suspected to be caused from myonecrosis, (creatine phosphokinase (CPK) = 65,142 IU/L; normal 363 ± 407 IU/L), shock, and heart damage. Treatment consisted of ampicillin sodium (Ampicillin Sodium, Lilly, IN USA) administered at 170 mg i.v. b.i.d., together with amikacin sulfate (Amiglyde-V, Fort Dodge, IA USA) at 12.5 mg i.v. t.i.d., and digoxin (Digoxin injection, Elkins-Sinn, NJ USA) at 6.8 µg i.v. once followed by 17.22 µg i.v. s.i.d. q 48 days. However, the organism was later found to be resistant to ampicillin, so only amikacin sulfate was continued. The gorilla was further maintained with a percutaneous indwelling catheter and total parenteral nutrition.

The clinical weakness and motor impairment gradually improved from a generalized severe paresis to a state resembling cerebral palsy motor development in humans. These similarities include delayed developmental skills and physical findings such as abnormal muscle tone, abnormal movement, and abnormal reflexes.[14]

A Haberman nipple was used to aid in feeding the gorilla since it initially lacked the ability to suckle properly. Once it began eating, treatment changed from crisis care and therapy to long-term management and supportive care. Daily physical therapy was used to aid in the development of motor skills. However, the age of development of her skills was considerably delayed in comparison to previous Toledo Zoo gorilla neonates and other neonatal gorillas (Table 1).

Cerebral damage was monitored using CAT scan, MRI, and periodic neurologic examinations. The initial CAT scan at 31 days of age showed possible temporal lobe attenuation. At this point the gorilla had an improved grip and no suckle reflex. An MRI at 45 days of age showed diffuse brain swelling and global hypoxia of the cerebral hemispheres. There was also extensive atrophy in the parietal area and bilateral subdural hygroma. Cortical function impairment was anticipated due to these findings. The motor development at this age included an asymmetric posture, decreased tone of the right arm and leg, and continued inability to grasp. Assistance with feeding was still required. The MRI at 94 days of age showed focal subdural collection of fluid on the left side of the brain. The gyri and the sulci were prominent and there was more generalized subarachnoid space present. The gorilla was having generalized twitching, was apraxic or dyskinetic of the right arm, and used the left arm less than the right arm. The gorilla was able to scoot at this age. An MRI at 287 days of age showed possible shrinkage of the brain in areas associated with cognitive function. Motor

development by this age included rolling over, reaching randomly, and placing objects in the mouth. The lack of normal gorilla brain scans available for comparison made diagnosis difficult, but human pediatric neurologists assisted in interpretation of the scans by comparing them to human neonates.

At 1 yr of age the neonate started to seizure and the antiseizure medication phenytoin (Dilantin, Parke-Davis, Morris Plains, NJ USA) was prescribed at a dose of 20.42 mg p.o., b.i.d. The medication ceased the seizures, but due to the continued lack of motor development and need for physical assistance with many skills, a decision was made by a committee named the "Sindiswa Care Committee" after the gorilla infant, to euthanatize the gorilla. The Sindiswa Care Committee was formed early on due to the high profile nature of this case (an endangered species and an infant gorilla that was very endearing to the public and staff). The committee was formed to assess this gorilla's progress, treatment, goals for recovery, and prognosis. The Sindiswa Care Committee consisted of the veterinary staff, mammal curatorial and gorilla keeper staff, human medical specialists, the zoo's director and assistant director, and several members of the Toledo Zoological Society's Board of Directors.

Necropsy revealed the posterior bilateral cerebral convexities to be severely atrophic with focally corresponding diminished white matter and dilated posterior horns of the lateral ventricles and subarachnoid spaces. There was also evidence of a 0.5-cm depressed area consistent with an old infarct in the superior aspect of the left cerebellar hemisphere. Histopathology showed neuron loss and gliosis with the most severe atrophy in the depths of the sulci in the posterior parietal occipital cortex. The affected areas had mineralized neurons and macrophages. The lesion in the left cerebellar hemisphere shows a loss of cerebellar cortical neurons.

Discussion

Collaborations were made with numerous human and medical specialists, including a neonatologist, pediatric neurologist, speech therapist, occupational therapist, and a physical therapist. These individuals worked closely with the zoo veterinary, curatorial, and keeper staff and numerous volunteers to care for the infant gorilla. They all worked in conjunction to develop plans for treatment and goals for motor development. Comparisons of human infant MRI and CRT images with those of the gorilla from this report proved very valuable for diagnostic interpretation due to lack of "normal" gorilla infant images available.[21] The establishment of a physical therapy regimen based on those used in people was felt to have aided the progress and development of the motor skills this neonate did achieve. A motor development chart (Table 1) was created by the zoo's gorilla keeper staff for comparison of this gorilla's motor development progress versus that of a normal infant gorilla. These comparisons helped in assessment of clinical progress and also became prognostic indicators for the zoo's veterinarians and staff.

This case was unique in light of the consensus-building approach and numerous people involved in making decisions on the gorilla's care and ultimate outcome. Anthropomorphization is a natural tendency when humans and gorillas or other apes interact significantly, especially a gorilla infant under human care. Having a larger decision-making body made up partially of animal care staff

very knowledgeable of normal gorilla behavior, development, and social structure helped to temper this tendency. Quality of life, animal welfare, and human safety issues were primary considerations in the Sindiswa Care Committee discussions regarding long-term condition and care. The final decision to euthanatize this animal was the result of a consensus from the Sindiswa Care Committee and the Toledo Zoological Society's Board of Directors together. The high profile and sensitive nature of this case also required the involvement of the zoo's public relations department in dealing with local media and a special reporter from the local newspaper.

This case demonstrates the potential severe septicemic capabilities of *Aeromonas hydrophila* in a neonatal western lowland gorilla, beginning with an initial insult of septic shock requiring intensive care and resulting in cerebral damage with long-term therapy. All of these led to a gorilla that never attained full sensory-motor development and was incapable of existing without human assistance. This condition has never been reported in a gorilla before, nor has the resulting encephalopathy been reported.

The gorilla was slowly making improvements, but probably would not have attained the ability to walk until at least 4-5 yr of age, if ever. This would have led to a situation where the animal would not have been able to be assisted safely by humans, and would not have been able to maintain itself within a gorilla group.

The fact that this animal was being reared by a first-time mother in a socially unstable group in an outdoor enclosure may have led to increased stress for the mother and infant, subsequent development of weakness in the infant, compromised immunity, and increased susceptibility to infection.

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Table 1. Motor development of Western Lowland gorillas (*Gorilla gorilla gorilla*).

Behavior	Age of normal gorilla	Age of subject gorilla
Cling/Grasp onto mother, surrogate object, or person	Birth	Birth, after septicemia Never achieved
Roll onto stomach and back	4-6 days old	114 days old
Scooting	14-32 days old	85 days old
Focusing on an object	26-28 days old	165 days old
Reaching randomly	50-98 days old	165 days old
Putting objects in mouth	50-52 days old	180 days old
Sitting up unaided	84-107 days old	Never achieved
Attempt to and/or pulling up to stand	62-100 days old	Never achieved
Rolling back to front	66-106 days old	Never achieved
Walking on all fours	97-266 days old	Never achieved

PATELLAR LIGAMENT RUPTURE AND TIBIOTARSAL CREST FRACTURES IN WHOOPING CRANE (*Grus americana*) CHICKS: POTENTIAL RISK FACTORS AND TREATMENT CONSTRAINTS

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Abstract

Pelvic limb injuries involving the long bones are common in cranes, and are frequently associated with traumatic injury.[3] The basis for the injury is often unknown, but is suspected to frequently involve collision with enclosure materials during direct attacks from conspecifics, or reaction to outside disturbances such as automobile, aircraft, predator, or human voice noise. Capture and restraint episodes may also induce iatrogenic injury to the pelvic limbs of cranes, including fractures. In 1996, the Whooping Crane Health Advisory Team identified musculoskeletal problems, specifically pelvic limb fractures, as a significant source of morbidity and mortality for the captive population of whooping crane (*Grus americana*) chicks used in reintroduction efforts. Among cases of pelvic limb fracture, we have identified seven cases of tibiotarsal cnemial crest fracture with varying degrees of patellar ligament rupture in whooping crane chicks from records of the International Crane Foundation (ICF, n = 4), Calgary Zoo (n = 1), and a release project conducted at Grays Lake, Idaho[2] (n = 2) between 1993-2000.

