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## A RETROSPECTIVE STUDY OF DISEASE IN MONITOR LIZARDS (*Varanus spp.*)

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### Abstract

Retrospective studies of case files pertaining to monitor lizards have been limited to specific disease processes.<sup>1-3</sup> To the author's knowledge, a retrospective study specifically addressing the diseases of monitor lizards has not been published. This report summarizes the disease processes in 333 monitor lizards representing 39 species submitted to Northwest ZooPath from 1995-2007. Infectious/inflammatory disease processes (73%) and deposition disorders (37%) were the most prevalent disease classes. The prevalence of cardiovascular, degenerative, nutritional, neoplastic, traumatic and reproductive disease was approximately equal (10-13%).

Within the various classes the most common diseases were as follows: cardiovascular (47 cases): cardiac fibrosis (21%); degenerative (37 cases): chronic renal disease (24 %) and chronic hepatopathies (24%); deposition disorders (127 cases): gout (33%), hepatocellular lipidosis (32%), and metastatic mineralization (23%); nutritional (37 cases): inanition (76%); neoplastic (41 cases): soft tissue sarcomas (20%), squamous cell carcinoma (17%), and lymphoma (12 %); inflammatory diseases (243 cases): bacterial (44%), unknown cause (35%), parasitic (15%); and reproductive (40 cases): yolk coelomitis (33%), oophoritis (30%), and follicular degeneration/rupture (20%).

Species with higher than average prevalence of specific diseases were as follows: Asian water monitor (*Varanus salvator salvator*) (6 cases): osteomyelitis (50%). Black throated monitor (*V. albigularis ionidesi*) (13 cases): meningoencephalitis and myenteric ganglioneuritis (23%). Black tree monitor (*V. beccarii*) (29 cases): various neoplasia (24%). Crocodile monitor (*V. salvadorii*) (39 cases): metastatic mineralization (21 %). Desert Monitor (*V. griseus*) (12 cases): osteomyelitis (34%). Dumeril's monitor (*V. dumerilii*) (3 cases): gout (100%). Green tree monitor (*V. prasinus*) (25 cases): sepsis (36%). Komodo dragon (*V. komodoensis*) (15 cases): Amyloid-like deposition disorder (38%), metastatic mineralization (31%), yolk embolism (31%), various female reproductive disorders (40%). Peach throated monitor (*V. jobiensis*) (6 cases): hepatic lipidosis (50%), gout (50%). Savannah monitor (*V. exanthematicus*) (52 cases): cryptosporidiosis (10%), various neoplasms (20 %). Storr's monitor (*V. storri*) (5 cases): emaciation (40%), hepatic lipidosis (40%). "Water" monitor (*V. Salvator*) (11 cases): gout (36 %). White throated monitor (*V. albigularis albigularis*), (5 cases): gout (40%), inanition (40%). Rough necked monitor (*V. rudicollis*) (15 cases): various female reproductive disorders (40%).

Diseases of special concern for which the etiology, significance to conspecifics/penmates, or zoonotic potential was unknown, or for which the disease presentation or histologic lesion was considered unique, where as follows: Degenerative encephalomyelopathy (five cases),

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ganglioneuritis/meningoencephalitis (five cases), hypertrophic osteopathy (one case), megacolon (two cases), and nonsuppurative myocarditis (three cases).

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## ENDOSCOPIC DETERMINATION OF GENDER IN NEONATE AND JUVENILE CHELONIANS

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### Abstract

There are nearly 300 species belonging to the order Testudines. Over half are listed by CITES as either threatened or endangered, many are maintained in captivity as pets, zoological exhibits or research animals, and a few are farmed for commercial purposes. The gender of most species is determined by temperature dependent sex determination during incubation, with males predominating at lower and females at higher temperatures (although genetically-determined gender has been documented for a couple of species).<sup>1,2,5-13</sup> Unfortunately, precise incubation parameters have only been determined for a handful of species. For most hatchlings gender will not be reliably known until secondary characteristics become obvious which may take several years. This delay in identifying gender prevents the refinement of incubation practices, and may result in gender-biased clutches. Such phenotypic bias could have obvious deleterious effects on head-start or conservation programs, and so a means of confirming gender in neonates and hatchlings is beneficial.

Unlike most birds there is no commercially available DNA probe to identify gender for chelonians. However, endoscopy has proven effective in determining gender in monomorphic birds, and more recently, similar techniques have been developed for reptiles.<sup>3,4</sup> Under anesthesia, prefemoral insertion of 1.9 mm or 2.7 mm rigid telescopes combined with CO<sub>2</sub>, air, or sterile saline insufflation enables visualization of the immature reproductive tract, even in hatchling turtles. Given the small size of the animals involved, a single injection can often achieve sufficient insufflation. In some species, the ovary or testis may be differentiated at a very early age, especially when the terminal telescope lens is held against the gonad to facilitate a 15-20x magnification of the tissue. However, in those species that appear to undergo significant post-hatching gonadal differentiation, the identification of the immature oviduct can also lead to accurate gender identification. Endoscopy offers a fast, reliable and immediate means of determining gender that can be used to monitor and adjust incubation parameters more quickly than waiting for secondary characteristics to develop. In addition, the advent of portable equipment makes this system appropriate for use in the field as well as the hospital.

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## AN OVERVIEW OF ERYTHROCYTIC IRIDOVIRUSES IN REPTILES AND FIRST MOLECULAR CHARACTERIZATION OF AN ERYTHROCYTIC IRIDOVIRUS

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### Abstract

Erythrocytic inclusions ultrastructurally consistent with iridoviruses have been identified in reptiles, amphibians and fish. In reptiles, these inclusions were formerly misidentified as parasitic protozoa and named *Toddia* sp. and *Pirhemocytos* sp. Clinically significant anemia has been documented in association with erythrocytic viruses in several squamate species, and experimental infections have been fatal.<sup>1,2</sup> These viruses have never been further characterized. A wild peninsula ribbon snake (*Thamnophis sauritus sackenii*) in Florida was found to have hypochromic erythrocytes containing two different types of inclusions: purple granular inclusions, and pale crystalline inclusions that were round or rectangular in shape. Transmission electron microscopy revealed homogenous albuminoid vacuoles and enveloped particles morphologically consistent with a member of the Iridoviridae. Histopathology of the animal revealed necrotizing hepatitis. Consensus polymerase chain reaction (PCR) was used to amplify a region of iridoviral DNA dependent DNA polymerase for sequencing. Bayesian and maximum likelihood phylogenetic analysis found that this virus was distinct from other known iridoviral genera and species, and may represent a novel genus and species.

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## HYPOVITAMINOSIS A IN A CAPTIVE COLLECTION OF AMPHIBIANS

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### Abstract

#### Overview

Hypovitaminosis A is a disease of malnutrition or dysnutrition. There have been references to this disease for thousands of years and the ancient Egyptians treated xerophthalmia (night blindness) with liver from cattle or poultry that is rich in vitamin A.<sup>1</sup> Today hypovitaminosis A is routinely diagnosed in humans, domestic livestock, and recently with more regularity in captive amphibian species.<sup>1</sup>

#### Case Report

Over a 4-mo period there was a significant increase in the number of amphibian clinical cases submitted to the hospital. This was unusual as this group of animals had been relatively problem free in the past. On physical examination clinical signs included, inability to use the tongue, gastrointestinal bloat, and dermal ulceration, static growth rates in juveniles, as well as septicemia and acute death. The following species were affected, Yellow and blue poison arrow frog, (*Dendrobates tinctorius*), New Guinea tree frog (*Litoria infrafrenata*), African foam nesting frog (*Chiromantis xerampelina*), Puerto Rican Crested Toad (*Peltophryne lemur*).

This etiology of clinical signs was traced to a possible mixing error in the powdered amphibian supplement used to dust invertebrate food items fed to the frogs. The base of the amphibian nutritional supplement normally has a concentration of 230,000 IU/kg of vitamin A. Crickets fed and dusted with this supplement contain approximately 4,000 IU/kg of vitamin A (3/4' crickets). It was determined by analysis that the "new" supplement being used during the time period of this case report contained only 44,000 IU/kg of vitamin A which was roughly 20% of the concentration normally provided or 800 IU/kg of vitamin A (3/4' crickets).

The first clinical signs were noted approximately 3-4 mo after the "new" supplement was fed. A small number of amphibians (10 individuals) showing clinical signs were currently being treated with vitamin A, however with this information, the remainder of the collection (790 frogs) were placed on prophylactic treatment of vitamin A as described below.

#### Diagnostic Sampling

Blood was collected from the New Guinea tree frogs and 0.3-0.4 ml of plasma was obtained and submitted to the Michigan State University Diagnostic Center for Population and Animal Health

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for vitamin A analysis. Most of the other frogs were too small (< 20 g) to be able to collect the required volume of plasma for analysis.

### **Treatment**

The total number of amphibians to be treated was exceeded 800 individuals, which included over 600 juvenile African foam nesting frogs. Frogs with clinical signs were treated based on the severity of signs using the following protocols:

#### Drug

Aquisol A (Mayne Pharma (USA) Paramus NJ, 07652) 50,000 USP/ml  
1:10 dilution = 5,000 USP/ml (with sterile saline)

#### Dose

Small frog dose 1 drop (< 20 g frog) = 50-100 USP per dose  
Large frog dose 2 drops (20-120 g frog) = 100-150 USP per dose

#### Frequency

- 1) No clinical signs – Topically, once a wk for 2 mo
- 2) Mild clinical signs – Topically, every 3 days for 2 mo
- 3) Severe clinical signs – Injection for first does, then topical, every 3 days for 2 mo

### **Results**

Prior to treatment, two blood samples from the New Guinea tree frogs were analyzed and were found to have concentrations of vitamin A of, 11 and 20 ng/ml. Plasma vitamin A levels drastically increased from 5-20 ng/ml to 142- 217 ng/ml over the course of the 2 mo treatment. However in many cases clinical improvement was seen within days. Skin lesions were seen to be improving within 2 wk but many required up to 2 mo to completely heal. Frogs with “short tongue syndrome” also showed clinical improvement.<sup>2</sup> Acute cases of short tongue syndrome successfully resolved with topical treatment and supportive care in the form of hand feeding and routine weighing.

Within 3 wk of cessation of treatment, plasma concentrations of vitamin A ranged from 46-295 ng/dl. Currently a published value for serum vitamin A in wild frogs or toads could not be located. Samples from wild toads will be obtained this summer to determine baseline levels of plasma vitamin A concentration.

Topical therapy appears to increase plasma levels of vitamin A, and to produce improvements in the clinical signs of hypovitaminosis A; however duration of therapy has not been established. A comprehensive nutrition plan that includes vitamin A supplementation (minimum of 230,000 IU vitamin A/ kg of supplement) should be implemented in captive anuran collections. Current clinical trails for dose and duration of therapy are ongoing.

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## **REPTILE TRAINING AND ENRICHMENT: A USEFUL TOOL FOR THE ZOO VETERINARIAN**

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### **Abstract**

Both the words training and enrichment appear to have been lost when used in conjunction with the words reptile or amphibian. Even though enrichment is attempted in many species, reptiles are often kept in small sterile environments. Enriching captive reptiles will allow them to exhibit their natural array of behaviors. Similarly, we rarely hear the word training used in association with reptiles and amphibians. When veterinarians hear the word training, we think of large mammals such as hoof stock or primates that have been trained to allow for passive diagnostic sampling, ultrasound and radiographs. These same techniques can be used to train reptiles and amphibians to assist with medical procedures and to facilitate everyday keeper/animal interactions.

### **Enrichment**

Enrichment can be defined as a process for improving or enhancing animal environments and care within the context of their inhabitants' behavioral biology and natural history.<sup>1</sup> It is a dynamic process in which changes to structures and husbandry practices are made with the goal of increasing behavioral choices available to animals and drawing out their species-appropriate behaviors and abilities, thus enhancing animal welfare.

The enrichment framework developed at Disney's Animal Programs provides us with a process to ensure that our enrichment program meets the needs of the animals, and provides them with the opportunity to experience enhanced animal welfare. Animal welfare involves both the physical health of the animals (e.g., preventing and treating illnesses and injuries), as well as their psychologic well-being. As an important aspect of welfare, an animal's psychologic well-being is influenced by whether it can:

- Perform its highly motivated behaviors;
- Respond to environmental conditions using its evolutionary adaptations;
- Develop and use its cognitive abilities;
- Effectively cope with challenges in its environment.

Thus keeping a snake in a plastic box with newspaper and a water bowl is not sufficient and we should attempt to provide a more complex environment to watch and enjoy the animals we keep in captivity. Of course balancing proper husbandry, thermoregulation and veterinary concerns becomes more difficult, however I think it is our obligation to do this for the animals we keep in captivity.

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For more comprehensive information please take a look at [www.animalenrichment.org](http://www.animalenrichment.org) for a full description and how to develop and enrichment plan.

## **Training**

When I think of animal training I think of the old parrot shows or circuses where animals were trained to complete a variety of unnatural acts for our entertainment. Training now has a different context which includes “training” animals to exhibit a variety of behaviors for husbandry, education, and yes, entertainment purposes. Sometimes we influence (train) animals’ behavior inadvertently through our actions, our husbandry routines, or through other stimuli present in the captive environment. In effect, animal care staff are always training and they need to be aware of that fact. Training is all about associations. The key to an optimal captive environment is to facilitate animals’ opportunities to make associations that enhance their well-being.

Following the “SPIDER” system of training. Setting Goals, Planning, Implementing, Documenting, Evaluating and Readjusting, it is possible to train reptiles for a variety of behaviors. For example at Disney’s Animal Kingdom Nile crocodiles (*Crocodylus niloticus*) are trained to shift and crate on command, Dendrobatid frogs (*Dendrobatid* spp.) will station on a weigh scale, Komodo dragons (*Varanus komodoensis*) will target, follow a laser pointer, station in a crate and station to have their nails trimmed.

We suggest here that in order to select the most effective and appropriate techniques to shape (train) behavior, it is necessary to consider three things:

- 1) The animal’s natural history – it’s important to consider the animal’s predispositions. For example, it may make more sense to ask an arboreal animal to station off the ground/on a perch.
- 2) The animal’s individual history – it’s important to consider the early rearing/life experiences of the animal being trained. For example, an animal that’s imprinted on humans may be trained substantially differently than a wild-caught animal brought in as an adult.
- 3) The animal’s function or “role” in your collection – the animal may be in the collection as part of a breeding program or part of an education program. The type of training and your level of interactions with that animal may differ depending on the function this animal serves in your collection.

Of course everyone has different goals for training and as a veterinarian our goals may differ from private owners and zoos alike. The scope of this talk cannot scratch the surface of how to implement a training program however to get more information on this topic go to [www.animaltraining.org](http://www.animaltraining.org) which has a full plan to start your own training program.

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## USE OF THE COMBINATION EMODEPSIDE-PRAZIQUANTEL IN REPTILES: A NEW DRUG FOR ENDOPARASITE MANAGEMENT?