All cases occurred in whooping crane chicks being reared in isolation from human contact for reintroduction efforts. The relevant history for each case is summarized in Table 1. All cases occurred in hatch-year birds, with ages ranging from 62-138 days. All weights were considered normal for the chick's stage of development, except for Case #6 which experienced developmental limb abnormalities that required treatment prior to the injury. All chicks were captured and restrained for transport crate loading, pen transfer, or medical examination within the previous 24 hr of injury. Each injury was characterized by one or more of the following lesions: (1) patellar ligament rupture with patellar luxation, (2) avulsion fracture(s) of the cnemial crest of the tibiotarsus, (3) severe muscle and joint capsule trauma, and (4) extensive subcutaneous and intra-articular hemorrhage.

Surgical repair was attempted in four of the six cases. Each surgery was successful in achieving an anatomic restoration, but two chicks died during anesthetic recovery with severe lymphoid necrosis attributed to excessive stress. Case #2 died 68 days post-surgery during transport to a new release site. This bird had originally sustained a minimally displaced right tibiotarsal crest fracture with little patellar ligament trauma; the fracture of the tibiotarsal crest had healed normally by the time of death. Case #7 survived to 18 days post-surgery, including 11 days in a modified sling to

diminish weight bearing on the repaired leg, but was ultimately euthanatized due to additional muscle trauma and wound healing complications.[1] Thus, the mortality associated with this type of injury in captive reared whooping crane chicks is 86%. A similar case in a female 120-day-old Brolga crane (*Grus rubicunda*) chick from ICF in 1997 survived surgical repair and post-operative therapy; she eventually died from visceral gout and systemic amyloidosis 10.5 mo post-surgery possibly due to chronic inflammation at the surgical site. At necropsy, a severe and chronic synosteoarthritis in the repaired joint was noted, suggesting complete rehabilitation was not achieved in this case.

The underlying risk factors in these cases are difficult to identify. To date, some form of handling by husbandry or veterinary staff preceded each injury, but there were four or more different handlers involved in the seven cases. Case #1 died in its transport crate from severe hemorrhage and shock due to bilateral patellar ligament rupture and tibiotarsal crest fracture. Upon release of the remaining cases, each bird was noted to be lame or non-weight bearing on the affected limb, but handlers did not perceive a significant injury during the preceding restraint. Whooping crane chicks do not appear to be predisposed to this type of injury compared to chicks of other crane species fledged during the same period at ICF (4/72 vs. 1/79, respectively; $\chi^2_1 = 2.15$, $P = 0.14$). Hatching date, age, sex, diet, weight gain curves, long bone growth (specifically tibiotarsal length), body weight at injury, rearing method, social status when in cohorts, pen types, and concurrent health problems were also not associated with the development of this injury in whooping crane chicks. None of the chicks appeared to have primary morphologic or histologic abnormalities in the affected structures postmortem.

We believe the basis of these injuries was due to increased biomechanic stress from the femorotibialis/iliotibialis muscles on the insertion at the cnemial crest. In particular, the restraint method chosen by the handler may have compounded this stress. To our knowledge, each bird was restrained with the pelvic limbs folded under the crane's body. This common handling method, an alternative technique to keeping the legs in an extended position, is used only when the pelvic limbs are easily folded with little resistance.[4] Based on goniometry performed during several recent handling events using whooping cranes at ICF, the stifle joint remains in a moderately flexed state in either restraint position, suggesting the femorotibialis/iliotibialis mechanism is not in a neutral position and may experience loading with muscle contraction. However, with the limbs folded, resistance to extension (isometric loading) is likely to be extreme during struggling, whereas minimal loading should occur with the legs held extended. We believe it may have been the resistance to movement that created conditions favorable for the development of forces sufficient to fracture the cnemial crest in these cases.

Future investigations will include biomechanic stress testing on archived specimens and simulation modeling to support or refute our hypotheses.

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Table 1. Characteristics of whooping crane chicks with patellar ligament ruptures (1995-2000).

Case	Location	Year	Sex	Age (days)	Weight	Handled within 24 hr	Outcome
1	Idaho	1993	M	89	5.2	Yes	Died during transport
2	Idaho	1993	M	87	Unknown	Yes	Died during transport 2 mo after successful surgical repair
3	ICF	1995	F	70	4.4	Yes	Post-operative anesthetic death
4	ICF	1995	F	138	5.5	Yes	Post-operative anesthetic death
5	Calgary	1996	F	63	3.6	Yes	Euthanasia after surgical exploration
6	ICF	1999	F	62	3.4	Yes	Euthanasia at diagnosis
7	ICF	2000	F	137	5.6	Yes	Euthanasia 18 days after surgical repair

LEPTOSPIROSIS IN A MEXICAN GREY WOLF (*Canis lupus baileyi*): SURVEY FOR *Leptospira* INFECTION IN CAPTIVE AND LOCAL FREE-RANGING WILDLIFE AND FERAL ANIMALS AT AFRICAM SAFARI, PUEBLA, MÉXICO

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Abstract

A 3.5-yr-old, captive-born, male Mexican grey wolf (*Canis lupus baileyi*) housed in a facility with two other Mexican wolves was reported to have voided dark urine; urinalysis revealed hematuria, hemoglobinuria, and bilirubinuria. The animal was anorexic. Complete blood count (CBC) and serum biochemistries revealed leukocytosis, mostly due to absolute neutrophilia, azotemia, hyperbilirubinemia, and elevated aspartate transferase (AST), alanine transferase (ALT), and alkaline phosphatase (ALK); plasma was icteric. Treatment was initiated with oral doxycycline, and changed to intramuscular oxytetracycline and fluid therapy 3 days later. The animal's condition progressively deteriorated and it died 6 days after presentation. This wolf had been vaccinated for leptospirosis (Nobivac Lepto, Intervet) once yearly since its birth (twice in 1998). At necropsy, body weight was 20 kg; gross findings consisted of depleted fat stores, icterus, ascites, reddened enlarged lymph nodes, hemorrhages in the intestinal serosa and endocardium, and reddened kidneys and gastric mucosa. Histopathology revealed moderate to severe, chronic lymphocytic interstitial nephritis with severe, acute, diffuse tubular necrosis and hemoglobin casts within tubules. Warthin-Starry stain on renal tissue revealed low numbers of organisms morphologically compatible with spirochetes in the lumina of tubules. There was severe intracanalicular cholestasis in the liver. Immunohistochemistry for leptospiral antigen on renal tissue using previously described procedures[6] was negative. Serologic testing for 15 *Leptospira interrogans* serovars performed on serum collected 3 days after presentation at two different laboratories revealed positive titers (1:200) to *L. icterohaemorrhagiae* and borderline titers (1:50) to *L. canicola*. The two cage mates remained clinically unaffected, but hemogram revealed hemoconcentration and leukocytosis in one animal (blood urea nitrogen and creatinine were within normal limits in this wolf). A few days later the leukocytosis had resolved and serum biochemistry for hepatic and renal function was normal. One month later, this animal had hyperbilirubinemia. Serologic testing in the two cage mates revealed positive titers (1:100 or 1:200) to both *L. canicola* and *L. wolffi* at the time of death of the cage mate. The wolf with leukocytosis was seronegative for *L. wolffi* 1 mo later. This animal died of unrelated causes 3 mo later. Histopathology revealed mild multifocal plasmacytic interstitial nephritis that was negative for tubular spirochetes with Warthin-Starry stain.

The clinical, pathologic, and histochemical findings in the deceased Mexican grey wolf are characteristic of leptospirosis. This animal had previously been vaccinated against *L. canicola* and *L. icterohaemorrhagiae*, the two serovars for which borderline and positive titers were found respectively at the time of clinical presentation. Therefore, these titers were most likely vaccine-induced and the serovar involved in this case undetectable with the serovar panels used. The lack of seroconversion to *L. wolffi* (not included in the vaccine) in the two cagemates makes the interpretation of positive titers to this serovar difficult.

A preliminary serosurvey for leptospirosis has been performed at two different laboratories using the microscopic agglutination test for 11, 12 or 15 *Leptospira interrogans* serovars in captive carnivores (felids, hyenas, bears, and red pandas) with banked sera, in trapped wildlife (*Didelphis virginiana, Spermophilus mexicanus,* and *Bassariscus astutus*), and in feral domestic animals (dogs, and cats). All captive carnivores except the Mexican grey wolves were tested serologically prior to vaccination for leptospirosis. Additionally, urine from trapped wildlife and feral domestic animals was tested for leptospiruria by dark-field microscopy. Overall, this serosurvey has revealed seropositivity for 10 different serovars. All tested *D. virginiana* and *B. astutus* were negative; however, leptospiruria associated with chronic tubulointerstitial nephritis was found in a *D. virginiana*. Dark-field microscopy of all other urine samples was negative with the possible exception of a cat. Four of five *S. mexicanus* had borderline titers to *L. pyrogenes* and/or *L. grippotyphosa*. Two dogs had borderline or positive titers to multiple serovars (8 between the two dogs) and a third dog not tested serologically had chronic plasmacytic interstitial nephritis; the vaccination history for these dogs is unknown. *Leptospira* immunohistochemistry in three selected opossums (including that affected with nephritis) and this last dog was negative.

Seropositivity to seven serovars has been found in captive felids included in this survey with titers up to 1:800; in contrast, only one of five feral domestic cats had borderline titers to *L. pomona*. Ten of 10 wild cats from the conventional zoo (CZ) were seropositive to one or more serovars in contrast to 1/10 positive captive felids from the wild animal park (WAP). Six of 10 seropositive felids at the CZ had positive titers both to *Leptospira hardjo, L. tarassovi*, and *L. wolffi*. Leptospiremia had been diagnosed in October 1996 in four *P. onca* by dark-field microscopy, and two had abnormalities in the urinalysis (hematuria, bilirubinuria, proteinuria, pyuria ,and/or positivity for urobilinogen) and normal CBC and biochemistry panels for renal and hepatic function. A single hyena and black bear accounted for most of the positive and borderline titers found in these species. The only captive carnivore species serologically negative was the red panda.

These preliminary findings suggest that leptospirosis may be an important infectious disease at the primary authors facility as at other zoologic institutions.[1,3,4] These findings also highlight not only the problems with the diagnosis and control of leptospirosis in zoo animals, mostly due to the existence of more than 200 pathogenic serovars (and, thus, the limited value of serologic testing, immunohistochemistry, and vaccination programs)[3-5] but also the difficulties in continuously controlling the direct and indirect interactions of zoo animals with free-ranging wildlife and feral domestic animals. The use of a more sensitive diagnostic test, such as the PCR assay, may help to better characterize the epizootiology of leptospirosis in zoos and thus prevent it.[2]

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USE OF CELL CULTURE INVASION ASSAYS TO DETERMINE PATHOGENICITY OF *Salmonella arizonae* ISOLATES FROM A COLONY OF RIDGENOSE RATTLESNAKES (*Crotalus willardi*)

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Abstract

Seven cases of progressive osteomyelitis associated with *Salmonella enterica* ssp *arizonae* were identified in a colony of ridgenose rattlesnakes (*Crotalus willardi*) at the Knoxville Zoological Gardens.[2] The *Salmonella arizonae* serotype 56:Z4,Z23 was consistently isolated from all bony lesions but was isolated only once from cloacal swabs. Twelve other *S. arizonae* serotypes were isolated from cloacal swabs but not from the bony lesions. The present study was initiated to see if it was possible to use cell culture invasion assays to compare the pathogenicity of the 56:Z4,Z23 serotype with four of the intestinal isolates. Our hypothesis was that an *in vitro* assay could differentiate the pathogenic 56:Z4,Z23 serotype from the intestinal isolates. Two cell lines, human HeLa cells and viper heart cells, were used in the invasion assays. Multiwell plates with a confluent monolayer of cells were incubated with each of the five *S. arizonae* serotypes. After incubation, the cells were treated with antibiotics, rinsed, and then lysed to release any bacteria which had invaded the cells during the incubation period.[1,3] The results of the HeLa cell invasion assay supported our initial hypothesis. The number of colony forming units recovered was significantly higher ($P < 0.05$) for the 56:Z4,Z23 serotype compared to the intestinal isolates. The results of the viper heart assay varied from this pattern. One intestinal isolate, serotype 48:I-Z, was recovered in significantly higher ($P < 0.05$) numbers than the 56:Z4,Z23 serotype.

Based on these results, the HeLa cell invasion assay appears to be able to differentiate between the pathogenic 56:Z4,Z23 serotype and the apparent commensal intestinal serotypes. Evaluation of the invasion assay with other known pathogenic and non-pathogenic *Salmonella* sp. seems warranted. The cause of failure of the viper heart cell assay to differentiate between the 56:Z4,Z23 and other Arizona serotypes is unclear. The two cell lines are grown and maintained at different ambient temperatures, which may contribute to the observed differences. Viper heart cells have not, to our knowledge, been previously used for invasion assays and intrinsic cell properties are another potential cause for the observed results. Development and investigation of other reptile cell cultures, such as snake macrophages, might prove more useful as an in vitro test for differentiating between these *Salmonella arizonae* serotypes.

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MELIOIDOSIS IN NINE PSITTACINES

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Abstract

Burkholderia pseudomallei, the etiologic agent of melioidosis, is a bacterium found in soil and surface water throughout the main endemic area of Southeast Asia and northern Australia.[1] It can cause acute septicaemia, mild or asymptomatic, subacute, and chronic disease in humans. Risk factors such as diabetes mellitus, chronic renal failure, alcoholism, and steroid therapy have been recognized. Cases have mainly been described in mammals. There has been two reports in the literature of cases in psittaciformes.[2,3] Both occurred in Australia. This is a description of nine cases of meliodosis in psittacines at a zoologic facility in Hong Kong.

The nine cases occurred between August 1996 and September 2000. Three male and six females, 2-25 yr of age were affected. Cases were found dead or presented with anorexia and lethargy followed by death within 24 hr. Gross lesions at necropsy predominantly showed multifocal miliary abscesses throughout the liver and spleen as well as enlargement of these two organs. A definitive diagnosis was obtained by isolation of the gram-negative organism *Burkholderia pseudomallei* from multiple organs including blood. Histopathology revealed that acute septicemia was the main presentation in this case series. One bird presented with gross and histologic lesions of chronic active pericarditis.

Melioidosis should be considered as a differential diagnosis in psittacine patients in endemic areas or if imported from these areas. The veterinarian should take precautions to avoid infection during necropsy. Carcasses of birds which die of this disease should be disposed of in such manner to prevent a potential public health risk through dissemination of *Burkholderia pseudomallei* into the environment.