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### Abstract

Many anthelmintic compounds have been used in herpetologic medicine. However treatment of nematodiasis in reptiles can be very challenging, especially in stressed, non cooperative or dangerous species. Recently, a combination of emodepside and praziquantel (Profender<sup>®</sup>, Bayer Health Care group, Leverkusen, Germany) for topical application may be a promising alternative treatment option for the veterinarian. This presentation summarizes all the current information on the various nematocides known to be efficient in reptiles and describes the mode of action of Profender<sup>®</sup> in the cat as well as its potential use in reptiles.

### Introduction

Intestinal helminths, pentastomids, intestinal and blood protozoans are common parasites of captive or wild reptiles (reptiles may serve as definitive, intermediate, accidental or paratenic hosts).<sup>1,3,4,6-8</sup> Generally, these parasites are non-pathogenic or commensal in wild animals. However, they may become pathogenic when present in high concentrations or if the host is weakened by inadequate environmental conditions in captivity (Table 1).

Most deworming protocols for reptiles (suffering from nematodiasis, cestodiasis, trematodiasis, and acanthocephalidiasis) originated from domestic animal medicine experiences. Over time, some of these compounds were tested and became empirically recommended because of their efficacy and safety (Table 2).<sup>1-3,5-9,12</sup> Many references promoting the use of these anthelmintic compounds in reptiles are currently available.<sup>1,6-9</sup>

### Emodepside and Praziquantel

Since the 1960s, there have predominantly been three classes of broad-spectrum anthelmintic drugs used to control helminths in mammals: benzimidazole/probendimidazole (such as fenbendazole and febantel), tétrahydropyrimidines/imidazothiazoles (such as pyrantel, morantel/lévamisole), and macrocyclic lactones (such as ivermectine and moxidectine). Emodepside belongs to a relatively new class of anthelmintics (depsipeptides) which achieve their antiparasitic effect against nematodes by a novel mechanism of action. Emodepside acts at the neuromuscular junction by stimulating presynaptic receptors. Emodepside has been shown to be highly effective against a number of nematodes affecting a wide range of animal species. Its efficacy against gastrointestinal nematodes of cats has been extensively studied. Additional studies in sheep, cattle, horses, and dogs have shown emodepside to display effective

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anthelmintic activity against trichostrongylids, ascarids, hookworms, trichocephalids, large and small strongyles as well as respiratory strongyles. Importantly, emodepside was reported to show some efficacy against nematodes resistant to ivermectine, benzimidazoles, and levamisole in sheep, and nematodes resistant to ivermectine in cattle.

The anthelmintic activity of praziquantel was discovered in 1972 in the laboratories of Bayer AG, and was followed up at the German pharmaceutical manufacturer E. Merck. The mechanism of action is through the induction of tetanic muscular contraction and vacuolisation of the integument in cestodes and trematodes. The severe contractions observed in cestodes appear within seconds after exposure to praziquantel and this is soon followed by the loss of sucker concavity. As rapidly as 5 min after exposure, major changes in the syncytial integument are present in the area of the scolex and strobila. Integumental lesions are likely the result of the interaction of praziquantel with cutaneous phospholipids and proteins. Ultimately, these changes lead to the reduction of glucose intake and accelerated depletion of energy reserves. Praziquantel is a broad-spectrum anthelmintic drug used in human and veterinary medicine; it ensures reliable control of trematode infestations [blood fluke, liver fluke (except *Fasciola hepatica*), and lung fluke] and all stages of intestinal growth in cestodes.

### **Use of Profender® in Reptiles**

A new treatment for internal parasites was shown to be safe and effective when administered to a wide range of reptile species.<sup>10</sup> Marketed under the tradename Profender®, this preparation is a unique combination of two active substances, emodepside and praziquantel: emodepside comes from a new class of active anthelmintic substances, while praziquantel, a convenient spot-on formulation for cats, controls cestodes.

Positive results were seen after administration of Profender® in a wide range of reptiles (snakes, aquatic turtles, agamids, varanids, and geckonids) found to be harboring high concentrations of nematodes belonging to the families Oxyridae, Ascaridae, Strongylidae, Trychostrongylidae, and Capillaridae.<sup>10</sup> Because of the thick *stratum corneum* of the epidermis of reptiles, it was necessary to administer four times the dosage of Profender® compared to that used in cats; approximately 0.56 ml for an animal of 1 kg body weight (equivalent to 22 drops/kg or 2 drops/100 g body weight).<sup>10</sup> In a published report, it was recommended by the authors to apply the product to areas where the epidermis is relatively thin (snakes: gular region or between scales; lizards: under armpits or skin folds of the groin area; turtles: prefemoral and gural fossa).<sup>10</sup> In one report, dosages of 15, 30 and 50 times greater than those recommended for cats were administered to a variety of reptile species (snakes, geckos, and anolids) with no visible adverse effects.<sup>10</sup>

Profender® is distributed in three different sizes as single-dose pipettes (spot-on solution for small, medium, and large cats respectively). They all contain the same concentration of active ingredients. Only the dose volumes dispensed are different. The product has a shelf life and is effective for several weeks after opening if properly sealed

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This new treatment method and product may find a wide range of applications in herpetologic medicine: the “spot-on” administration is relatively simple, less traumatizing and reduces the stress generated by repeated orogastric intubation in “shy” lizards and turtles. Despite the initial encouraging results, caution in use of this medication should be exercised. Further research should be undertaken in a wide range of reptile species to verify the safety and efficacy of this promising drug, especially in affected animals.

**Note:** Toxicosis associated with fenbendazole (Panacur<sup>®</sup>) has been reported by Neiffer et al.<sup>11</sup> In this study, six male Hermann’s tortoises (*Testudo hermanni*) were treated orally with two 5-day courses of fenbendazole 2 wk apart at a dosage of 50 mg/kg. In addition to health monitoring and stool examination, blood samples were collected to assess hematologic and plasma biochemical changes before, during and after the treatment period. Although the tortoises remained clinically healthy, blood analysis revealed several changes such as transient heteropenia, hypoglycemia, hyperuricemia, and hyperphosphatemia. Therefore, the authors recommended to 1) determine the hematologic and biochemical status of reptiles prior to administer fenbendazole and 2) monitor hematologic and biochemical parameters of treated animals.

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**Table 1. Helminths affecting reptiles.**<sup>3,4</sup>

Parasites	Hosts	Target Organ	Pathogeny	
Trematodes	Monogenean ( <i>Aspidogaster</i> , <i>Monogenea</i> )	Aquatic turtles	Digestive tract Blader, Nostrils Oropharynx Oesophagus Oropharynx	None
	Digenean ( <i>Renifer</i> , <i>Spirorchis</i> , <i>Styphlodora</i> )	Snakes Aquatic turtles	Oesophagus Trachea Lungs Kidneys	Pneumopathy Vascular disorder Nephropathy
Cestodes	( <i>Proteocephalus</i> , <i>Spirometra</i> , <i>Bothridium</i> , <i>Bothriocephalus</i> )	Varans Snakes Aquatic turtles	Digestive tract Liver Coelomic cavity	Subcutaneous parasitic cysts
		Terrestrial turtles	Subcutaneous Tissues	Enteropathy
Nematodes	Oxyurids ( <i>Oxyurus</i> sp.)	Lizards Turtles	Large intestine	None
	Ascarids (e.g., <i>Angusticaecum</i> )	Lizard	Small intestine	Gastroenteritis
		Turtles	Lungs	Pulmonary lesions
	Rhabdiasids ( <i>Rhabdias</i> , <i>Strongyloides</i> )	Snakes	Oesophagus Stomach	Secondary gastritis (larvae)
		Lizards	Small intestine Lungs Trachea	Pneumopathy Cutaneous lesions (larvae)
	Strongylids ( <i>Kalicephalus</i> sp.)	Snakes	Small intestine	Enteritis
		Aquatic turtles		
Filarids ( <i>Foleyella</i> , <i>Cardianema</i> )	Lizards			
	Turtles	Circulatory System	Oedema Vascular necrosis	
Trichurids ( <i>Capillaria</i> , <i>Eustrongyloides</i> )	Snakes	Small intestine	Larval migrations	
	Crocodiles			
Spirurids ( <i>Dracunculus</i> , <i>Spirura</i> )	Lizards			
	Turtles	Subcutaneous Tissues	Pustulous dermatitis	
Acanthocephalids ( <i>Acanthocephalus</i> sp.)	Snakes	Small intestine		
	Aquatic turtles	(Aquatic turtles) ; Mesentery Viscera	Subcutaneous nodules (cysts)	
	Terrestrial turtles			

**Table 2.** Anthelmintic compounds for control of internal parasites of reptiles.<sup>1-3,5-8,12</sup>

Molecule	Posology (mg/kg)	Frequency	Method of Administration	References
Albendazole	50	1 ×	p.o.	Stein, 1996
	25	1 ×/day for 7 days	p.o.	Klingenberg, 1996
	50	1 ×/day for 3-5 days	p.o.	Gillespie, 1994
Fenbendazole		1 ×/day for 2 days		Boyer, 1992
	100	1 ×/wk for 3 wk	p.o.	Messonnier, 1996
Ivermectin	0.2 (except chelonian)	1 × 2 ×/day for 15 days	per cloacal	Innis, 1995
			s.c, i.m.	Barten, 1993
	5 (chelonian)-10 (Squamata: snakes, lizards)	2 ×/day for 15 days		Jacobson, 1993
Levamisole			s.c.	Wilson, 1993
	10-20	1 ×		Klingenberg, 1996
Mebendazole	50	1 ×	s.c, i.m, intracoelomic	Jenkins, 1991
	20-100	2 ×/day for 15 days	p.o.	Zwart and Ham, 1972
Milbemycin	0.25-0.50	1 ×	s.c, i.m, intracoelomic	Divers, 1999
			s.c. (us) p.o. (eu)	Bodri et al, 1992
Oxfendazole	68	2 ×/day for 15 days	p.o.	Divers, 1999
		2 ×/day for 14 days		Jenkins, 1992
Praziquantel	8		s.c, i.m, p.o.	Barten, 1993
	5-10	2 ×/day for 14 days		Jacobson, 1993
Pyrantel pamoate	5	2 ×/day for 14 days	p.o.	Klingenberg, 2000
			p.o.	Frye, 1994

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## RENAL DISEASE IN CAPTIVE FROGS: A RETROSPECTIVE STUDY OF AMPHIBIAN HISTOPATHOLOGY

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### Abstract

Pathology reports from Johns Hopkins University's veterinary pathology database were examined to determine the prevalence and types of kidney disease in amphibians. The submitted amphibian cases originated from in-house laboratory animal research facilities, the National Aquarium in Baltimore (NAIB) and the Maryland Zoo in Baltimore (MZIB). Kidney lesions were present in 150 of 408 (36.8%) cases in the database. Anurans with renal disease from NAIB and MZIB were examined in more detail. This subset was comprised of multiple frog species from NAIB submitted in 2006 and 2007 (26 cases) and Panamanian golden frogs (*Atelopus zeteki*) from MZIB submitted from 2001 to 2007 (37 cases). Within this subset of 63 cases, the most common morphologic diagnosis was interstitial nephritis (42.9%), followed by mineralization (30.2%), tubular degeneration (17.5%), tubular dilation/ectasia (15.9%), protein casts (14.3%), unqualified nephritis (14.3%), and tubular cysts (11.1%). Concurrent skin disease, typically bacterial or fungal dermatitis, was present in 49.2% of animals. Correlation of ante-mortem findings with histology findings showed 15 animals (25.4%) to have edema/hydrocoelom. Twelve of these 15 frogs (80%) had tubular dilation or degeneration. Among the frogs that showed mineralization in the kidneys, 26.3% were reported to have experienced hind limb paralysis/paresis. No clinical correlations could be drawn in regard to the occurrence of tubular cysts. The pathogeneses of these histopathologic findings are currently not well understood and further investigation into the role of renal disease in amphibian mortality and morbidity is warranted.

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## CRYPTOSPORIDIOSIS IN REPTILES: DIAGNOSIS AND THERAPY

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### Abstract

Cryptosporidiosis is an emerging disease in reptiles. In past years, prevalence of this protozoal infection has risen, increasing the need for practical methods of diagnosis and therapy. Some methods for diagnosing mammalian cryptosporidiosis can be adopted for use in reptiles and some can not.

In a long-term study, fecal samples from 30 infected geckos of the species *Eublepharis macularius* were tested with various methods such as immunofluorescence, koproantigen ELISA (*ProSpecT*®, Remel), PCR, immunochromatographic Striptests (*Crypto-Strip*, CORIS BioConcept) and Karbol-fuchsine-stain. These individuals were kept under identical quarantine conditions and treated with different medications, including azithromycine, spiramycine, metronidazole, nitazoxanide, paromomycine and preparations containing colostral antibodies or herbal extracts (thymol and creosote).

In combination with a direct detection technique (Karbol-fuchsine-stain) the ELISA has proven to be a sensitive diagnostic test which can be performed in a clinical practice setting. These results correlate with results from prior studies.<sup>1-3</sup> According to the manufacturer's statements this ELISA can detect 20 ng/ml of cryptosporidium specific antigen (CSA). Other methods were ineffective and/or demanding unusual equipment. Results from the PCR evaluation are pending.

Most drugs used were ineffective towards the elimination of the parasites. Paromomycine (100 mg/kg for 7 days then once every 7 days) did show effectiveness in resolving clinical symptoms and caused negative testing of fecal samples. If treatment with paromomycine was not continued fecals were positive after 6 wk and symptoms recurred. Individuals treated with the other named agents including nitazoxanide (25 mg/kg for 7 or 28 days) remained positive for *Cryptosporidium*. Results from treatments with high doses of nitazoxanide including the blood chemistry assay for measuring renal pathologic side-effects are pending.

With further study of cryptosporidiosis and evaluation of available diagnostics, treatment regimes and disinfection methods, a standard regime for managing cryptosporidiosis can be developed. A useful web site on *Cryptosporidia* can be found at [www.cryptosporidien.de](http://www.cryptosporidien.de) with English translation.

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## AN EPIZOOTIC IN SULAWESI TORTOISES (*Indotestudo forstenii*) CAUSED BY A NOVEL SIADENOVIRUS

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### Abstract

The Sulawesi (*Indotestudo forstenii*) or Forsten's tortoise (name no longer valid but still commonly used) inhabits the islands of Sulawesi and Halmahera in Indonesia and is currently listed in CITES appendix II. A group of *Indotestudo forstenii* (a large percentage of which were in poor health) was obtained by the Turtle Survival Alliance and subsequently placed in multiple facilities where veterinary care was readily available. Clinical signs included anorexia, lethargy, mucosal ulcerations and palatine erosions in the oral cavity, nasal and ocular discharge, and diarrhea.

Initial diagnostic tests included physical examination, fecal testing for parasites, complete blood count and plasma biochemistries, mycoplasma serology, and polymerase chain reaction (PCR) testing for intranuclear coccidia and chelonian herpesvirus. Tissue samples from animals that died were submitted for histopathology. Samples from the cloaca and various organs were submitted for bacterial culture and sensitivity. Treatment included the administration of antibiotics, antiparasitic medications, parenteral fluids, and nutritional support.

The histopathologic evaluation showed severe systemic inflammation and necrosis associated with intranuclear inclusions consistent with a systemic viral infection. Fecal testing results and histopathologic findings revealed intestinal and hepatic amoebiasis in some animals. *Aeromonas hydrophila* and *Escherichia coli* were cultured from multiple organs. The mycoplasma serology

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and PCR results for intranuclear coccidia and chelonian herpesvirus were negative. Polymerase chain reaction testing of tissues and choanal swabs from affected animals were positive for an adenovirus, which was characterized as a novel adenovirus of the genus *Siadenovirus*.

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## ASSESSMENT OF FECAL VIRAL SHEDDING AS AN ADJUNCT DIAGNOSTIC TEST DURING QUARANTINE OF SNAKES

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### **Abstract**

Negative staining electron microscopy (EM) of feces is an underutilized methodology for testing snakes in quarantine for viruses. Polymerase chain reaction (PCR) testing may be performed as follow-up on positives. This testing was initiated to allow viral screening in snakes too small to safely collect a blood sample for paramyxovirus serology, with the rationale that paramyxovirus presence in feces could be significant.

EM examination was adopted by the Jacksonville Zoo and Gardens as a trial quarantine screening test. Fecal samples were collected from snakes in quarantine and frozen in two aliquots at  $-80^{\circ}$  C. One aliquot was submitted to Kissimmee (Florida) Diagnostic Laboratory for EM. The second sample was retained for PCR to discern the identity of the virus. In snakes of sufficient size for blood sampling, paramyxovirus serology was performed as well.

Thirty-eight samples were screened from 18 species. Although no paramyxovirus was identified on EM, an adenovirus, a coronavirus and a parvovirus were seen. The adenovirus proved to be a novel atadenovirus by PCR at the University of Florida, College of Veterinary Medicine. No PCR was available for parvovirus. The coronavirus was PCR negative, although this assay has not been validated for any reptile coronavirus to date. Each of the respective snakes in which virus was identified have died and on necropsy had pathology grossly and histopathologically.

This testing methodology has potential for identifying individuals with viral infections during quarantine or as clinical concerns. This information can be used as a basis for management decisions.

### **ACKNOWLEDGMENTS**

The authors wish to thank Dr. Elliott Jacobson for his inspiration to add to the database of information relative to viral diseases and his recommendation to utilize negative staining EM. Also, thanks are extended to Woody Fraser and his staff at Kissimmee Diagnostic Laboratory, Florida Department of Agriculture and Consumer Services, Division of Animal Industry for their electron microscopy work and April Childress at the University of Florida for her assistance with the PCR.

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## DIAGNOSIS AND SURGICAL TREATMENT OF A CHIARI I-LIKE MALFORMATION IN AN AFRICAN LION (*Panthera leo*)

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### Abstract

A 13-mo-old intact male African lion (*Panthera leo*) presented with a 3-mo history of lethargy, ventral flexion of the neck, abnormal vocalization, and ataxia. Hemogram and serum biochemistries were within normal limits except hypokalemia (2.7 mEq/L) and hypochloridemia (108 mEq/L).<sup>5</sup> When no improvement was noted with oral potassium gluconate supplementation, a computed tomography scan was performed of the brain and skull and no abnormalities were noted. However, magnetic resonance imaging detected occipital bone thickening, crowding of the caudal cranial fossa with cerebellar compression and herniation, and cervical syringohydromyelia which was consistent with a Chiari I-like malformation. Foramen magnum decompression was performed to relieve the compression of the cerebellum. The animal recovered well with subsequent resolution of clinical signs. Hypovitaminosis A has been proposed previously as the underlying etiology for this malformation in lions with similar clinical presentations.<sup>1-4,6,7</sup> This lion's serum and liver vitamin A concentrations were low (100 ng/ml and 25.31 µg/g, respectively) as compared to concentrations reported for domestic carnivores and suggest hypovitaminosis A as the underlying cause of this animal's Chiari I-like malformation.

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**VITAMIN AND MINERAL COMPARISONS BETWEEN ZOO-BASED AND FREE-RANGING KOALAS (*Phascolarctos cinereus*) AND THE IMPLICATIONS FOR THE DEVELOPMENT OF HIP AND SHOULDER DYSPLASIA**

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**Abstract**

Hip and shoulder dysplasia is a prevalent problem in the San Diego Zoo koala colony. While the cause of the dysplasia appears genetic, abnormal bone development due to nutritional insufficiencies needed investigation. It is unknown if vitamin D deficiency occurs in koalas managed indoors without access to direct, unfiltered sunlight. Few plants have significant concentrations of vitamin D<sub>2</sub> and D<sub>3</sub> and therefore koalas may be reliant on exposure to UVB light. Serum samples from 22 zoo-based and 19 free-ranging koalas were analyzed for calcium, phosphorus, and vitamin D. Calcium values differed between zoo-based and free-ranging koalas [means of 10.6 and 10.3 mg/dl, respectively ( $p = 0.047$ )]. Phosphorus values differed between zoo-based and free-ranging koalas [means of 3.48 and 5.25 mg/ml, respectively ( $p < 0.0001$ )]. Initial testing for 25-dihydroxyvitamin D<sub>3</sub> questioned the validity of the testing method (RIA kit for humans) when 17 of the 19 free-ranging koalas failed to register a value, while the mean of the zoo-based koalas was 8.9 nmol/l. A second laboratory was consulted and preliminary testing, using liquid chromatography mass spectroscopy, is suggestive that zoo-based and free-ranging koalas have similar 25-dihydroxyvitamin D<sub>2</sub> values, but zoo-based koalas have non-detectable 25-dihydroxyvitamin D<sub>3</sub> values, while free-ranging koalas have values approximately half that of their 25-dihydroxyvitamin D<sub>2</sub> values. Further sampling and testing is required before conclusions can be made.

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**IMMUNE-MEDIATED ERYTHROID HYPOPLASIA AND ANEMIA IN AN AFRICAN CLAWLESS OTTER (*Aonyx capensis*)**

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**Abstract**

An estimated 9-yr-old adult male African clawless otter (*Aonyx capensis*) presented with acute lethargy, ataxia, and tachypnea. A full diagnostic examination revealed severe, regenerative anemia (PCV = 9%), elevated prothrombin time (35.6 sec), hypoproteinemia (TS = 4.0 mg/dl), and an elevated blood urea nitrogen (59 mg/dl). Radiographs, abdominal ultrasound, abdominocentesis, and upper gastrointestinal endoscopy failed to identify the source of the anemia. Five days later, when the anemia had not improved, the animal was transfused with Oxyglobin® (250 ml), a bone marrow aspirate and biopsy were collected, and prednisone therapy (1.06 mg/kg p.o. s.i.d.) was initiated. The bone marrow aspirate confirmed erythroid hypoplasia associated with erythrophagocytosis, consistent with a consumptive process, likely due to an immune-mediated and/or infectious process.

Infectious disease testing for *Dirofilariasis*, *Borreliosis*, and *Erlichiosis* was negative and the animal was continued on the prednisone for 9 mo while the PCV gradually increased to a high of 54%. By that time, the animal had developed a thinning hair coat, presumably due to induced hyperadrenocorticism. The otter was tapered off the steroids over 3 mo, the presumed hyperadrenocorticism resolved, and the animal is currently in remission.

**ACKNOWLEDGMENTS**

The authors would like to thank the veterinary staff, curators, and keepers of the Toledo Zoo for their assistance in the care and management of the otter in this report.

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## MORBIDITY AND MORTALITY IN FEATHERTAIL GLIDERS (*Acrobates pygmaeus*) AT THE CALGARY ZOO

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### Abstract

Named for its long feather-shaped tail, the feathertail or pygmy glider (*Acrobates pygmaeus*) is the world's smallest gliding mammal.<sup>4</sup> The Calgary Zoo has displayed this charismatic mouse-sized marsupial since 1999, with 125 recorded individuals, 101 mortality or presumed mortality events, 18 morbidity events, and 78 pathology records occurring during the last 8 yr. In adult gliders the two most common causes of morbidity and mortality were breeding exhaustion (26% of recorded events) and trauma (16% of recorded events), while mismothering with subsequent presumptive hypoglycemia/hypothermia was the most common cause of morbidity and mortality in neonates (100% of recorded events). At necropsy, aerobic culture of lungs yielded growth of *Sphingobacterium* sp., *Escherichia coli*, coagulase negative *Staphylococcus* sp., *Klebsiella* sp., *Bacillus* sp., *Serratia* sp., and *Morganella morganii*, while hepatic cultures yielded *Proteus mirabilis*, *Enterobacter* sp., *Enterococcus* sp., *Klebsiella* sp., *E. coli*, coagulase negative *Staphylococcus* sp., *Streptococcus viridans*, *Citrobacter freundii*, *Serratia* sp., *Pseudomonas* sp., and *Morganella morganii*. The significance of these isolates was generally considered equivocal in the absence of histologic changes. In cases where cystitis and endometritis were noted, the only two isolates recovered were *E. coli* and *Enterococcus* sp. Two noteworthy mycotic infections were diagnosed histologically- enteric or tracheal candidiasis (six cases), which is often associated with stressed or immunocompromised individuals,<sup>2</sup> and pulmonary and nasal cryptococcosis (two cases), which is considered an important emerging zoonotic disease.<sup>1</sup> Neoplasia is of significant concern in the species with two hepatic tumours (hepatocellular carcinoma and a metastatic cholangiocarcinoma), and eight cases of microchip-associated soft tissue sarcomas which have been noted in both domestic and exotic mammalian species.<sup>3</sup> Of special note, hepatic hemosiderosis was noted in 35% of cases evaluated histologically with no associated clinical or pathologic significance.

### ACKNOWLEDGMENTS

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## CRYPTOCOCCAL INFECTIONS IN AUSTRALIAN MAMMALS IN A ZOO

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### Abstract

In mammals cryptococcal infections are caused by two species; *Cryptococcus neoformans* (var. *grubii* and var. *neoformans*) and *C. gattii* (formerly *C. neoformans* var. *gattii* or *Cryptococcus bacillisporus*). Infection is acquired mainly by inhalation from the environment<sup>5</sup> with high inoculum levels of fungal particles being the key factor in the etiopathogenesis of the disease.<sup>1</sup> In Australia there is a close relationship between *C. gattii* infections and the presence of certain species of eucalyptus, specifically *Eucalyptus camaldulensis* and *E. tereticornis* in the environment. Dead wood and decaying vegetation are also known to be an important substrate for *Cryptococcus* spp.<sup>2</sup>

Cryptococcosis is a significant infectious disease usually impacting upon koalas; the respiratory system being the main focus of disease. Local extension to the surrounding tissues can also occur in cryptococcal infections of the upper respiratory tract. Dissemination, especially to the central nervous system, can develop in the advanced stage of the disease.<sup>1</sup>

Increasingly the condition is being seen in other captive native Australian mammal species.<sup>5</sup> Over a 10-yr period at Taronga Zoo cryptococcal infections have been reported in quokkas (*Setonix brachyurus*), koalas (*Phascolarctos cinereus*), red-necked wallabies (*Macropus rufogriseus*), yellow-bellied gliders (*Petaurus australis*), feather-tailed gliders (*Acrobates pygmaeus*), brush-tailed phascogales (*Phascogale tapoatafa*), greater stick-nest rats (*Leporillus conditor*), plains rats (*Pseudomys australis*) and an eastern barred bandicoot (*Perameles gunnii*). Affected animals suffered from a range of clinical conditions including sinusitis, pneumonia, pleuritis, and the disseminated form in three cases. The latter manifestation occurred more commonly in cryptic and nocturnal species housed indoors.

Diagnosis is based on the presence of clinical signs such as nasal discharge, facial asymmetry, weight loss, dyspnea, coughing, ataxia or seizing and ancillary testing, namely cytology and the latex cryptococcal antigen agglutination test (LCAT). Cases of cryptococcosis are definitively diagnosed on the basis of (i) culture of *Cryptococcus* from a normally sterile site, (ii) demonstration of characteristic organism morphology in specimens submitted for cytology or histopathology, (iii) visualization and culture of *Cryptococcus* from nasal swabs plus a positive LCAT (titre > or =2) (to rule out nasal colonization) or (iv) a positive LCAT in an animal with characteristic signs.<sup>4</sup>

Recently a multimodal approach to therapy has been developed involving surgical debulking of lesions, and concomitant medical therapy using an oral triazole such as itraconazole or fluconazole in concert with amphotericin (M. Krockenberger *pers. comm.*). In an attempt to limit

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possible spread to other organs during debulking, antifungal therapy is usually commenced prior to surgery. Regular testing of kidney and liver analytes, and LCAT levels should be performed to monitor the response to treatment and guard against possible side effects of therapy.

At Taronga Zoo animals housed in the nocturnal exhibit appeared to be particularly prone to cryptococcal infections. Control measures to decrease the incidence of *Cryptococcus gattii* in the environment include regular substrate changes and surface treatment of cage furniture with antifungal preparations. There is an ongoing need to examine initiatives to help decrease the environmental presence of *C. gattii* in animal enclosures through surface-acting environmental antifungal agents and the choice of appropriate substrates.<sup>3</sup>

#### **ACKNOWLEDGMENTS**

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## ACUTE PRESENTATION OF SEVERE PANCREATITIS IN SLENDER-TAILED MEERKATS (*Suricata suricatta*)

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### Abstract

Four adult sibling meerkats (*Suricata suricatta*) presented with acute severe pancreatitis. The underlying cause was undetermined, but dietary changes were implicated and genetics may have influenced disease progression. The first meerkat presented with lethargy and anorexia 2 wk following changes to a higher fat content diet.

In this animal, abdominal guarding and a cranial abdominal mass were identified. Blood chemistry revealed elevated cholesterol, triglycerides, amylase and lipase. Exploratory surgery revealed peritonitis and a saponified spleno-duodenal mass. A partial pancreatectomy, splenectomy, and mass excision were performed. Histopathology revealed severe, multifocal subacute necrotizing and granulomatous pancreatitis, peritonitis and pyogranulomatous mesenteric steatitis.

Two additional sibling meerkats presented within 12 days of each other with almost identical clinical and histologic abnormalities at subsequent surgical procedures. Complete physical examinations of all unaffected collection meerkats were performed. Based on physical and hematologic findings, the fourth sibling meerkat was diagnosed with pancreatitis. Medical rather than surgical treatment was initiated, as this animal was diagnosed before clinical signs presented.

Pancreatitis is serious, often fatal, and difficult to definitively diagnose. Several hematologic tests used in domestic animals have not demonstrated high sensitivity and specificity for pancreatitis. In recent years, pancreatic lipase immunoreactivity (PLI) has shown to be highly sensitive and specific for diagnosis of pancreatitis in both cats and dogs. However, PLI tests are species specific. PLI testing was performed on meerkats from multiple institutions to test its validity in *Suricata*.