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ENDOSCOPIC AND ULTRASONOGRAPHIC ANATOMY IN AMPHIBIANS

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Abstract

Like in reptiles, diagnostic imaging methods are of great value in clinical evaluation of amphibians. In amphibian medicine the use of diagnostic imagining methods is described in only a very few articles. The use of radiologic methods in amphibians has been described[5] and the use of endoscopic techniques has been explained in detail.[4,6] In contrast to endoscopic techniques, ultrasound examination is non-invasive and therefore does not require anesthesia.[7,8] Like in other imaging methods, the large number of species is problematic and demand special knowledge of the anatomic situations. Amphibians do not possess horny scales, the skin is leather-like or mucous.[1] Ultrasonography has been described as a tool for determination of gender in giant salamanders[3] and a general tool in amphibian medicine.[9,10]

For endoscopic examination amphibians have to be anesthetized. Endoscopic equipment and technique are described in detail.[4,6] Endoscopic examination produces three-dimensional pictures of inner organs in normal size, shape color and surrounding. Endoscopic examination of the coelomic cavity in amphibians permits to visualization of heart, lung, liver, spleen, intestines, gonads, kidneys, bladder and fatbody from on singly point of entry.

For ultrasonographic examination of amphibians we used a fully digitized real-time ultrasound unit, Logique 200 Pro (General Electric, Glattbrugg, Switzerland), equipped with two scanners, a 5.5-6.5 MHz microconvex-scanner and a 6-9 MHz linear-transducer, both shiftable frequencies. Even though resorption through the skin is well known in amphibians, no negative side effects occurred using coupling gel. In none of the examinations amphibians where sedated or anesthetized. Careful handling and slow movements of the examiner caused calm patients. The patient was placed in a cryo-plastic container filled with water. The scanner was either placed directly on the frogs surface (no coupling gel) or on the bottom of the container. Stand-offs were not used. The whole coelomic cavity was scanned in transverse and longitudinal sections from cranial to caudal.

Real-time ultrasonography produces a two-dimensional sectorial picture in graduated black and white depending on the different reflexability of the inner organs. It has no self-proofing method. To furnish proof of the diagnoses, post-mortem examinations with measurements, cryo-cuttings, and waterbath examinations of fresh organs were performed. For waterbath examination, inner organs (heart, liver, kidney, gonad, intestine, and fatbody) of recently deceased animals were divided into a plastic container filled with distilled water (to prevent air bubbles which jeopardize ultrasonographic examination). While dissecting the animals, photographs were taken and removed organs were measured. In addition, cryocuttings of frozen carcasses were performed.

The coelomic cavity of amphibians does not developed a diaphragm. The only separated compartment is the pericardial sac with the heart.[2] This undivided pleuro-peritoneal cavity displays the heart far in the rostral part, between the two shoulder joints. The lobulated liver covers the mid-third of the ventral part of the body cavity. In anurans the cranial parts of the left and right lobes are placed left and right to the ventricle of the heart. The stomach is situated dorsally in the left part of the coelom. The intestines fill the contralateral right part of the pleuro-peritoneal cavity. In pregnant female amphibians the follicles and/or ova could occupy large parts of the coelom and therefore distend the abdomen. Gonads and kidneys are placed dorsal and caudal, left and right to the aorta abdominalis.

The amphibian skin is a hyperechoic thin line and permits easy penetration of the ultrasound beam. The lack of horny or calcified scales like in reptiles prevents from the well known problems of coupling the scanner to the skin. Even though the skin is moist in most species (not in some toads) the use of coupling gel is preferred for homogenous transmission. The underlying layers of body muscle appear as hypoechoic structures as in mammals and reptiles. Often there is a anechoic fluid visible in the pleuro-peritoneal cavity of amphibians. Like in some reptile species, this is of physiologic nature and could be used for easier visualization of organs, such as a fluid-filled abdomen in mammals.

Endoscopy allows to examine the pericardium and the surface of the heart and to assess the quality of the heart action. The ultrasonographic examination reveals diagnosis concerning the inner structure of the heart while it is working (real time).

During endoscopic examination, liver and gallbladder can be identified immediately adjacent and caudal to the liver. The lobes of the liver encircle the heart caudally, left and right. Endoscopic examination permits the visualization of the liver and gallbladder. In addition biopsy under endoscopic control could easily be performed.

The liver parenchyma is hyperechoic and displays only very few, small vessels. The gallbladder is situated on the caudodorsal aspect of the liver. It is characterized by anechoic contents and thin hyperechoic wall. The liver of anura is more hyperechoic than described for reptiles. Like explained for Cryptobranchidae the liver of anurans display less larger vessels than in reptiles or birds.

The stomach is placed caudally to the liver and can be demonstrated endoscopically. Size and shapes varies within a wide range depending on the species (size) and the food-intake. In ultrasonographic examination the stomach displays a wall with three layers: hypoechoic, anechoic, hypoechoic. Small and large intestines are difficult to differentiated.

The gonads of amphibians are placed far dorsally, adjacent to the kidneys. The ovaries could easily be demonstrated endoscopically, independent from size, sexual status, and age of the animal. The ovaries filled with developing follicles display ultrasonographically as large nonhomogenous structures caudal to the liver. The single ovum is separated into hypoechoic (anechoic, yolk) and hyperechoic (embryo) structures. The very large amount of ova leads to a nonhomogenous

connected mass. Growing follicles of sexual active giant salamanders are described as anechoic bubbles within the ovary, whereas the ones in sexual inactive animals are hyperechoic. Ultrasonographically, testis could only be described for giant salamanders as lobulated hypoechcoic ovoid structures.[3] The endoscopic visualization of testes can easily be performed.[4,6]

The fat bodies of anura can easily be demonstrated endoscopically. They display as yellow, fingerlike organs. Ultrasonographically they display as hyperechoic structures. They emerge from the basis of the mesovarium and mesotestis and protrude into the pleuro-peritoneal cavity, between the other organs.

The kidneys of amphibians are cigar-shaped cylinders located caudal to and adjacent left and right to the spine. Endoscopically they display as dark red, cigar-shaped structures. The sonographic appearance is homogenous and hypoechcoic. In Cryptobrachidae, testes and kidneys are separated from each other (testes cranial to the kidneys) and could therefore easily been differentiated. In anura the testes are placed adjacent to the kidneys and are difficult to differentiate.

The bladder is situated far caudal in the coelom, ventral to the large intestine. Endoscopically it displays as a translucent gray to blue-colored bowl. Ultrasonographically the wall of the bladder can be demonstrated as a hypoechoic surrounding of the unechoic contents. The size and shaped can vary in a wide range, depending on the contents.

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LYMPHOBLASTIC LEUKEMIA IN A DIAMONDBACK TERRAPIN (*Malaclemys terrapin*)

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Abstract

This report details the diagnosis of lymphoblastic leukemia with suspected B-cell differentiation in a diamondback terrapin (*Malaclemys terrapin*). There are only nine reports of hemic tumors in turtles.[1-5] Antineoplastic treatment was not attempted in any of the cases. A thorough diagnostic investigation was performed to evaluate the extent of the disease, as this is the first reported case of neoplasia in a diamondback terrapin (J. Harshbarger, personal communication). The diagnostic procedures performed were a complete blood count (CBC), serum chemistry, routine blood films, a bone marrow biopsy, radiographs, immunocytochemistry to type the leukemic cell line, magnetic resonance imaging (MRI), and computed tomography (CT). An antineoplastic treatment protocol of prednisolone, cytosine arabinoside, and chlorambucil was devised (CGC). This abstract describes the investigation of the leukemic disease in the patient, elaborating on the use of immunocytochemical staining as part of the diagnostic procedures that were performed.

The diagnosis of lymphoblastic leukemia was made based on cytologic examination of blood films that revealed a preponderance of large mononuclear cells with intense cytoplasmic basophilia; increased nuclear:cytoplasmic ratio; mild anisokaryosis; round, cleaved, or lobulated nuclei; and rare bi-nucleation. Small well-differentiated lymphocytes, heterophils, basophils, and thrombocytes were scant. Occasional immature erythrocytic cells were also detected. Heterophils occasionally were immature.

Leukemic involvement of the extra-sinusoidal regions of the bone marrow was detected histopathologically. Increased osteoclastic activity was present in some areas of the trabecular bone. Extrasinusoidal myeloid cells and intrasinusoidal erythroid cells were scant. Fragmentation of the samples (attributed to collection and processing artifact) obscured architectural detail in some areas.

Immunocytochemistry is a technique which is able to determine the cell of origin by detecting various membrane and cytoplasmic antigens. The specific set of antigens expressed by a cell population constitutes a unique phenotype. This technique has been applied to a variety of animal species, including reptiles.[5] The BLA36 antigen is known to stain a subset of B-cells in humans and has been shown to stain cells in many mammals which correspond to CD79a positive cells, a component of the B-cell receptor. In addition, one of the authors has shown that BLA 36 stains similar B-cell populations in several reptiles (Wojcieszyn, unpublished observations). Results of

immunocytochemical staining revealed that the neoplastic cells were positive for the BLA36 antigen, demonstrating a 3-4+ membrane stain. The neoplastic cells were negative for CD3 antigen, although few circulating small well-differentiated lymphocytes were positive for this marker.

The antineoplastic treatment consisted of prednisone 2.5 mg p.o. given every 48 hr with cytosine arabinoside 5 mg s.c. and chlorambucil 1 mg p.o. given 7 days apart (Table 1; T = 0, T = 7 days). The patient's response to the therapy was monitored with a weekly CBC, chemistry, and cytology. The white blood cell (WBC) estimate underwent a steady decline during the first 24 days of treatment. At the start of therapy the WBC estimate was 200 Thd/CMM (reference range 3-8 Thd/CMM), while on day 24 the WBC estimate reached a nadir of 10 Thd/CMM. There were no mitotic figures observed on the cytologic examination performed on day 24. Concurrent increases in blood urea nitrogen (BUN), AST (aspartate transferase), and creatinine phosphokinase (CPK) were observed during the course of treatment. These elevations were likely due to the by-product of the tumor lysis. Tophaceous gout was diagnosed prior to the start of chemotherapy, and was treated with a regime of probenecid, colchicine, and allopurinol.

The anticancer therapy achieved a survival of 46 days. At necropsy a generalized lymphoma and lymphoid leukemia were diagnosed. Acute myocardial necrosis and focal pericardial fibrosis were present along with moderate renal tubular gout. Areas of tumor necrosis were observed, indicating a treatment effect; however, a toxemia with resultant myocardial necrosis and disseminated intravascular coagulation may have occurred from the drug therapy. There was no evidence that the necrotizing event had an effect on the myeloid cell line.

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Table 1. Hematologic parameters of anticancer therapy in a diamondback terrapin.

		T = 0	T = 7 days	T = 14 days	T = 24 days	T = 34 days	T = 46 days
Hematocrit	%	22	29	23	24	29	18
WBC estimate	3-8 Thd/CMM	200	65	33	10	16	28
Het/Poly	40-60 %	4	12	21	48	43	16
Absolute poly		8000	7800	6930	4800	6880	4480
Lymphocytes	40-60 %	96	88	18	27	30	80
Absolute lymphs		192000	57200	5940 b	2700	4800	22400
Monocytes	0-1 %	0	0	61	25	20	3
Absolute monos		0	0	20130	2500	3200	840
Basophils	0	0	0	0	0	0	1
Absolute basophils							280
Glucose	40-120 mg/dl	31	47	163	131	177	23
Urea nitrogen	10-70 mg/dl	89	139	181	106	88	69
Total protein	3.3-5.5 g/dl	3.3	3.2	3.2	3.5	3.8	2.4
Albumin	1.0-1.7 g/dl	1.2	0.7	0.7	0.7	0.7	0.6
AST(SGOT)	10-80 u/l	1396	1948	1332	652	537	496
Calcium	8-13 mg/dl	9.3	9.5	8.7	9.1	8.3	8.4
Phosphorus	5-8 mg/dl	6.1	4	5.4	4.2	2.9	2.1
Sodium	130-150 mEq/L	118	133	134	142	128	135
Potassium	2.0-5.5 mEq/L	5.7	6.1	4.4	4.1	2.6	2.1
Chloride	90-120 mEq/L	84	100	96	111	94	102
Globulin	1.7-2.9 g/dl	2.1	2.5	2.5	2.8	3.1	1.8
CPK	u/l	1916	8562	4480	764	950	3119
Uric acid	2-7 mg/dl	14.7	14.3	3.9	1.5	12	10.9
				Lipemia 2+			

A SIMPLE TECHNIQUE FOR BLOOD COLLECTION IN UNRESTRAINED EMUS (*Dromaius novaehollandiae*)

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Abstract

Manual and chemical restraint of ratites, including emus (*Dromaius novaehollandiae*) is universally employed to obtain blood specimens for analysis.[1-5] Restraint often alters the results of the complete blood count (CBC), creatinine phosphokinase (CPK), and aspartate aminotransferase (AST) in emus and other avian species.[1-5] In addition, safety concerns arise for the handler and the bird when restraint is employed.[1]

We devised a method for obtaining blood samples from emus without restraint. Blood specimens obtained in this manner may accurately reflect true blood values rather than those influenced by restraint. In our case, we obtained blood from the right jugular vein in three clinically healthy, manually restrained emus, and in the same three emus more than 6 mo later with this new technique. In each case, values for CPK, AST, and CBC were significantly different from those values obtained without restraint.

With restraint, blood is obtained from the right jugular vein or medial metatarsal vein.[1-5] The basilic vein in emus is difficult to access due to the size of the vestigial wing.[3,4]

We utilized a standard 19 or 23 ga vacutainer system (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ 07417-1885 USA) for obtaining blood samples. A lithium heparin tube or an EDTA tube is taped in an upright position on the medial aspect of the tarso-metatarsus along with the butterfly catheter. Lithium heparin is the preferred collection tube for plasma chemistries, and EDTA is preferred for hematology, as lithium heparin may interfere with leukocyte counts, and EDTA can interfere with calcium levels.[2] Some laboratories have better success analyzing ratite samples stored in citrated tubes.[3]

The medial metatarsal vein is visualized, cleansed, and accessed with the butterfly needle. After a step or two by the emu, the opposite end of the extension set is inserted in the vacutainer and blood freely flows into the tube. An added benefit is that the emu usually walks away, thereby mixing the blood adequately. After filling, the tube is removed, and the sample processed routinely.

We have found that values in unrestrained emus are statistically different from manually restrained emus, and may reflect more accurate baseline values in emus. We believe this method can also be employed in ostrich (*Struthio camelus*).

Advantages of this technique are generally increased safety, less manpower, and a more accurate reflection of blood values. Disadvantages include uncooperative patients and some risk for being kicked by the emu. Fractious birds should only be handled by experienced handlers or sedated for blood sampling.[1]

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SERUM HYDROCORTISONE LEVELS IN A MANUALLY RESTRAINED AFRICAN ELEPHANT (*Loxodonta africana*) PRE- AND POST-SEMEN COLLECTION

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Abstract

The African elephant (*Loxodonta africana*) is an endangered species with a poor history of successful breeding in captivity. Recent advances in artificial insemination provide hope for maintaining this species in captivity.[4] The manual collection of semen in this species compared to electroejaculation negates the use of anesthetics, is generally more acceptable by elephant managers, and in at least a few instances has been demonstrated to be of higher quality than with electroejaculation methods (Loskutoff, personal communication).