### ACKNOWLEDGMENTS

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## **DISEASE SYNDROMES IN THE ROCK HYRAX (*Procavia capensis*)**

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### **Abstract**

Rock hyrax (*Procavia capensis*) are unusual small mammals and appear susceptible to a range of medical problems, yet are poorly represented in the veterinary literature. This paper will be based on the author's experiences with two colonies of this species in a UK zoo over the past two decades (Table 1). Post-mortem diagnoses will be described and various disease presentations discussed. In the oral presentation sample taking sites and a summary of "normal" hematology/biochemistry results will also be provided

### **Soft Tissue Mineralization**

This was a feature in three cases. In two animals, calcinosis circumscripta lesions between the toes of all feet had been investigated prior to euthanasia. A cause was not found in two cases. There was no exposure to excess Vitamin D<sub>3</sub> either in the diet or by accidental ingestion of rodenticide. In one case there was significant renal pathology. In another there was hemochromatosis. The kidneys of this animal were too autolysed for full analysis but the nature of lesions in other organs may enable some conclusions to be drawn regarding possible renal pathology. In two cases there was evidence of renal disease but the pathology was not felt to be severe enough to cause such lesions. Only one animal (not with calcinosis circumscripta!) showed uremia in pre-mortem blood sampling

### **Hemochromatosis**

This was found as a subclinical finding in two animals and as suspected cause of disease in one other. However, it has not been a frequent finding in these animals and blood screening has failed to show significantly high levels of unbound iron. While testing procedures in this species are not fully evaluated, it is possible that hemochromatosis results from an individual's faulty metabolism of iron rather than excessive dietary levels. Nonetheless further research is indicated.

### **Mesenteric Torsion**

This was a feature in two cases of acute death. In both cases there was an underlying enteritis (presumed bacterial).

### **Renal Disease**

In addition to the cases of soft tissue mineralization, three cases of renal disease were found. Two appeared as significant primary problems: one was believed to be subclinical.

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## **Steatitis**

Two cases were seen. In both cases vitamin E/selenium deficiencies were proposed as causes though no proof was found

## **Yersiniosis**

This was a major problem in the colonies over a period of 4 yr. An autogenous killed vaccine was made and administered annually thereafter. Rodent control was also increased. Deaths from yersiniosis stopped at this time. Vaccination was continued for a further 6 yr but has not been given for the past 6 yr. No further cases have been seen even though rodents have been a problem from time-to-time. Blood sampling of two animals failed to find any evidence of antibody production in response to the vaccine. It is therefore not known what the decisive factor was in terms of controlling (and possibly eradicating) this infection. Lack of antibody production doesn't necessarily show vaccine failure and it is possible that cell-mediated immunity could be relatively longlasting. However, some animals present in the colonies now will never have been vaccinated. It is also possible that the period of rodent control and vaccination was long enough to allow the *Yersinia pseudotuberculosis* organism to become less prevalent in the local wild rodent population.

## **Parasites**

It is surprising that so few internal parasites were found. Parasitic gastroenteritis was found in one animal though the parasite was not identified. An unknown lung mite was found in another. In one live animal (that presented thin) lungworm was identified on lung washes. The condition responded to anthelmintic therapy. However, lungworm has not been identified in any post-mortem investigation

## **Thin, Starving Animals**

Two animals were identified as starved with no other findings. One was juvenile. A "wasting syndrome" is apparent in adult animals, usually in subordinate females. There are rarely any significant findings on clinical examination or on hematology/ biochemistry and, apart from one case of lungworm infestation, parasitic burdens have not been found. At post-mortem there are rarely significant findings other than terminal acute conditions (e.g., septicemia) or diseases that one would suspect to be related to reduced immunity (e.g., fungal oesophagitis). It is felt that the problem may be linked to group dynamics with subordinate animals being "rejected" from the colony and prevented from feeding. External wounds are rare so it does not seem to be related to physical aggression.

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**Table 1.** Post-mortem findings in rock hyrax at Marwell Zoo 1989-2008.

Post-mortem Finding	Number of Cases	Comments
Soft tissue mineralisation	4	Calcinosis circumscripta in two cases
Nephropathy	1	
Hemochromatosis –subclinical	2	
Hemochromatosis - clinical	1	
Pyometra/ Metritis	2	
Mesenteric torsion	2	
Renal disease	3	1.Fibrosis and tubular deterioration and cyst formation 2.Subclinical chronic mild 3.Acute nephritis
Gastritis	1	Lymphocytic -? <i>Helicobacter</i> -related. Also oesophagitis
Steatitis	2	
Oesophagitis – fungal	1	
<i>Yersinia pseudotuberculosis</i> infection	12	
Parasitic gastroenteritis	1	Type unknown
Septicemia	1	
No diagnosis	5	Two starved

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## GRANULOMATOUS ARTERITIS IN BIRDS WITH MYCOBACTERIOSIS

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### Abstract

Avian mycobacteriosis is an important cause of morbidity and mortality in captive exotic, wild and domestic birds and can affect numerous organ systems. Avian mycobacteriosis has rarely been reported to cause granulomatous arteritis or to affect the myocardium.<sup>2,3</sup>

In a retrospective study of birds with mycobacteriosis, 14 individuals with vascular mycobacterial infection were identified. Thirteen species were affected, all were adults and no sex predilection was noted. Disseminated mycobacteriosis was noted in 9 of 14 birds, however in 5 birds histologic evidence of mycobacteriosis was only present in the vessels. Vascular lesions were categorized according to the severity of mononuclear cell infiltrate and degree of architectural distortion.

Affected vessels were predominantly the aorta, brachycephalic trunk, coronary arteries and pulmonary trunk. Vascular lesions included mild to extensive accumulations of macrophages, lymphocytes, plasma cells and rare heterophils with variable distortion of vascular architecture. Intralesional acid-fast positive bacilli were generally abundant. Vascular changes were considered mild in four cases, moderate in seven and severe in three. Associated arteriosclerotic and/or atherosclerotic lesions were noted in six cases.

Granulomatous arteritis is an uncommon lesion. An immune-mediated response to mycobacteria antigens is implicated in the human syndrome Takayasu's arteritis.<sup>1</sup> A similar mechanism has been suggested in some cases of human atherosclerosis.<sup>4</sup> Interestingly, the distribution of granulomatous inflammation in Takayasu's arteritis is similar to that noted in these birds. The pathogenesis of these lesions may also be similar, however, intralesional acid-fast bacilli, have not been documented in either human disease.

### ACKNOWLEDGMENTS

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## HEMOSIDEROSIS IN MICRONESIAN KINGFISHERS AT THE NATIONAL ZOO

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### **Abstract**

The livers from 35 Micronesian kingfishers necropsied at the National Zoo between 1985 and 2007 were examined histologically with hematoxylin and eosin stain for iron content. Livers with significant pigment clusters were further examined with Perl's iron, Hall's bile, lipofuscin and acid fast stains. Seventeen kingfishers (49%) had significant accumulations of hepatic iron as demonstrated by Perl's stain. Thirteen of these kingfishers were male and males were identified as having triple the risk of developing hemosiderosis. Affected livers varied in gross appearance. Some livers were enlarged with rounded edges and scattered tan to yellow foci and others were normal to enlarged with diffuse, tan or yellow-orange pallor. The histologic pattern of distribution of iron pigment was consistent from case to case, and the degree of pigmentation varied from moderate to marked in all cases. Throughout the parenchyma, stainable iron was diffuse and granular within hepatocytes and granular to globular within macrophage clusters. Densely staining hemosiderin-laden macrophages were distributed in multifocal, periportal and random clusters, or singly within sinusoids and parenchyma. Minimal stainable iron was also detected in other organs including the heart, renal tubules, lung, spleen, thyroid gland and brain. Ten cases had moderate to marked, multifocal birefringence within pigment clusters. Eleven cases had minimal to moderate portal and periportal, lymphocytic and heterophilic inflammation often surrounding pigment clusters. Two cases had scant to few acid fast bacilli identified within pigment clusters. A single case had moderate bridging fibrosis and two other cases had marked, diffuse vacuolation of hepatocytes. The cause of death was attributed to liver disease in only one case.

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## RHABDITOID OPHTHALMITIS AND MENINGOENCEPHALITIS IN CAPTIVE ASIAN HORNED FROGS (*Megaphrys montana*)

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### Abstract

Ocular and neural nematodiasis in amphibians is sporadic and typically an extension of visceral larval migrans.<sup>2,4</sup> Two genera of rhabditoid nematodes, *Rhabdias* and *Strongyloides*, are usually involved. These nematodes have a direct life cycle and can be free-living or parasitic. Low numbers cause little impact; however, high numbers can cause considerable morbidity and mortality, particularly in confined captive populations.<sup>3</sup>

At the San Diego Zoo, an Asian horned frog (*Megaphrys montana*) was necropsied after prolonged treatment for bilateral corneal ulceration and perforation and acute onset of neurologic signs. Histopathologic findings confirmed the corneal perforations and revealed granulomatous ophthalmitis and meningoencephalitis with intralesional nematodes. The small size, thin cuticle, platymyarian musculature and uninucleate intestinal cells identified the nematodes as rhabditoids.<sup>1</sup> Samples of frozen, ethanol-preserved and formalin-fixed tissue and samples of the enclosure substrate were submitted for nematode speciation. Comparison of the 28s rRNA and internal transcribed spacer region gene sequence of two nematodes isolated from formalin-fixed tissue resulted in absolute homology to *Caenorhabditis elegans*. Though much is known about *C. elegans* as a research model, its potential as a pathogen has not been thoroughly investigated. To this date, there are no reports of *C. elegans* associated with disease in vertebrates. After this case was documented, three additional confirmed and potential cases from the same exhibit were identified. Heavy environmental contamination with free-living nematodes and subsequent direct corneal invasion was suspected. Management changes included disinfection of the exhibit, increased frequency of substrate changes and anti-helminthic treatment of the remaining frog.

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AN UNUSUAL MORTALITY EVENT IN CAPTIVE COQUI FROGS (*Eleutherodactylus coqui*)

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**Abstract**

Wild caught coqui frogs (*Eleutherodactylus coqui*) from Hawaii were received by multiple AZA-accredited zoos in 2007. Upon arrival frogs were quarantined and separated into multiple tanks containing 5-10 animals. Environmental temperatures and humidity ranged from 70-85°F and 75-95%, respectively. All frogs were fed a diet of pin-head crickets and fruit-flies. Intestinal nematodiasis was diagnosed at multiple facilities and one or more anthelmintic treatments (fenbendazole, praziquantel, levamisole) were administered. Several animals at one zoo were polymerase chain reaction positive for *Batrachochytrium dendrobatidis*. Mortality as high as 96% was experienced at all facilities within several weeks of animal arrival. Frogs were generally found dead with no premonitory signs. Supportive care and prophylactic therapy (amphibian ringer's solution, enrofloxacin, itraconazole) was initiated in response to increasing animal deaths. Significant lesions in dead animals regardless of facility included hepatitis and hepatic necrosis, proliferative glomerulonephritis, splenic histiocytosis, vascular thrombosis and intestinal nematodiasis. *Ranavirus* polymerase chain reaction was positive in liver samples from several animals. All examined animals were histologically negative for *B. dendrobatidis*. These findings highlight the inherent risks of animal movement, intentional or unintentional, and the ease with which known, novel or emerging diseases can spread.

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## PREVALENCE OF *Coxiella burnetii* IN HOOFSOCK PLACENTAS IN ZOOLOGICAL COLLECTIONS

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### Abstract

Many species of mammals, birds, and ticks are reservoirs of *Coxiella burnetii*, the cause of Q fever in humans, but ruminant species are the predominant zoonotic source. Animals infected with *C. burnetii* can be asymptomatic. This retrospective study evaluated prevalence of *Coxiella burnetii* in placentas obtained from 88 animals representing 32 different ungulate species between 1993 and 2007 from seven different zoos, and in two known positives from one of the facilities. Tissues were from normal births, abortions, or stillbirths in which *C. burnetii* was not considered the main differential diagnosis. Archived paraffin embedded tissues were examined for microscopic lesions and for DNA from *C. burnetii*, using a real-time PCR assay. Infection was confirmed by immunohistochemistry. The PCR assay detected *C. burnetii* in placental tissues from four additional animals: two bongos (*Tragelaphus eurycerus*), a Cuvier's gazelle (*Gazella cuvieri*), and an Indochinese sika (*Cervus nippon pseudaxis*), as well as the two known positives, a Malayan sambar (*Rusa unicolor equine*) and a Javan rusa (*Rusa timorensis russa*); all cases were from one of the seven facilities. Histology showed mild to severe necrotizing placentitis in all infected placentas. Immunohistochemistry demonstrated organisms within the trophoblast cells and free within the overlying necrotic debris. Evaluating *C. burnetii* prevalence in the zoological environment may aid in determining the potential for occupational exposure and aid in the design of biosafety training of zoo employees in contact with infected animals. This study also highlights the value of ancillary testing which may not have been previously available, such as PCR, in the diagnosis of *C. burnetii* even in healthy births or from grossly normal placentas.

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## SYSTEMIC CORONAVIRUS INFECTION IN DOMESTIC FERRETS, *Mustela putorius*

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### Abstract

In the last 6 yr, several ferrets from Europe and the United States have been diagnosed with systemic pyogranulomatous inflammation resembling feline infectious peritonitis (FIP).<sup>1</sup> Most ferrets are young adults, and have a clinical course of approximately 2 mo duration. Common clinical findings can include anorexia, weight loss, diarrhea, and large palpable intra-abdominal masses; less frequent findings include hind limb paresis, CNS signs, vomiting, and dyspnea. Hematologic findings are not specific, and most often include mild anemia, thrombocytopenia and hypergammaglobulinemia. Grossly, whitish nodules are found in numerous tissues, most frequently in the mesenteric adipose tissue and lymph nodes, visceral peritoneum, liver, kidneys, spleen, and lungs. Rarely, a serous abdominal effusion may be present. This condition is diagnosed histologically and lesions include pyogranulomatous inflammation in the visceral peritoneum, mesenteric adipose tissue, thoracic and abdominal viscera, and blood vessels. In tissue sections, viral antigen cross reacts with FIP antibody using monoclonal antibody FIPV3-70. Electron microscopic examination has confirmed the presence of viral particles with coronavirus morphology in the cytoplasm of macrophages. Partial sequencing of the coronavirus spike gene indicates that the virus is most closely related to ferret enteric coronavirus.

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**PRELIMINARY RESULTS OF A SURVEY OF UNITED STATES AND CANADIAN ORANGUTAN MORTALITIES IN THE NORTH AMERICAN SSP POPULATION FROM 1980 TO MARCH, 2008**

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**Abstract**

Using data from the North American Orangutan SSP Stud Book, 293 deaths (97 *Pongo pygmaeus* (Bornean), 135 *Pongo abelii* (Sumatran), 60 *P. pygmaeus* x *abelii* hybrids) were identified in US and Canadian zoos and private collections from January 1980 to March 2008. Thirty-one deaths were perinatal (10.6%), 18 neonatal (6.14%), 33 infants (11.3%), 11 juveniles (3.8%), 38 adolescents (13%), 21 subadult males (7.2%), 127 adults (43.4%) and 14 (4.8%) old adults (40-55 yrs). Necropsy diagnoses (n=35) or full reports (n= 107) were available for 142 (48.5%).