An in-house study was undertaken to indirectly evaluate a physiologic "stressful" event (manual semen collection from an elephant in an elephant restraint device or ERD) through the pre-and post-collection evaluation of serum hydrocortisone levels. Corticoid levels are routinely correlated with stress in numerous species of animals.[1,2,5,6] Complete blood counts (CBC) and select biochemical profiles were evaluated for any underlying disease process. Over the course of 1 yr, 25 pre- and post- semen collection blood samples were collected and analyzed.

The elephant, a 22-yr-old African bull elephant, was conditioned to enter the ERD on a routine basis. Once stationary, cargo straps were placed to prevent injury to the legs and hydraulically operated sides were closed to restrain the elephant. Ultrasonic evaluation of the reproductive tract[3] had been performed prior to routine manual semen collection.

Blood was obtained from the saphenous vein immediately after the animal was restrained. The collection of semen was subsequently performed, and once completed (on an average of 20-40 min) blood was again obtained from the saphenous vein, placed in a serum separator tube, and processed routinely for analysis. Semen samples were cultured for aerobic and anaerobic bacteria and screened for viral particles with electron microscopy.

Serum hydrocortisone values were evaluated using a commercially available immunoassay (Dade IAC-TRI immunoassay, DPC Coat A-count cortisol kit). Results of serum hydrocortisone levels demonstrated a significant disparity initially but over time, pre- and post-collection samples correlated very closely. The CBC and serum chemistry results did not differ significantly over the course of the evaluation. No correlation with semen collection time was observed. Bacterial cultures of semen were negative. No viral particles were observed.

Over time, serum hydrocortisone levels demonstrated similar levels pre- and post-semen-collection. Reasons for this are unknown but may reflect accommodation to the procedure, diurnal or seasonal

variations, changes in reproductive status, or other unknown influences. Long term effects on semen quality are unknown. However, it has been noted with increasing frequency that the volume of sample has decreased with in-house manual semen collection. No other underlying cause of disease has been observed and no clinical illness has been observed. Further investigation of apparent accommodation by the elephant is necessary before additional conclusions can be made.

The first successful artificial insemination of an African elephant with extended semen was performed 3 yr ago at the Indianapolis Zoo, with semen obtained from the Kansas City Zoo's African bull elephant. As of this writing, the Kansas City Zoo has confirmed a natural pregnancy in one of its six African cow elephants. The due date is September, 2001.

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ASSESSMENT OF DICLAZURIL TOXICITY IN NEONATAL DOMESTIC CATS (*Felis catus*) AND INITIAL APPLICATION FOR PREVENTION AND TREATMENT OF TOXOPLASMOSIS IN NEONATAL PALLAS' CATS (*Otocolobus manul*)

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Abstract

Between 1995 and 1999, a total of 24 (12 male, 12 female) wild-caught Pallas' cats (*Otocolobus manul*) were imported into North America from Russia and Mongolia to establish a founder population of this small-sized felid species. Over the past 5 yr, breeding of these Pallas' cats has resulted in the birth of 45 kittens in 13 litters; however, more than 50% of these kittens have died within 4 mo of birth. In almost all kittens subjected to necropsy, the cause of death has been attributed to infection with the protozoal parasite *Toxoplasma gondii*. As a consequence of toxoplasmosis, only eight of the 24 potential Pallas' cat founders have surviving offspring in the captive population, with many of the original wild-caught cats approaching reproductive senescence (based on reproductive patterns derived from studbook data). If Pallas' cats are to be maintained in captivity as a self-sustaining, genetically viable population, then it is imperative that preventive and treatment modalities be established for reducing neonatal losses caused by toxoplasmosis.

One promising new drug, diclazuril, is marketed as an oral anticoccidial agent in Europe, Canada, and South Africa and has proven very effective in a number of domestic species for prevention and treatment of coccidiosis. Diclazuril also has been effective in mice for both prophylaxis and treatment of acute toxoplasmosis. Although diclazuril has established efficacy and a wide margin of safety in other species, there are no published reports of this drug being tested in any felid species. Therefore, the objectives of the present study were to (1) assess the relative toxicity of diclazuril when administered at prophylactic dosages to neonatal domestic cats and (2) begin evaluating the safety and efficacy of diclazuril for preventing and treating toxoplasmosis in neonatal Pallas' cats.

For drug evaluation in domestic cats, adult queens in the Cincinnati Zoo Center for Research of Endangered Wildlife (CREW) research colony were monitored daily for behavioral estrus and then naturally mated to one of two proven breeder males. Three females became pregnant, subsequently giving birth to a total of 13 viable kittens. Ten kittens were randomly assigned to one of two treatment groups (diclazuril or control). Beginning at 3 days after birth and then every 3 days until 8 wk of age, kittens were treated orally either with diclazuril (1 mg/kg body weight; Vecoxan, Janssen Animal Health, United Kingdom; 2.5 mg diclazuril/ml) or distilled water (0.4 ml/kg). Blood samples were collected from each kitten beginning at 10 days of age and then weekly thereafter to assess hematologic parameters. Every 3 days, kittens were weighed and measured to monitor body growth. Domestic cat data were evaluated using repeated measures ANOVA.

For initial drug assessment in Pallas' cats, neonates were treated with diclazuril at two institutions (Cincinnati Zoo and Botanical Garden and the Oakhill Center for Rare & Endangered Species). For evaluation of prophylaxis, five kittens in one healthy litter were treated with diclazuril (1 mg/kg p.o.) beginning at 7 days after birth and then every 7 days until 12 wk of age. Two of these kittens were hand-raised whereas the other three kittens remained with the dam throughout the study. To evaluate effectiveness as a treatment option, one kitten with suspected congenital toxoplasmosis was treated daily with diclazuril (10 mg/kg p.o.) beginning on the first day after birth. This kitten was the only surviving offspring from a litter of six (four stillborn kittens with the fifth kitten dying approximately 12 hr after birth) that were born to a dam with a previous history of in utero toxoplasmosis transmission.

Our preliminary results suggest that diclazuril is safe to administer at low dosages to neonatal domestic cats. Through 3 wk of age, mean (± SEM) body weight (diclazuril, 460 ± 85 g; control, 406 ± 30 g) and crown rump length (diclazuril, 22.6 ± 1.0 cm; control, 22.5 ± 0.6 cm) did not differ ($P > 0.05$) between treatment groups. Hematologic assessment revealed no differences ($P > 0.05$) over time in white or red blood cell counts or hematocrit values. Biochemical profiles also did not differ ($P > 0.05$), with similar values for serum enzymes (alanine transferase, aspartate transferase, alkaline phosphatase), total bilirubin, cholesterol, total protein, blood urea nitrogen (BUN), BUN/creatinine ratio and glucose levels. In Pallas' cats, neonatal studies have just been initiated (as of April 2001) and complete findings will not be available until mid-2001. To date, the five kittens receiving diclazuril prophylaxis appear to remain healthy whereas the single kitten receiving diclazuril as treatment for acute toxoplasmosis continues to survive and gain weight slowly (after 7 days of treatment). In conclusion, diclazuril appears to have no adverse effects when administered at a prophylactic dosage every 3 days to newborn felids. Furthermore, we anticipate that our ongoing studies may establish this drug as an effective preventive or treatment option to help reduce the devastating impact of toxoplasmosis on Pallas' cat conservation efforts.