Perinatal causes of death (COD) included placentitis, umbilical strangulation, congenital deformity, and maternal pre-eclampsia. Neonatal deaths were attributed to maternal factors, infections, low birth weight/prematurity, and one birth defect. Strongyloidiasis was the most important COD of infants, followed by pneumonia, trauma and one smoke inhalation. Juvenile deaths included strongyloidiasis, intestinal torsion, enteritis, respiratory infection, and autoimmune disease. Among adolescents, respiratory infection was the most reported COD; others included trauma, drowning, amoebic encephalitis, acute viral myocarditis, and balantidiasis.

For 83 of 163 orangutans over 15 yr old (subadult males and adults/old adults of both sexes), 24 (28.9%) deaths were due to cardiovascular disease, 13 (15.7%) to respiratory infections, 12 (14.5%) to chronic renal disease, 5 (6%) to GI disease, 4 parturition-related, 4 neoplasia, 3 liver disease, 2 multifactorial or undetermined, 2 abdominal abscesses, 3 anesthetic associated with obesity, 2 stroke, 1 environmental, 1 complication of endocrine disease, and 1 osteoarthritis leading to euthanasia. Osteoarthritis and aortic atherosclerosis were incidental findings in several adults.

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## EFFECTS OF SHORT- AND LONG-TERM HYPOXIA ON HEMOLYMPH GAS VALUES IN THE AMERICAN HORSESHOE CRAB (*Limulus polyphemus*)

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### Abstract

In order to evaluate the effect of short and long-term hypoxia in the American horseshoe crab (*Limulus polyphemus*), 22 adult, healthy crabs were evaluated for this study. In group 1, baseline pH, PO<sub>2</sub>, PCO<sub>2</sub>, HCO<sub>3</sub>, base excess, TCO<sub>2</sub> and lactate concentrations were determined from hemolymph samples collected from 10 horseshoe crabs submerged in water. Baseline results were compared to values obtained following removal from water for 5 min, and following recovery in water for 10 and for greater than 60 min (range: 61-221 min). In group 2, hemolymph gas parameters were determined in 12 horseshoe crabs following shipment out of water for 24 hr and compared to values obtained from group 1 animals. Following removal from water for 5 min, all crabs developed severe hypoxia with PO<sub>2</sub> levels below the detectable limit of the analyzer. No significant changes in PCO<sub>2</sub> concentrations were seen over time in group 1 animals. Group 2 crabs had pronounced respiratory acidosis (pH = 6.7; PCO<sub>2</sub> = 46 mmHg) following transport, and PO<sub>2</sub> concentrations were significantly below baseline values of Group 1 animals. Baseline hemolymph gas values of the American horseshoe crab were within range reported for other aquatic vertebrates. Short term removal of crabs from their aquatic environment causes severe hypoxia and metabolic changes. While crabs have the ability to compensate for a changing hypoxic environment if removed from water for long periods of time (24 hr) severe hypercapnia and respiratory acidosis will still be present.

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## NOVEL ANELLOVIRUS-ASSOCIATED DEMISE IN THREE CALIFORNIA SEA LIONS (*Zalophus californianus*)

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### Abstract

Three aged California sea lions (*Zalophus californianus*) died within 1 yr of each other; two animals died within the same week. Two of three animals demonstrated histopathologic evidence of lymphocytic/plasmacytic pleuritis and mediastinitis. The third animal presented with epistaxis and hypertension when comparing indirect oscillometric blood pressures to clinically healthy sea lions. Histopathology revealed a non-suppurative rhinitis. Toxicity assays, bacterial, parasitic, and fungal testing were negative for pathogenic organisms. All three animals tested negative for West Nile virus. Further diagnostic evaluation using viral metagenomic sequencing<sup>1</sup> documented a novel sea lion anellovirus (SLA). The virus has a 2.1 kilobase, single-stranded, circular genome, with amino acid level similarity to a feline Anellovirus. Anelloviruses are known in humans, domestic dogs (*Canis lupus familiaris*), domestic cats (*Felis silvestris catus*), pigs (*Sus scrofa*), tree shrews (*Tupaia belangeri chinensis*), and non-human primates<sup>2-5</sup> but have not been documented in marine animals.

Polymerase chain reaction (PCR) primers designed to specifically amplify SLA were positive in pulmonary tissues from all three affected sea lions and negative in the unaffected tissues. Abdominal fluid, blood, oral and nasal swabs from the affected sea lions were negative, as were nasal and oral swabs from clinically healthy conspecifics. To determine if this virus was involved in the mortality event, pulmonary tissues from four other sea lions that died of unrelated causes (neoplastic disease, renal failure, etc.) were also tested. All of these sea lions were negative for SLA. It is reasonable, but not proven, to believe that this newly identified virus played a role in the demise in these aged sea lions and may be an emerging disease. Further work is being performed on additional sea lion pulmonary tissues to determine the prevalence of SLA in wild and captive sea lions and characterize the pathology of the virus. This is the first Anellovirus discovered in marine animals, and it demonstrates the ability of metagenomics sequencing to discover novel pathogenic viruses from animal tissues.

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## A REVIEW OF ADULT STINGRAY MORTALITIES AT THE NATIONAL AQUARIUM IN BALTIMORE (1997-2008)

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### Abstract

A 260,000-gallon artificial saltwater system at the National Aquarium in Baltimore houses stingrays, sharks, teleosts, and sea turtles. The majority of animals are southern stingrays (*Dasyatis americana*) and cownose rays (*Rhinoptera bonasus*). Twenty adult stingray mortalities were reported from 1997 to 2008: five southern stingrays, four cownose rays, three spiny butterfly rays (*Gymnura altavela*), two pelagic stingrays (*Pteroplatytrygon* [*Dasyatis*] *violacea*), two rough-tail stingrays (*Dasyatis centroura*), two spotted eagle rays (*Aetobatus narinari*), one blunt-nose stingray (*Dasyatis say*), and one banded guitarfish (*Zapteryx exasperata*). All animals had unknown birth dates and were assigned either estimated birth dates upon arrival into the collection or minimum ages at death. Mean longevities from arrival date to death date in years  $\pm$  one standard deviation (SD) were  $8.2 \pm 6.6$  yr (range 0.3-18.5) for the group and  $13.0 \pm 6.2$  yr (range 2.0-18.5) for only the southern and cownose rays. Mean longevities from minimum estimated birth date to death date  $\pm$  SD were  $10.3 \pm 7.4$  yr (range 1.0-23.0) for the group and  $15.4 \pm 7.4$  yr (range 2.0-23.0) for only the southern and cownose rays, these values likely being underestimations. Presenting clinical signs included anorexia (38.9%), coelomic distension (16.7%), neurologic signs (16.7%), generalized edema (5.6%), and cloacal prolapse (5.6%). Gross necropsy and histologic analyses showed skin pathology (50.0%), suspicion of sepsis (47.1%), suspicion of disseminated intravascular coagulation (27.8%), reproductive pathology (17.6%), and neoplasia (5.9%). The majority of southern stingray and cownose ray mortalities reflect changes expected in an aging population of animals.

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**TREATMENT OF *Dolops nana* INFESTATION OF SAN FRANCISCO PIRANHA (*Pygocentrus piraya*)**

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**Abstract**

Eighteen recently imported San Francisco piranha (*Pygocentrus piraya*) were found to have mobile cutaneous parasites. Fish were anesthetized with 75 ppm tricaine methanesulfonate (MS-222) (FINQUEL, Argent Chemical Laboratories, Inc., Redmond, WA 98052, USA) and two to seven parasites per fish manually removed. To prevent conspecific aggression, fish were maintained @ 15 ppm MS-222 in individual enclosures until simultaneously re-introduced. Parasites were identified as branchiuran fish lice, *Dolops nana*. Gill biopsies and skin scrapes of four randomly selected fish had abundant *Dactylogyrus* sp. trematodes. One fish had a single *Braga* sp. cymothoid isopod removed from under the operculum.

Treatment for immature *D. nana* and *Dactylogyrus* sp. was performed with 0.5 ppm trichlorfon [DyLox (Masoten), Bayer Environmental Science, Research Triangle Park, NC 27709, USA] weekly for 8 wk. A 25% water change was performed prior to treatment, and as necessary to maintain water quality. The day after the last treatment most fish developed incoordination and depression. A 50% water change was performed, aeration increased, and carbon filtration added. Affected fish were treated once or twice with atropine (1 mg/kg i.m.) and once with dexamethasone sodium phosphate (1 mg/kg i.m.); eight died and the rest improved. The next day fish were normal, a 50% water change was performed, clinical signs recurred, atropine and dexamethasone were repeated, and another fish died. Over the course of 48 hr the two 50% water changes and continuous carbon filtration should have removed the trichlorfon from the system, yet clinical signs recurred after the water change. Tank and replacement water parameters measured (pH, ammonia, nitrites, nitrates, copper, and chlorine) were within expected ranges.

Dead fish were examined grossly and one autolyzed fish was not further evaluated. Histopathology was performed on five and tissues from three were pooled for ancillary diagnostics. All were in good body condition with moderate to abundant coelomic adipose. Gross changes included erythematous and congested gill filaments partially covered by excessive mucoid secretions. No parasites were present. Histologically, there was mild to moderate acute branchitis and mild blunting and swelling of secondary lamellae. There was mild diffuse hepatic accumulation of lipid vacuoles and hydropic degeneration. Hepatic organophosphate levels were undetectable.

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It could not be determined if the morbidity and mortality were related to a water parameter abnormality or treatment complication, but surviving fish were normal and parasite free.

#### **ACKNOWLEDGMENTS**

The authors thank the Prospect Park Zoo and New York Aquarium staffs for their care and management of these fish, Dr. Tim Georoff for assistance with the procedure, and Nina Palmer of the Queens Zoo for technical support.

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## COMPARISON BETWEEN COELIOSCOPY AND COELIOTOMY FOR LIVER BIOPSY IN CHANNEL CATFISH (*Ictalurus punctatus*)

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### Abstract

A comparison of liver biopsies by endoscopy versus standard coeliotomy in 30 channel catfish (*Ictalurus punctatus*) was performed.<sup>1</sup> Ten fish were randomly assigned into either the control, coeliotomy, or coelioscopy groups. All fish were anesthetized using buffered tricaine methanesulfonate and maintained using a re-circulating anesthesia machine.<sup>2</sup> Body weight, packed cell volume (PCV), total protein (TP), aspartate aminotransferase (AST), creatinine phosphokinase (CPK), lactate dehydrogenase (LDH), and sorbitol dehydrogenase (SDH) were measured pre- and post-operatively. A standard ventral coeliotomy or saline-infusion coelioscopy was performed,<sup>3</sup> and collected biopsies were scored histologically. Both the coeliotomy and coelioscopy procedures were well tolerated without acute mortalities. The PCV and TP decreased post-operatively in the coelioscopy group due to intracoelomic fluid administration during the procedure to aid visualization. Minor changes in activities for hepatic and muscular enzyme activities were apparent, but were not statistically significant between the coelioscopy and coeliotomy groups. Coelioscopy and coeliotomy yielded biopsies of similar diagnostic quality on the basis of histopathology. However, coelioscopy permitted a more extensive evaluation of the viscera. This study indicated that all surgical wounds (10/10) for coelioscopy healed completely, while the surgical wounds (3/10) of the coeliotomy group had severe wound dehiscence. Surgeon skill, suture material and pattern, and environmental factors can affect surgical outcome. In conclusion, both coelioscopy and coeliotomy are capable of yielding ante-mortem liver biopsies of diagnostic quality in catfish. Coelioscopy permits a more detailed examination of the coelomic viscera through a smaller surgical incision, is less traumatic, results in decreased wound dehiscence, and is recommended for visceral examination and biopsy in fish.

### ACKNOWLEDGMENTS

This project was supported by a grant from Geraldine R. Dodge Foundation. The authors would like to thank Karl Storz Veterinary Endoscopy for supporting endoscopy research and development at The University of Georgia. Additional assistance was provided by Dr. David Martinez, Mr. Dan Regan, Mr. Jason Norman, Mr. Raj Joshia, and Dr. Deborah Keys.

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**ATTEMPTED SURGICAL CORRECTION OF MULTI-YEAR EGG STASIS IN IRISH LORDS (*Hemilepidotus jordani* AND *H. hemilepidotus*)**

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**Abstract**

Egg stasis, the inability to complete ovulation and expel eggs, is a poorly characterized syndrome in fish. We used a modified ovariohysterectomy to remove large egg masses from three Irish Lords, with 1 to 3 yr accumulation of eggs.

Anesthesia was induced with 100 mg/L tricaine methanesulfonate and maintained at 50 mg/L in sea water. Fish were positioned in dorsal recumbency; the ventral midline was disinfected with iodophor solution, covered with a sterile transparent drape and the skin and body wall incised from the pectoral fin arch to anterior to the vent. Exteriorization of bulky egg masses was blocked by the colon ventral to the connection between lateral lobes. Rochester-Carmalt forceps were clamped across one oviduct at a time. The ovarian blood vessels were ligated, and the oviduct was transfixed and ligated with 3-0 PDS. Forceps were drawn under the colon, the procedure was repeated on the other side, and the egg masses were excised and removed. The abdomen was flushed and filled with saline. The body wall and skin were closed separately with 3-0 PDS using a simple continuous pattern. Coelomic air was removed by coelomocentesis and the fish were recovered.

The Irish Lord with only 1 yr of eggs began eating within a week, was returned to its display tank 4 mo later, and continues to do well. The fish with multi-year eggs never resumed eating and died after 1 and 4 wk. We recommend performing surgery once egg stasis is recognized, before multi-year accumulation develops.

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## THE CLAM EXAM: CLINICAL PATHOLOGY OF FRESHWATER MUSSELS

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### Abstract

Freshwater mussels are the most imperiled taxon of animals in North America with more than two-thirds of nearly 300 identified species considered endangered, threatened, or extinct.<sup>2</sup> These bivalves are important in maintaining our waterway health and species diversity and serve as valuable indicators of environmental quality. While captive propagation and translocation of freshwater mussels are widely supported as conservation measures, a high proportion of these normally long-lived animals die within the first year of translocation.<sup>1</sup> Furthermore, the ability to evaluate the health of freshwater mussels has been limited and has relied primarily on behavioral changes, mortality rates, histopathology, and non-survival assays.

Our goal is to develop better biomarkers of health and disease in wild, captive and translocated freshwater mussels, specifically to: 1) develop techniques for identification of hemocyte cell types in freshwater mussel hemolymph; 2) identify hemolymph chemistries of value in health assessment; and 3) identify catecholamines and monoamines present in hemolymph and assess their response to stressors. We compared six hemolymph collection and processing strategies in *Quadrula* sp. and assessed the effects on cell yield and morphology, characterizing key morphologic features for the development of a mussel hemogram. We have assessed hemolymph chemistries and hemocyte cell function in control wild mussels and those brought into captivity and monitored closely. Finally, using pericardial catheterization, we have evaluated five hemolymph catecholamine and monoamine reactions to specific stressors in an attempt to identify the mechanisms of the captive stress response in these species.

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## MEDICAL AND SURGICAL MANAGEMENT OF SEVERE BLEPHARITIS AND DACRYOADENITIS IN A GIANT PANDA (*Ailuropoda melanoleuca*)

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### Abstract

A 10-yr-old male giant panda presented for severe, acute proliferation and protrusion of the right third eyelid, non-responsive to topical therapy. Excisional biopsy of the third eyelid and associated lacrimal gland was elected due to necrosis and friability of the tissue. Histopathology revealed suppurative, necrotizing blepharitis and suppurative dacryoadenitis. Culture grew *Enterococcus* spp. and *Stenotrophomonas maltophilia* with extensive antibiotic resistance. Topical aminoglycoside treatment was discontinued based on sensitivities. Treatment was initiated with neomycin/polymyxin B/gramicidin ophthalmic solution (Neocidin, Major Pharmaceuticals, Livonia, MI, 48150 USA) o.d. b.i.d. × 10 days, 25 mg/ml doxycycline ophthalmic solution (Franck's Pharmacy, Ocala, FL, 34474 USA) OD b.i.d. × 10 days, and doxycycline (West-ward Pharmaceutical, Eatontown, NJ, 07724 USA) 4.9 mg/kg p.o. b.i.d. × 10 days. All treatments were well tolerated. Healing was uncomplicated with no recurrence of the lesion and no clinical evidence of keratoconjunctivitis sicca. Ophthalmic exam performed opportunistically 3 mo post-operatively was unremarkable with Schirmer tear test results (9 mm OS, 11.5 mm OD) similar in both eyes and within the satisfactory range for domestic animals, confirming that excision of the lacrimal gland did not cause appreciable detrimental effects.<sup>3</sup>

The etiology of this third eyelid lesion remains undetermined, but trauma is suspected. Ocular changes described in the giant panda include keratoconjunctivitis, corneal ulceration, retinal degeneration, lenticular sclerosis, and neoplasia.<sup>1</sup> A non-invasive hemangiosarcoma was excised from the ocular limbus of a giant panda.<sup>2</sup> To the authors' knowledge, this is the first report of third eyelid excision in the giant panda.

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## CARDIAC DISEASE IN A SADDLE-BILLED STORK (*Ephippiorhynchus senegalensis*)

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### Abstract

A 20-yr-old, female saddle-billed stork (*Ephippiorhynchus senegalensis*) was diagnosed with grade III/VI heart murmur and mitral regurgitation after osteomyelitis and resultant toe amputation. Treatment was attempted with antibiotics and enalapril, but medicating was unsuccessful. Four years later the mitral regurgitation and heart murmur had worsened (grade IV/VI). Treatment with enalapril (0.5 mg/kg s.i.d. p.o.; Wockhardt Ltd., Mumbai, India) and furosemide (0.2 mg/kg s.i.d. p.o.; Vedco, Inc., St. Joseph, Missouri 64507 USA) was again attempted, and medications were successfully delivered 60% of the time. The bird was clinically normal for 8 mo, but then showed acute lethargy and anorexia. Cardiac consultation revealed atrial fibrillation, pulmonary edema, and hepatic congestion. Digitalization (15 µg/kg b.i.d. p.o.; Jerome Stevens Pharmaceuticals, Inc., Bohemia, New York, 11716 USA) and increased diuresis initially improved the bird's condition, but the animal was found dead 10 days after first signs of decompensated heart failure. At necropsy dilation of the left ventricle and atrium and thickening of the mitral valve were noted. Histologic examination revealed myocardial degeneration and fibrosis.

Cardiac disease is not uncommon but infrequently diagnosed in birds.<sup>2</sup> Several institutions holding this species have recently noted heart murmurs and cardiomegaly. Infection with the filarid *Paraonchocerca ciconarum* has been previously reported<sup>1</sup> but this individual did not appear to be infected. Full cardiac examination should be considered to screen individuals of this species and to help clarify findings and etiologies to enable early treatment of cardiac disease, since several cases such as this one have anecdotally responded to medical treatment.

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## GENERALIZED DEMODICOSIS IN THREE SIBLING JUVENILE ROCK HYRAX (*Procavia capensis*)

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### Abstract

One female and two male 4-mo-old sibling rock hyrax (*Procavia capensis*) presented with severe generalized dermatitis characterized by non-pruritic, non-alopecic, mildly encrusted, focally ulcerated, pustular nodules over the entire body surface. The dorsum, limbs and dorsal cranium were most severely affected. Skin scrapings and histopathology revealed a novel *Demodex* spp. mite in various life stages. Scanning electron microscopy was used to further characterize the mites. Due to the generalized nature of infection treatment was initiated with ivermectin (Ivercide, Phoenix Pharmaceutical, Inc., St. Joseph, MO 64507 USA) then changed to doramectin (Dectomax, Pfizer, New York, NY 10017 USA) at a dose of 0.6 mg/kg s.c. every 7 days.<sup>1</sup> Weekly skin scrapings and intermittent blood work including serum protein electrophoresis were performed to evaluate treatment response. A progressive reduction in the number and life stages of mites were observed over the course of treatment as well as the number and prominence of skin nodules. Complete resolution, as determined by two consecutive negative skin scrapings,<sup>2</sup> was obtained in all three hyrax within 10-14 wk. These cases highlight the use of an alternative antiparasitic that allows for longer duration of activity and therefore decreased patient handling.

### ACKNOWLEDGMENTS

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## SUB-TOTAL GLOSSECTOMY IN A RETICULATED GIRAFFE

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### **Abstract**

A 29-yr-old female Reticulated Giraffe presented with an acutely swollen tongue. This animal habitually allowed a majority of the tongue to hang out of the right side of its mouth but on presentation, the tongue was markedly swollen with dried hay and debris adherent to the surface due to oozing of serous fluid. After placement in a restraint chute, the giraffe was immobilized with 200 mg xylazine (Lloyd Laboratories, Shenandoah, IA 51601 USA) followed by 4.0 mg etorphine (Zoopharm, Fort Collins, CO 80522 USA) for exam. The distal two-thirds of the tongue was obviously devitalized, hard, and malodorous and a glossectomy was deemed the only salvage procedure possible. Towel forceps were placed in the tongue, allowing it to be extended until healthy tissue could be identified. A series of closely spaced vertical mattress sutures was placed across the width of the tongue for hemostasis. Approximately 29 cm of tongue was amputated, near the rostral edge of the frenulum, leaving an unknown length of tongue remaining. A V-shaped incision into the muscular layer of the tongue facilitated apposition of the cut edges. By the third day post-surgery, the giraffe was noted to be picking up feed and trying to manipulate it with its mouth, tipping the nose vertically upward to allow feed to fall into the back of the mouth. Switching to larger alfalfa cubes and leafy browse allowed better prehension of the feed and the giraffe was able to maintain or gain weight over the next 10 mo. At that time progressive, severe lameness due to chronic osteoarthritis of the left front fetlock developed and humane euthanasia was elected. At necropsy, there was approximately 7.5 cm of healthy appearing tongue remaining.

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**USE OF NEUPOGEN® (FILGRASTIM) IN A BOTTLENOSE DOLPHIN (*Tursiops truncatus*)**

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**Abstract**

In December 2006 all the dolphins from L'Oceanogràfic were removed from their exhibit and separated into several other minor enclosures during pool remodeling. When the work was finished 3 mo later, all the animals were placed back in the dolphinarium. During the previous week one young male dolphin (body weight was 126 kg) was already experiencing a social stress situation in his own small group. According to the unstable blood work, some relevant rake marks and the concern this animal was potentially immunocompromised, antibiotic treatment with amoxicillin + clavulanic acid (Augmentine®, Glaxo Smith Kline, S.A. P.T.M. C/ Severo Ochoa, 2. 28760 Tres Cantos, Madrid, Spain) at 10mg/kg PO BID was initiated and this animal was separated from the aggressive pool mate.

Once in the dolphinarium, after 5 days of controlled regrouping of the entire original group, trauma from conspecifics resulted in new injuries, group isolation and eventually shock of this juvenile dolphin.

The day of the crisis, although the animal was performing a show in the morning, at noon the dolphin was completely stuporous with tremors, became unable to swim and eventually unable to keep position in the water. Emergency support treatment was applied to stabilize the animal. Six hr later, he was able to swim without assistance, especially under the effect of pain relievers such as metamizol (Nolotil®, Boehringer Ingelheim España, S.A. Prat de la Riba s/n, Sector Turó de Can Matas, 08173 Sant Cugat del Vallés, Barcelona, Spain) at a total dose of 1 g i.m. The initial total leukocyte count was 2100 cells/ $\mu$ l with 94% neutrophils and 2% band cells (absolute leukopenia). Although there was an evident drop in ALKP, albumin and iron, other relevant markers of inflammation such as ESR and fibrinogen were within normal ranges. A decision was made to institute among others, a more aggressive antibiotic therapy including amikacin (Biclin®, Bristol-Myers Squibb, S.L., C/Almansa, 101, 28040 Madrid) at 14 mg/kg i.m. s.i.d.) to cover the potential septic shock or septicemia.

Twelve hours after the initiation of the stabilizing therapy the WBC increased slightly up to 2720cells/ $\mu$ l. Six hours later another blood sample showed a decrease to 1400 WBC/ $\mu$ l and another 6 hr later it fell to 1040 WBC/ $\mu$ l. At this point the animal had greatly improved in attitude, was swimming almost normally and was eating on its own. A decision was made to remove the clavulanic component of the antibiotic therapy and to decrease as much as possible, the metamizol as these two drugs have been previously described to potentially cause granulocytopenia and leukopenia in humans.<sup>4,6</sup> Additionally, dexamethasone (Fortecortín®,

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Merck Farma y Química, S.A., Polígono Merck, 08100 Mollet del Vallés, Barcelona, Spain) was used at a total dose of 30 mg i.m. to revert the shock. It was then substituted by progressive decreasing dosages of prednisone (Dacortín®, Merck Farma y Química, S.A., Polígono Merck, 08100 Mollet del Vallés, Barcelona, Spain). Finally a recombinant human granulocyte colony-stimulating factor, G-CSF, the filgrastim (Neupogen®, Amgen Europe B.V., Minervum 7061, NL-4817 ZK Breda, Holland) was administered at a total dose of 30 MU (0,0025 mg/kg) i.m. in an attempt to increase neutrophil production in this animal.

Twelve hours after the first filgrastim injection the WBC dramatically increased to 5160 cells/μl with 96,9% neutrophils and a band and proleukocyte count of 76%. A second dose of the same amount of Neupogen® was administered 24 hr after the first one.

After these two filgrastim doses, the WBC kept gradually increasing during the next few days up to a maximum of 16400 cells/μl on the sixth day after the first administration. Meanwhile band cell counts kept decreasing, reaching normal values for this individual (1,7%) on the tenth day. The hemogram and blood chemistry was completely normal regarding baselines for this individual 19 days after the first filgrastim injection.

The viability of this initial increase of highly immature leukocytes to combat bacterial infection could be controversial. Furthermore the potential host antibody production could inactivate the filgrastim in case of prolonged or successive treatments in allospecific patients. In this case there was no decline in the WBC series suppression after the treatment as described by other authors in other species.<sup>1,2,5</sup> In fact, they kept increasing during the 5 days following the second filgrastim dose. Additionally in this particular case, filgrastim seemed to increase proliferation much faster than the previously reported experience in a killer whale (*Orcinus orca*),<sup>3</sup> rising up not only granulocytes but all other leukocyte populations and even red blood cell precursors.

In the authors' opinion, this case suggests that filgrastim is potentially useful to treat neutropenic bottlenose dolphins at a dose of 0,0025 mg/kg i.m. s.i.d. for 2 days, aiding to overcome infection especially in the event the bone marrow is not properly responding.

#### **ACKNOWLEDGMENTS**

The authors would like to particularly acknowledge Mr. Pedro Malabia and Mr. Ezequiel Martí of the Central Hospital of Valencia for the support provided to this case, providing the Neupogen® when we required it. We wish to thank as well all the Marine Mammal team of L'Oceanogràfic and all the students involved in the Animal Care for their daily efforts to save this dolphin. Finally thanks to Dr. James McBain, Dr. Géraldine Lacàve and Dr. Andrew Greenwood for their expertise.

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## BABESIOSIS IN A CAPTIVE HERD OF MUSK OXEN (*Ovibos moschatus*): TREATMENT AND A NOVEL APPROACH FOR PREVENTION

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### Abstract

*Babesia odocoilei* is an intraerythrocytic parasite transmitted by Ixodid ticks that is endemic in wild whitetail deer populations in various geographic locations within the United States.<sup>1,4</sup>

In 2003, two acute fatal cases of *Babesia odocoilei* occurred in musk oxen at the Minnesota Zoological Garden (MZG). A 6-yr-old bull recently acquired from a zoo in Colorado succumbed to the disease in late September after being housed at the MZG's quarantine facility for 13 days. A 1.5-yr-old resident male succumbed to the disease approximately 2 wk later. Both animals presented with sudden lethargy and hematuria and died within 36 and 72 hr respectively. The diagnosis of babesiosis was based upon seeing characteristic intraerythrocytic inclusions on blood smears. The second animal received one treatment of the babesiocide imidocarb (Imizol®, 12%, Schering –Plough Animal Health, Union, New Jersey 07083, USA) at 3 mg/kg i.m., s.i.d., but the first animal died prior to treatment. Postmortem examination of both animals revealed icterus, anemia, hemoglobinuric nephrosis, hematuria, splenic red pulp hyperplasia, abomasal mucosal edema and hemorrhage, and small intestinal intraluminal hemorrhage. *Babesia odocoilei* infection was confirmed by 18S ribosomal RNA gene sequence analysis.<sup>3</sup>

The herd has subsequently been fed Mazuri ADF-16 with ivermectin 10 g/ton (Land O'Lakes Purina Feed, St. Louis, MO 63166-6812, USA) to provide an estimated daily intake of 0.09 mg ivermectin/kg body weight. A previous study on an isolated population of white-tailed deer (*Odocoileus virginianus*) indicated feeding ivermectin treated corn may be beneficial in reducing disease transmission by *Ixodes scapularis*.<sup>2</sup>

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## FUNGAL KERATITIS IN A CONGO PEAFOWL (*Afropavo congensis*) AND A SATYR TRAGOPAN (*Tragopan satyra*)

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### Abstract

Fungal keratitis is an uncommon finding in avian species.<sup>1,2</sup> Cases of confirmed unilateral *Aspergillus fumigatus* keratitis were diagnosed in a 12-day-old Congo peafowl (*Afropavo congensis*) and a 1.5-yr-old Satyr tragopan (*Tragopan satyra*). Both animals were treated with oral antibiotics and antifungals. The Congo was treated, in addition, with topical ocular antibiotics and antifungals. The Satyr was initially treated with a temporary tarsorrhaphy and then later with antifungal nebulization. Both animals had moderate to severe leukocytosis characterized by a heterophilia and a monocytosis. Neither case resolved with medical treatment. The Congo peafowl's affected globe started to collapse during treatment so the bird was immobilized and intraocular natamycin (Natamycin ophthalmic suspension 5%, Alcon Laboratories, Inc., Fort Worth, TX 76134 USA) was administered to decrease the chance of fungal invasion of the surrounding tissue as the globe became phthisical. The Satyr tragopan was anesthetized and the affected globe was surgically enucleated. Both animals remained on oral antifungals post-operatively. White blood cells counts normalized rapidly after the intraocular treatment and the enucleation. These cases represent presumptive localized aspergillus infection with leukocytosis but without systemic or neurologic involvement. Treatment of the affected eye with either an intraocular antifungal or enucleation was curative with no negative long term sequela.