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STRUCTURAL DIFFERENCES OF PHARYNX AND LARYNX AMONG MEMBERS OF THE FAMILY FELIDAE (MAMMALIA)

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Abstract

Evidences in literature dealing with pharyngeal or laryngeal anatomy of lynxes, big cats, and the cheetah are only scarce. Whereas morphologic features of the upper neck region are well described in the domestic cat, very few investigations examine the larynx[1] or the hyoid[2] of other members of the felids more closely. Providing anatomic data for diagnostic examination and surgical treatment we investigated upper neck regions or skulls of four jaguars (*Panthera onca*), two tigers (*Panthera tigris*), two lions (*Panthera leo*), two cheetahs (*Acinonyx jubatus*), and two Eurasian lynxes (*Lynx lynx*) by means of gross anatomic dissection, histologic staining techniques, and radiographic examination. The most important difference between domestic cats, lynxes, or cheetahs and jaguars, lions, or tigers on the other hand is, that the roaring-species reveal an elastic and collagenous ligament instead of a bony epihyoid. In the examined tigers and jaguars the epihyal ligament bridges the long distance between the delicate and curved stylohyoid and the short ceratohyoid, which, in adult individuals, is situated together with the basihyoid, the thyrohyoid, and the entire larynx far caudal from the position usual in domestic cats. The stylohyoid is connected with the cartilaginous tympanohyoid, which is situated close by the lateral wall of the bulla tympanica. In comparison with the domestic cat, the lynx, and the cheetah, the highly elastic pharyngeal wall and the soft palate of the *Panthera*-species appear elongated in caudal direction. This elongation of the pharynx and the mid-cervical position of the basihyoid and the larynx is only apparent in adults, whereas in newborn individuals of *Panthera onca* we found the basihyoid near the border between the upper neck and the intermandibular region. Together with special morphologic features of the vocal folds[1] the elongation of the pharynx seems to play an important role in sound production of roaring cats.

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HEALING AND REPAIR IN TUSK, INCISIVUM AND MAXILLA OF AN AFRICAN ELEPHANT (*Loxodonta africana*)

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Abstract

Tusk disorders are common in elephants.[1] The exposed position of this hypsodont incisor, its special shape and structure, and its use for digging, carrying and behavioral display[2] make it clear, that this tooth can easily be injured. Tusk disorders in free-ranging elephants, which play probably, from an evolutionary standpoint, a distinct role as a limiting factor, should, therefore, show a fast and complete healing. Tusk injuries of zoo animals, however, frequently require medical treatment. In order to provide information about the healing mechanisms in tusks and the surrounding bone tissue we investigated the remaining tusk and the skull of a female, approximately 40-yr-old African elephant (*Loxodonta africana*) by means of gross anatomic dissection and histologic staining techniques. This elephant had been kept at the Vienna zoo and was euthanatized in January 2000 because of severe disorders of the locomotor apparatus, which were resistant to treatment. The extra-alveolar part of its left tusk had fractured in September 1996 and the pulp cavity was opened. In the following days procaine penicillin and dihydrostreptomycin were administered intramuscularly and the distal part of the remaining tusk, which revealed a longitudinal cleft, was cut off. A distal section of the remaining pulp tissue was extracted and the pulp cavity was closed with a gauze wick drenched in lincomycin-solution. The gauze wick was removed 1 wk later. At the time of the elephant's death the pulp cavity of the left tusk appeared completely closed with secondary dentin and the alveolar part of the tusk did not show signs of a pathologic alteration. The dental pulp tissue nearly 15 cm from the distal end of the remaining tusk reveals large arteries and anisotropic collagenous fibers. The alveolar cavity of the right tusk, which was totally extirpated, is filled up with bone tissue, leaving only a small osseous duct on the bony palate. The prominence on the laterodorsal surface of the right incisivum and maxilla, which is normally produced by the dental root of a tusk, is completely reduced. The tusk of the elephant and its surrounding bony elements, therefore, reveal an enormous healing ability,[2,3] which is in striking contrast to the human tooth.

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ASSESSMENT OF DEHYDRATION AND EFFICACY OF REHYDRATION WITH SUBCUTANEOUS SALINE IN THE PIGEON (*Columba livia*)

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Abstract

Identification of key clinically applicable signs correlating directly with dehydration severity could allow clinicians to accurately define the degree of dehydration they are encountering in an avian patient at presentation, when fluid therapy is commonly initiated. Certain signs have been reported to indicate clinical dehydration, although their use in predicting severity has led to uncertainty. When delivering fluid therapy to a dehydrated debilitated bird, the risk of aspiration with oral administration and the stressful and often cumbersome maintenance of intravenous or intraosseous catheters might be avoided if subcutaneous administration were effective. Moreover, avian species may not follow the mammalian standard of 7% dehydration as the decision point for determining if subcutaneous fluids can be effectively absorbed. The purpose of this study was to assess the effect of controlled dehydration on the reliability of clinical sign development, and to determine if subcutaneous fluid administration rehydrates pigeons when dehydration progresses beyond 7.5%.

Thirty-six mixed sex pigeons were purchased from a local breeder and housed singly under controlled photoperiod, ambient temperature, and humidity. All birds had food withheld from 12 hr before, and water was provided until immediately before study. Once on study, all pigeons were deprived of water and weighed frequently until a target weight (percent dehydration) was reached, when corrected for maintenance energy losses. Target groups consisted of 0%, 4%, 7.5%, 9.5%, 11%, and 13.5% dehydration. Dehydrated birds in each group were assessed in regard to the following physical diagnostic characteristics: ulnar vein changes (diameter, shape-round or flattened, and refill time), body temperature changes (cloacal, carpal contact, and middle toe base contact), lower eyelid pinch recovery time, presence of oral mucous strings with repeated closed mouth opening, and clinical biochemical changes (hematocrit, blood urea nitrogen (BUN), albumin). Each bird had the same assessments repeated following 5% rehydration with 0.9% sodium chloride (NaCl) delivered subcutaneously divided equally into each medial leg web. Two 1-ml heparinized blood samples were collected from the medial tarsal or ulnar wing veins opposite to the site of sign assessment. After the hematocrit was determined, plasma samples for albumin (improved biuret for birds) and BUN were collected into o-ring tubes and stored at -70° C until analyzed on a Hitachi 917 chemistry analyzer.

The following clinical signs were found to be significantly influenced by dehydration: eye pinch time ($P < 0.0001$), presence of oral string(s) ($P = 0.0051$), albumin ($P = 0.0289$), and BUN ($P = 0.0031$). Carpal contact temperature ($P = 0.1349$), cloacal temperature ($P = 0.3354$), middle toe base

contact temperature (P = 0.2350), ulnar vein diameter (P = 0.0670), ulnar vein refill time (P = 0.0774), ulnar vein flattening (P = 0.0863), and hematocrit (P = 0.9344) were not reliable markers over the dehydration range studied. When 11-13.5 % dehydrated birds were compared to hydrated controls with regard to these same parameters, the same signs were significant. In addition, carpal contact temperature (P = 0.0280), ulnar vein diameter (P = 0.0010), ulnar vein refill time (P = 0.0010), and ulnar vein flattening (P = 0.0080) became significant markers, whereas, albumin (P = 0.3990) became nonsignificant. Delayed eyelid pinch time (P = 0.005), presence of oral strings (P = 0.008), ulnar vein diameter (P = 0.0010), ulnar vein refill (P = 0.0010), ulnar vein flattening (P = 0.008) and BUN (P = 0.001) were the best predictors of more severe dehydration. As previously reported, individual birds did not exhibit all dehydration signs at any one level of dehydration, necessitating the evaluation of greater than one sign during clinical assessment. Likewise, change rather than the absolute value of a parameter was often most indicative of dehydration, sometimes limiting its usefulness at presentation. Paired T testing revealed that all signs except oral string formation (P = 0.2930) were corrected toward normal by subcutaneous rehydration in birds 9.5% or more dehydrated, and in 2.5 hr or less. At all sites measured, body temperature reliably decreased in response to rehydration.