### ACKNOWLEDGMENTS

The authors would like to thank the Department of Ornithology at the Wildlife Conservation Society for their assistance in the care of these birds

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## CAUDA EQUINA NEURITIS IN A BAIRD'S TAPIR (*Tapirus bairdii*)

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### Abstract

A 12-yr-old, female Baird's tapir (*Tapirus bairdii*) presented with inappetance, lethargy, and perineal swelling and pruritis of one day's duration. Tenesmus and urine dribbling were noted within 2 days. Antibiotic, non-steroidal anti-inflammatory, and anti-histamine therapies were initiated with little to no clinical improvement. Immobilization and examination revealed impacted feces in the colon and a markedly distended, atonic bladder. Attempts to manage the atonic bladder with indwelling catheters and medical management were unsuccessful. Due to continued anorexia and lethargy, and poor prognosis, the tapir was euthanatized 19 days after initial presentation. Necropsy findings included a flaccid, distended bladder and urethra, and distended colon and rectum. Histologically, there was inflammation within the cauda equina and lumbar nerve roots consistent with cauda equina neuritis. No other causes for neurologic signs were found, and testing for potential infectious etiologies (e.g., *Sarcocystis neurona*, West Nile virus, and equine encephalitis virus) was negative.

### ACKNOWLEDGMENTS

The authors would like to thank the pachyderm animal care staff at Brookfield Zoo for their help with this case.

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## TREATMENT OF AN AMELANOTIC MELANOMA USING RADIATION THERAPY IN A LESSER MADAGASCAR HEDGEHOG TENREC (*Echinops telfairi*)

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### Abstract

Neoplasia is commonly reported in insectivores.<sup>1-5</sup> In this case, a 15-yr-old male lesser Madagascar hedgehog tenrec (*Echinops telfairi*) presented with a mass caudal to the right ear. Cytology suggested a sarcoma. Surgical removal was attempted. Histologically, the mass resembled a soft tissue sarcoma. The mass recurred at 331 days post operation. Radiation therapy was initiated. Computed tomography was used for staging and, in conjunction with three-dimensional computerized treatment planning software, to permit accurate lesion localization and optimize normal tissue sparing. A total dose of 6480 cGy was administered in 24 fractions over 46 days. Transient hind limb paresis developed during the course of the radiation therapy, but resolved after 7 days with prednisone treatment. Minimal acute radiation toxicity was observed. The mass responded with at least a 90% reduction in volume following radiation treatment. The animal survived 266 days from the initiation of treatment. Lesions at necropsy consisted of a small residual mass and granulation tissue at the site of the initial neoplasm, indicating good local regional control of the tumor, but there were extensive metastases to the spleen and liver. Immunohistochemically, the original, recurrent, and metastatic populations were strongly positive for HMB 45 and weakly positive for S-100 and the final diagnosis was metastatic amelanotic melanoma.

### ACKNOWLEDGMENTS

The authors would like to thank Potter Park Zoo Keepers and staff, as well as Michigan State University's College of Veterinary Medicine veterinarians, veterinary technicians, and students who assisted in this case. We would like to give a special thanks to Holly for performing anesthesia for this animal's radiation treatments.

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## MANAGEMENT OF AN ADULT MALE BORNEAN ORANGUTAN (*Pongo pygmaeus pygmaeus*) WITH CHRONIC AIR SACCULITIS AND PNEUMONIA

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### Abstract

A 21-yr-old male orangutan was acquired with a history of pneumonia at 16 mo, airsacculitis at 6 yr, and stereotypic behaviors. He was treated with marsupialization of the airsac at 7 yr, followed by airsac ostia closure, intermittent antibiotics, nebulization and bronchodilators.<sup>2-4</sup> Quarantine examination revealed nasal and airsac discharge, chronic cough, persistent bronchopneumonia, bronchiectasis, and pulmonary bullae. He was considered stable and continued bronchodilator therapy.

One year later he became lethargic, anorexic and depressed. Examination showed progressive pneumonia and airsacculitis. He was stabilized with antibiotics and airsac removal performed one mo later.<sup>1</sup> Surgery took 6 hr due to extensiveness of the airsac which extended over the chest wall, pectoralis major, into axillae, and invagination around the clavicles. The airsac ostia were 1cm diameter, with fat and muscle layers that were oversewn twice. The cavity was infused with tetracycline and lidocaine to sclerose the dead-space. The wound was closed without skin resection. A small opening was left to allow serosal drainage and residual air venting. He was managed post-operatively with opiates, antibiotics, and an antidepressant (Remeron<sup>®</sup>) for stereotypic behavior, depression and anorexia. He was considered medically stable 9 mo later and epithelialization of the surgical site had occurred.

His bedroom was fitted with a negative pressure air purification system (IQAIR<sup>®</sup>). He has been managed 5 yr post-operatively with periodic exams and antibiotics when necessary. His medical behaviors now include nebulization, i.m. injections and i.v. blood draw. His stereotypic behaviors resolved, antidepressants were discontinued and he has sired an offspring.

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**THE RADIOGRAPHIC DEVELOPMENT OF SEVERE HIP AND SHOULDER DYSPLASIA IN A JUVENILE KOALA (*Phascolarctos cinereus*)**

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**Abstract**

Hip dysplasia is a prevalent problem in the San Diego Zoo koala colony. In order to better understand the development of hip dysplasia, a longitudinal radiographic study of the hips was performed monthly on a juvenile koala from approximately 7 mo old (within 1 mo post pouch emergence) to 2 yr old. During the study, it was discovered that shoulder dysplasia was also prevalent in the San Diego Zoo colony and so a longitudinal radiographic study of the shoulders was performed monthly on the same koala from 21 mo old to 2 yr old. Single views of the shoulders were fortuitously obtained at 7 mo and 12 mo of age. Evidence of femur changes associated with hip dysplasia and humerus changes associated with shoulder dysplasia were radiographically evident as early as 7 mo of age in this case. Ossification of the greater trochanter started at 8 mo of age and was complete by 15 mo of age. Timing of ossification of the femoral head and neck was difficult to determine in this case because neither formed properly, but a review of historic radiographs at San Diego Zoo revealed that ossification should have begun prior to 12 mo and mostly completed by 17 mo of age. Closure of the acetabular growth plate occurred at 19-20 mo of age.

Hip and shoulder dysplasia can be radiographically evident as early as 7 mo of age. Survey radiographs would be recommended at 12 mo of age.

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## ORPHANED MOUNTAIN GORILLAS: NOW THERE ARE FOUR

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### Abstract

Few mountain gorillas (*Gorilla beringei beringei*) have ever lived in captivity. A few infants whose mothers were killed by poachers have been rescued, nurtured back to health, and reintroduced to wild gorilla families. Each orphan died or disappeared soon after its return to the forest. After an infant mountain gorilla was confiscated from poachers in December 2004, MGVP, Inc. and their main conservation partners, DFGFI (Dian Fossey Gorilla Fund International), ORTPN (Office Rwandais de Tourisme et Parcs Nationaux), ICCN (Institut Congolaise pour la Conservation de la Nature) and IGCP (International Gorilla Conservation Programme.) formed a scientific committee to make recommendations for the disposition of this gorilla. The partners ultimately agreed to provide short-term housing and veterinary care for orphaned gorillas representing two subspecies—mountain and eastern plains, or Grauer's, gorillas. The cases histories of this gorilla and three additional ones that have since been confiscated show that with proper veterinary care, this species can survive in captivity. But difficult questions remain regarding their future. After living in close contact with people and another gorilla subspecies, they may be carriers of infections not yet identified. Whether or not the orphans exhibit the full range of normal gorilla behaviors is another important consideration. Even if accepted by a wild gorilla family group, their reactions to human visitors may be unpredictable. The partnership has also established an orphaned gorilla scientific technical committee. The committee is considering all options for the orphans, including reintroduction and long-term sanctuary housing.

### Introduction

Few mountain gorillas (*Gorilla beringei beringei*) have ever lived in captivity. A few infants whose mothers were killed by poachers have been rescued, nurtured back to health, and reintroduced to wild gorilla families. Each orphan died or disappeared within a year after its return to the forest.

In December 2004, when Maisha, an approximately 3-yr-old female mountain gorilla, was confiscated by Rwandan authorities, the five major mountain gorilla conservation organizations agreed to work together and try a new approach. MGVP Inc. joined a committee with DFGFI (Dian Fossey Gorilla Fund International), ORTPN (Office Rwandais de Tourisme et Parcs Nationaux), ICCN (Institut Congolaise pour la Conservation de la Nature) and IGCP (International Gorilla Conservation Programme). The committee recommended that Maisha be housed with two other gorillas orphaned by poaching that were also under the care of the partners. These were Grauer's gorilla (*Gorilla beringei graueri*), a closely related subspecies

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found only in the Democratic Republic of Congo (DRC). Once Maisha reached adult age and showed her first signs of estrus, the plan calls for her return to the forest in the hope that wild gorillas would accept her as a potential breeding female. The other orphaned Grauer's gorillas would eventually be moved to a sanctuary, or reintroduced to the wild, in DRC. In the meantime, protocols were put in place to protect Maisha and the other orphans from exposure to disease.

A temporary quarantine facility was completed in Kinigi, Rwanda (the site of the former mountain gorilla veterinary clinic) in January 2007. The two Grauer's and four additional confiscated Grauer's orphans eventually became Maisha's family group, along with seven caretakers and a facility manager. The orphans have been vaccinated for polio, measles, tetanus, and rabies. They undergo routine TB testing, health exams under anesthesia once a year, and regular deworming. Visitors are not allowed, the vet staff visits the facility only when necessary, and the gorilla caretakers participate in MGVP's employee health program.

### **Case Report**

Maisha has grown substantially and remains generally healthy. However, she and the other orphans have suffered several episodes of respiratory tract illness characterized by malaise, sneezing, and coughing. The disease agents are presumably viral, with secondary bacterial infection, similar to respiratory disease observed in free-living wild mountain gorillas (MGVP research group, unpubl. data).<sup>4</sup> Treatments have included extra fluids, expectorants, and antibiotics (amoxicillin, amoxicillin trihydrate/clavulanate potassium).

A second mountain gorilla orphan, an approximately 3-yr-old male, Kaboko, was confiscated in March 2007. He presented with severe dehydration and a necrotic, maggot-infested wound at the site of a presumed snare injury to his right wrist. Unfortunately, the hand could not be saved. After amputation and quarantine, Kaboko recovered quickly, joining Maisha and the other orphans in May 2007. He has since developed respiratory tract illness similar to that described for Maisha.

In June and July 2007, two additional orphaned mountain gorillas, Ndakasi and Ndeze, were rescued from the Parc National des Virungas in the DRC after their mothers were killed. Both were infants, estimated at 2 and 6 mo old, respectively. They were moved to a private residence dedicated to their care in Goma, DRC. The two orphans are being hand-raised by four caretakers following health protocols similar to those in place at the Rwanda facility.

Shortly after her arrival, Ndakasi suffered a severe respiratory disorder, requiring intensive care with parenteral antibiotics (ceftriaxime), supplemental oxygen, and nebulization therapy for 2 wk. Clinically, her illness closely resembled influenza or respiratory syncytial virus infection of human infants. Serology results are not yet final.

Ndakasi and Ndeze have grown steadily, despite initial problems with recurring diarrhea. The orphans are bottle-fed commercially available human milk formula and have started eating small amounts of forest food. Initially, their stool color and consistency seemed normal for infant

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mountain gorillas (i.e., soft, light yellow). Several months later, both orphans developed white, pasty feces. The problem progressed to watery diarrhea in Ndakasi first. Fecal cultures, cytology, and parasitology screening were normal. She developed anorexia and mild dehydration despite oral and subcutaneous fluid therapy. Ndakasi responded immediately to oral antibiotic therapy (metronidazole.). Ndeze developed a similar condition a few weeks later.

The sudden change to watery stool recurred in each orphan, requiring repeat antibiotic therapy (ciprofloxacin.). Fecal gram stains were initiated on a daily basis. These revealed relatively few bacteria, with a predominance of gram negative rods and few gram positive bacteria. The diagnosis was inadequate fecal flora associated with premature weaning and an artificial environment. Because of the ongoing war in the DRC, there was no way to offer the orphans access to gorilla feces which might help establish normal flora. Each was begun on *Acidophilus* capsules (various brands), mixed in their milk formula twice a day, with excellent effect. Their diarrhea resolved; stool color and consistency changed to normal for 5- to 9-mo-old infant gorillas, brown and formed. When the treatment was discontinued, the white pasty stools recurred. The orphans continue on *Acidophilus*.

## **Discussion**

With proper veterinary care, mountain gorillas orphaned in the wild can survive in captivity. But difficult questions remain regarding the future of the orphans. After years of living in close contact with people and, in Maisha and Kaboko's case, another gorilla subspecies, the orphaned mountain gorillas may be carriers of infections not yet identified.

Samples collected opportunistically from wild mountain gorillas by MGVP field vets over the years reveal varying degrees of exposure and infection with known human pathogens<sup>1-6</sup>. This result is expected given the large percentage (estimated to be 70%) of wild gorilla groups habituated for research and ecotourism. Initial results from Maisha, Kaboko, and a previous orphan show some pathogen exposure, but analysis of the data for the newly arrived orphans continues (Dr. Chris Whittier, pers. comm.)

Whether or not the orphans exhibit the full range of normal gorilla behaviors is another important consideration. Even if accepted by a wild gorilla family group, their reactions to human visitors may be unpredictable.

As Maisha's potential reintroduction approaches, the orphaned gorilla scientific technical committee will continue to meet and make recommendations for her future—and that of the other orphans.

## **ACKNOWLEDGMENTS**

The authors thank all current and previous MGVP field vets, particularly Drs. Chris Whittier and Felicia Nutter who first accepted responsibility for orphaned gorilla care, the orphan gorilla caretakers in Rwanda and DRC, and the rest of the MGVP Africa and US staff.

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## THE BAGHDAD ZOO SURGE

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### Abstract

The Baghdad Zoo offers a rare opportunity for recreation and tourism in war-torn Iraq. The Zoo currently employs 14 full-time staff veterinarians; however, ongoing security issues, insufficient medical and equipment resources, and lack of continuing education pose significant challenges for animal care. A collaborative relationship was forged between the North Carolina State University (NCSU) College of Veterinary Medicine, North Carolina Zoo, the United States Army, and U.S Department of State to assist courageous and dedicated Iraqi zoo veterinarians.

The Baghdad Zoo Director, also a veterinarian, spends about half his day with animal-related activities and the other half with administrative responsibilities. He has been at the Zoo for 18 yr, graduated from Baghdad University in 1979, and holds an advanced degree in parasitology. The other veterinarians spend almost all of their time with animal-related activities, such as overseeing the control and treatment of animals, lab operations, and the maintenance of enclosures. Some of the veterinarians are on the zoo staff, and the rest are considered daily hires. Animal husbandry staff is limited to two keepers with 28 hired on a daily basis, but the veterinarians do most of the nursing care and animal restraint.