Delayed eyelid pinch time, presence of oral strings, decreased ulnar vein diameter, longer ulnar vein refill, increased ulnar vein flattening and elevated BUN were the best predictors of more severe dehydration from this study when used in combination. Dehydration up to 13.5 % percent can be readily corrected with relatively simple subcutaneous rehydration, without the need for more invasive procedures. Further work is needed in avian species to explore the use of subcutaneous fluid administration for dehydration of greater severity than reported here. However, from the present study, it appears the mammalian subcutaneous rehydration cutoff of 7.0% may not apply in avian species.

ELEPHANT BREEDING SOUNDNESS EXAMINATION FORMS AND DATABASE

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Abstract

Routine use of standardized breeding soundness examination forms for male and female elephants provides data for an inter-relational database. Through queries we may answer population-based reproductive questions essential for promoting self-sustaining populations. This poster presentation demonstrates breeding soundness examination forms and accompanying database used in a pilot project to evaluate 25 elephants.

Introduction

Asian (*Elaphus maximus*) and African (*Loxodonta africana*) elephants in North America are not self-sustaining. Within 50 yr, Asian elephants will be demographically extinct if fecundity does not increase four- to eight-fold.[5] Reproduction and juvenile survivorship in African elephants must increase significantly in the next 10 yr to promote a self-sustaining population.[4] Limited resources as well as an ever-aging population underscore the urgency to prioritize and breed reproductively sound animals. Major reasons for low reproductive rates are undiagnosed reproductive disorders in females[2] and reproductive dysfunction in males.[3] Endocrine evaluation in conjunction with ultrasound examination and behavioral assessment are needed to identify and treat causes of reproductive failure.[1] Routine breeding soundness examinations including trans-rectal ultrasound imaging, endocrine profiling, and semen analysis provide data for efficiently making breeding decisions for reproductively fit animals, as well as evaluating therapeutic interventions for reproductively unsound animals (e.g., flat-liners, poor semen quality).

Methods

A standardized approach for evaluating elephant reproductive potentials simplifies processing of data from many different animals. Information gathered from standard breeding soundness examinations, once entered into an inter-relational database, may through queries answer population based questions. Attendees at the first ultrasound workshop held by Dr. Dennis Schmitt at Riddle's Elephant Breeding Farm and Sanctuary created a breeding soundness examination (BSE) form for evaluating male and female elephants. The BSE form documents findings from trans-rectal ultrasound imaging, semen analysis, retrospective endocrine assays, and breeding history. An inter-relational database (Access-MS Office 2000) was created to organize data in multiple tables, permitting querying of data and tracking of trends.

Results

Data from more than 60 examinations of 25 elephants in North America currently populate the inter-relational database. In this presentation, we review each data element of the report form and provide related images of trans-rectal ultrasound scans and semen analyses. The utility of the breeding soundness examination forms and database will increase as we enroll more elephants. The report form may be accessed at www.senecazoo.org.

ACKNOWLEDGMENTS

Many individuals contributed to development of the Breeding Soundness Examination Form and collection of data, including all of the participants of the first, second, and third ultrasound workshops held at Riddles' Elephant Breeding Farm and Sanctuary. This project would not have been possible without Scott and Heidi Riddle promoting a unique learning environment at their sanctuary. Special recognition goes to Dr. Dennis Schmitt for his pioneering efforts to develop and teach other veterinarians the art of elephant trans-rectal ultrasound imaging of elephants. I thank April Rajca-Tiribassi, Assistant Director of Finance & Administration of University of Rochester Vivarium, for her expert knowledge used to create the database. Last but not least, grateful appreciation is extended to the many elephants we were privileged to examine in order to test drive the breeding soundness examination report forms and database with the ultimate hope of someday promoting self-sustaining populations.

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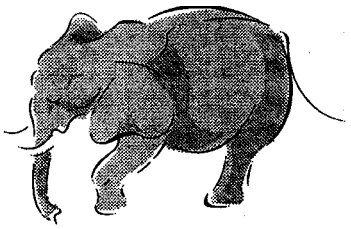

Date of Exam:_____ Institution Name:_____

Animal Name:_____ Institution Location:_____

Stud Book Number:_____ Purpose of Exam:_____

DOB:_____ Captive Born: Yes or No

Gender: Male or Female Weight (lbs.):_____

Body Condition:_____ Height (ft):_____

Contact: Free / Protected / Other Most Recent US Exam:_____

Herd Info:

Genders
Ages

Herd Comments:_____

_____

_____

Health Reproductive History:_____

_____

_____

_____

Institution Vet (Name and Phone Number):_____

Institution Vet (Email Address):_____

Institution Primary Contact (Name and Phone Number):_____

Institution Primary Contact (Email Address):_____

Female Exam

Date:_____ Animal Name:_____ Stud Book #:_____

External Exam

Vestibule/Vulva Examined: YES or NO V/V Comments:_____

Mammary Glands Examined: YES or NO MG Comments:_____

Endocrine Info

Endocrine Status: KNOWN / UNKOWN Most Recent Serum Hormone Testing Date:_____

Cycling Regularly: YES or NO If Abnormal Cycling: Flatline

Current State of Cycle:_____ Irregular

Other:_____

US Exam

Sonographer:_____ US Machine Make and Model:_____

Urethra Examined: YES or NO mHz:_____

Comments:_____

Bladder Examined: YES or NO mHz:_____

Comments:_____

Vagina Examined: YES or NO mHz:_____

Vagina Mucus: YES or NO

Comments:_____

Cervix Examined: YES or NO mHz:_____

Comments:_____

Uterus Examined: YES or NO mHz:_____

Uterine Increased Vascularity: YES or NO

Comments:_____

Ovaries Examined: YES or NO mHz:_____

L Ovary Height x Width: _____X_____ R Ovary Height x Width: _____X_____

Comments:_____

Probe Comments:_____

_____

_____

Overall Comments:_____

_____

_____

Male Exam and Semen Collection

Date:_____ Animal Name:_____ Stud Book #:_____

Engorged Penis Circumference:_____ External Base of Penis:_____

contractions before ejaculate:_____ # contractions before ejaculate:_____

Total # Contractions:_____ Total Time:_____

Urinate during collection: YES or NO Ejaculate Volume:_____

Sperm Count (x106/ml)_____ Sperm motility%:_____

% live:_____ Motility status (0-5):_____

% morphologically normal:_____ pH:_____

Semen Collector(s):_____ Collection Method:_____

Semen Collection Comments:_____

Ultrasonographer:_____ US Machine Make and Model:_____

Urethra Examined: YES or NO mHz:_____ Bladder Examined: YES or NO mHz:_____

Comments:_____ Comments:_____

Prostate Examined: YES or NO mHz:_____

L Prostate Height x Width: _____X_____ R Prostate Height x Width: _____X_____

Comments:_____

Seminal Vesicle Examined: YES or NO

mHz:_____

L Seminal Vesicle Height x Width: _____X_____ R Seminal Vesicle Height x Width: _____X_____

Comments:_____

L Seminal Vesicle Ventral Epith Thickness: _____ R Seminal Vesicle Ventral Epith Thickness: _____

Ampulla Examined: YES or NO mHz:_____

L Ampulla Height x Width: _____X_____ R Ampulla Height x Width: _____X_____

Comments:_____

Testes Examined: YES or NO mHz:_____

L Teste Height x Width: _____X_____ R Teste Height x Width: _____X_____

Comments:_____

Probe Comments:_____

_____

_____

Overall Comments:_____

_____

_____