The most significant obstacle to the improvement of animal care is the lack of accessible veterinary continuing education. Although there are now nine colleges of veterinary medicine in Iraq with widely variable capabilities, zoological medicine is not incorporated into the curricula, and students are not empowered to explore and create new professional niches outside of livestock medicine. Similarly, fish medicine is not highlighted in Iraqi veterinary schools where many of the graduates are unemployed or underemployed, yet aquaculture has been practiced in Iraq for 2,000 yr and is receiving renewed interest and emphasis. An initial onsite training mission and needs assessment was conducted on October 3, 2007, at the Baghdad Zoo. Included in the training was familiarization on diagnostic ultrasound and other laboratory equipment, hands-on instruction on fish diagnostics and therapeutics, health and husbandry assessment of

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the equine stables and aquarium, dietary recommendations, and examination of the pair of arthritic cheetahs that once belonged to Uday and Qusay Hussein.

We successfully linked the Baghdad Zoo veterinarians with the Advanced Topics in Zoological Medicine course at NCSU. This is a 3-yr-long course designed for graduate veterinarians preparing for careers in zoological medicine. The students taking the class onsite at NCSU number about 20 and include residents, graduate students, and a large number of veterinary students in the first 3 yr of their curriculum. These are primarily zoological medicine focus DVM students who can take the course as an elective. The course has linked with remote sites for several years and other simultaneous links include the North Carolina Zoo, Center for Marine Sciences and Technology (CMAST), the South Carolina Aquarium, and the North Carolina Museum of Natural Sciences. The connection to Baghdad was implemented using open source software, XMeeting that follows an H323 protocol. The North Carolina Zoological Society donated and sent two iMacs to the Baghdad Zoo to allow them to implement the software, and the Army was involved in stabilizing the internet connection. Incompatible bandwidths and early software issues caused initial delays, but this was corrected by reducing the video feed rate to lower bandwidth requirements at the Baghdad Zoo while still sending and receiving a good quality picture. As of March 2008, there was still an echo cancelation problem on the Baghdad side which was handled by muting their sound and transmitting questions and comments via instant messenger. This worked well because the Iraqi veterinarians are more proficient in reading and written English skills than their speaking and comprehension skills, and it also aided us in trouble shooting. Written translation engines are being incorporated into the instant messenger system.

The Baghdad Zoo faces a brighter horizon as opportunities for continuing education are afforded to the veterinarians and staff, along with improved equipment, supplies, and security. The Zoo Director attended the Iraqi National Animal Health Program Workshop in Damascus for a week in January, 2008, also speaking on the Zoo's perspective on control of the five diseases of interest (highly pathogenic avian influenza, foot and mouth disease, brucellosis, bovine tuberculosis, and echinococcosis). As of April, 2008, other planned international collaborative activities included one wk of intensive training at Chester Zoo and efforts to transfer two tigers from a sanctuary in the United States to the Baghdad Zoo. The expanding and diverse animal collection inventory now includes over 800 mammals and birds. The number of visitors has been unprecedented recently, with attendance in the hundreds of thousands over the Eid holiday in October, 2007 - more evidence that the surge is working.

#### **ACKNOWLEDGMENTS**

The authors thank Dr. Alan Nuradin (Multinational Corps – Iraq) and MAJ Jessica McCoy (U.S. Veterinary Corps) for their coordination and participation with the onsite training and assessment, Linda Dunn (IT specialist at the CMAST, Morehead City, NC) for software assistance, CPT Amy Cronin and CPT Jason Felix (U.S. Army) and their soldiers for their support missions to the Baghdad Zoo, and most importantly, the 14 tireless veterinarians and their support staff at the Baghdad Zoo. The authors also acknowledge the critical pioneering efforts of Mr. Lawrence Anthony (Thula Thula Game Reserve and Earth Organization, author of “Babylon’s Ark: The Incredible Wartime Rescue of the Baghdad Zoo” and responsible for passage of “Wildlife in War Zones: United Nations Resolution”) and MAJ William Sumner (U.S. Army) on behalf of the Baghdad Zoo during and immediately following the 2003

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U.S.-led invasion of Iraq. Missions were jointly funded and staffed by the United States Army and the Department of State.

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## AN INTEGRATED NATIONAL PLAN FOR ZOOS AND AQUARIUMS IN DISASTERS

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### **Abstract**

There are over 2000 captive wildlife facilities licensed by the USDA in the United States. Only about 200 are accredited by the Association of Zoos and Aquariums (AZA). The American Association of Zoo Veterinarians (AAZV) and the AZA are committed to advancing captive animal welfare. Preparation for catastrophic disasters is essential to ensuring the welfare of institutional animal dependents in the event of infrastructure collapse or national disease outbreak. Thousands of road side menageries, private wildlife sanctuaries, and undocumented personal collections of captive wildlife exist throughout the country.

The number and diversity of these institutions presents a number of animal welfare challenges. One area of animal welfare that has not received sufficient attention, even by the AZA and AAZV, is advance preparation for disasters. Consequently, an immediate need exists for collaborative efforts to plan, prepare for, and recover from a catastrophic disaster. The AAZV should be at the forefront of animal welfare planning for zoos and aquariums in disasters.

A local or regional catastrophic disaster far exceeds the emergency planning for an institutional emergency such as animal escapes, public injury, or episodic event. Disasters far exceed the capability of not only the institution, but the local or regional community. It may require weeks to months to overcome transportation interruptions, power loss and clean water unavailability. Animal evacuation may be the only alternative to mandatory animal collection depopulation in disease outbreaks or severe environmental conditions. Animal welfare will be at risk along with that of the victimized human population, including the institutional staff.

Dealing with the unique requirements of captive wildlife is as diverse and specialized as the species involved. Specialized diets, thermoregulatory requirements, USDA regulations and public health risks posed by wildlife must be considered in any Institutional Catastrophic Disaster Plan. Triage and resource allocation based on conservation status is an essential component that uniquely resides within the professional animal conservation community. However, no institution alone has the resources to address an organizational or regional infrastructure failure.

Only within the zoologic professional community itself, do the equipment and skills exist to provide animal welfare assistance for related institutions. The relevance, requirements, and collaboration of institutions and professionals acting collaboratively, through an Integrated National Plan for Zoos and Aquariums in Disasters, is the professional solution. Physical

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infrastructure destruction, victim staff displacement, food and supply chain disruption can affect even the most well prepared establishment.

In addition to the physical destruction of facilities in a disaster, information storage and data retrieval can pose greater challenges. Human resource records, payroll and financial information can be lost along with animal records. Assistance by professional colleagues and sister institutions can be the best resource to resolve these data management challenges.

The Incident Command System (ICS) is used by local, state and federal Emergency Management agencies when they respond to disasters. Communication and planning within the ICS structure will assist integration into existing governmental and NGO response groups in a disaster. Likewise, intra-institutional collaboration and assistance can be enhanced by communication through the same ICS management system. The ability to “speak the same language” through the ICS structure will enhance the zoologic professional ability to ensure animal welfare through the worst possible conditions. Inter-institutional wildlife veterinary collaboration is one essential operational component.

ICS is flexible enough to relate directly to zoologic and aquarium institutional needs. It can accommodate the unique concerns of any type of disaster, animal transportation requirements, staff assistance, evacuation and other distinctive wildlife considerations. It is the nexus and means of effective communication with non-animal response professionals.

The unique skill set required for zoo, aquarium and wildlife institutions in catastrophic disasters can only be found within the collaborative network of captive wildlife institutions and professionals. Only through an integrated national plan for zoos and aquariums in disasters can animal welfare catastrophe be mitigated.

This is an important animal welfare and institutional welfare topic that needs more attention and resources than has been devoted in the past. Zoological medicine veterinarians should lead the way, as they are often the primary advocates for animal welfare within their respective institutions. They are uniquely qualified to address the overview considerations for diet, containment, transport, capture and medical concerns for animal welfare in catastrophic disasters.

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## PINNIPED EXHIBIT DESIGN PITFALLS

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### **Abstract**

One of the most challenging and expensive zoological exhibits to build today are those designed for marine mammals. In addition to creating an attractive, functional exhibit, animal welfare issues are an important concern. In particular, a sophisticated life support system designed to maintain excellent water quality must be present. Historically cetaceans have required more stringent water quality parameters than pinnipeds, but it is clear that with the number of chronic pinniped eye problems seen in zoos and aquariums today, that water quality should be assigned a higher priority than it has been in the past. Life support systems should be designed to maximize the cleansing of the water while minimizing the amount of caustic chemical additives. Chlorine spikes and residual ozone levels have been blamed for the majority of eye problems seen in captive pinnipeds. Bright reflective pool colors and a lack of shade may exacerbate existing eye problems caused by chemical spikes or other etiologies. Pinniped exhibit design should include plans for shading at least a portion of an exhibit to offer animals with eye problems or other health issues relief from bright sunny conditions. Light reflective pool colors are often selected to enhance the public viewing of the animals as well as improve the appearance of the water. However, this color choice may result in exacerbating or even causing some of the more common eye problems seen in captive pinnipeds today.

Pinniped exhibit design is often a balance between functionality, practicality, appearance, and budget. Architects or building contractors with little marine mammal experience may err by designing an exhibit that has a pleasing appearance but does not result in an optimal living situation for the animals. Pinnipeds such as California sea lions (*Zalophus californianus*) or harbor seals (*Phoca vitulina*) have specialized physiology which requires careful planning when designing their exhibit. The use of gunite, especially hollow gunite-covered walls in the exhibit construction may render areas unusable to the animals on warm or hot days. The color of the walls may be chosen because it has a pleasing appearance, however some colors are more solar-reflective, and will reflect longwave and shortwave radiation back into the exhibit, causing the exposed and shaded beach areas to exceed the upper critical temperature for sea lions, which is defined as the temperature which requires heat loss for a species.<sup>1</sup>

Otariids, in general, and California sea lions, in particular, appear to be limited in their ability to thermoregulate at high ambient temperatures while they are on land. In natural habitats, sea lions use a variety of behavioral options to maintain a balance between heat loss and heat gain. When pinnipeds are housed in captive situations, their choices of how to thermoregulate may be limited by the health of the animal, the exhibit design and construction, housing constraints imposed on the animal(s), or social interactions with other animals in the exhibit. During periods of time when ambient temperatures reach over 30° C extra caution should be taken to ensure sea

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lions do not become hyperthermic. Providing full shade and a constant water spray to sea lions without access to pools may help to prevent hyperthermia. Adding shade to an existing exhibit, and/or altering the gunite surfaces to decrease both the surface temperature and the reflectance of shortwave radiation, as well as designing the interior of the gunite structures to optimize their properties of heating and cooling should be considered to help minimize the thermal burden on the sea lions.<sup>1</sup> More studies need to be done on harbor seals to determine their upper critical temperature and thermoregulatory abilities.

Hollow gunite rockwork has been utilized to create attractive underwater rock outcroppings and arches. On warm or hot days, the gunite will absorb the radiant energy and transfer that heat directly into the water, causing the water temperature to rise and placing more heat burden on the water chilling system, or if that is lacking, then on the animals themselves.

The ease of use of the exhibit for the animals should also be considered. Sloping beaches are optimal to allow young harbor seal or sea lion pups or geriatric animals to easily exit the pool. Several separate haul-out areas help to minimize male aggression. A visual block whereby one animal may rest out of view of another may also mediate male aggression during breeding season. Exhibits, especially walrus enclosures, should be designed so the public cannot drop or throw items into the pool.

Back areas should be designed to permit multiple functions. Large flat dry areas may be utilized for training purposes or medical procedures. Fully shaded flat pens with a built-in shallow pool are useful for housing mothers with young pups or allowing debilitated animals unsupervised access to water. Back areas should be large enough to accommodate specialized equipment necessary to move larger pinnipeds. Dry haul-out areas and back areas should be designed to be washed down and disinfected directly into sewer drains rather than into the pool.

Walrus exhibits require more planning and careful design than those for other species of pinnipeds. Due to their large size they must have reinforced gates or doors to their exhibit. Walruses are capable of disassembling nuts and bolts and are notorious for damaging or consuming window sealant material from around underwater viewing windows. Walruses may also cause damage to the exhibit when using their tusks. Walruses are more apt to consume foreign objects than other pinnipeds, possibly resulting in surgical intervention or death.

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## EVOLUTION OF A PEST CONTROL PROGRAM AT THE MARYLAND ZOO IN BALTIMORE

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### Abstract

Pest control is an important part of zoo animal welfare programs, as it can be effective in reducing infectious disease, preventing contamination of food, and improving exhibit maintenance and appearance. Several years ago, the Maryland Zoo in Baltimore (MZIB) was cited on its Association of Zoos and Aquariums (AZA) inspection for deficiencies in its pest control efforts. The zoo did not have a pest management program for the whole zoo; different departments were using different external vendors or department personnel for pest control and the effort was overall less than successful in many areas. Around the same time, the zoo was questioned by the Maryland Department of Agriculture (MDA) for ordering regulated pest control products (rodenticides) without a certified applicator on site. The original certified applicator had let his certification lapse several years earlier. Individual staff members were bringing in ant traps and wasp spray from home. Pest control logs were not being utilized to track application or use. The zoo had always had a policy that the veterinarians approved all chemicals, including pesticides, used on grounds but that was the extent of the veterinary department involvement in pest control at that time.

Fortunately, the MDA was very willing to work with us, and the AZA citation encouraged administration to allocate more financial and staff resources to pest control efforts. All areas with outside pest control vendors were consolidated into one vendor as the individual contracts ended. Following discussions with the MDA, it became apparent that certified applicators on site were necessary in order to put out rodent baits, insect growth regulators, spray wasps, treat ponds for mosquitoes and to use topical flea and tick products. Four staff members (two veterinarians, two animal department staff) became certified pest control applicators. The MDA allowed us to apply as Public Agency Applicators, saving the zoo hundreds of dollars in testing and registration fees. One veterinarian is in charge of mosquito control efforts. The other veterinarian is in charge of all chemical approvals, including pesticides, and with the operations manager of the animal department, conducts staff training classes to create "registered users." The operations manager monitors pest control in the animal department areas. The other animal department staff member directs most of the actual application of pesticides, oversees the registered users and works with the outside pest control vendor. The registered users are allowed to use regulated pesticides under the direction of a certified applicator. All regulated pesticides are kept in locked containers and may only be distributed by a certified applicator to the registered users. Every squirt of topical pesticide, ant trap, burst of wasp spray, rodent bait block, and line of insect growth regulator is logged. Horticulture maintains its own certified applicator and log books.

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The biggest surprise was that topical pesticides, such as imidacloprid, fipronil and fly sprays, are regulated pesticides. A veterinary prescription or a pest control log sheet must accompany these products. Different states may have different regulations.

The MZIB now has a well run, effective and legal pest control program. Learn your state regulations regarding pest control, especially topical pesticides. Becoming a certified applicator was not difficult, was very informative, and has been very useful. The MDA Pesticide Regulation section was very willing to work with us to help us become compliant with all regulations. Pest control is not taught in veterinary school, but is very important to the zoo veterinarian and zoo animal welfare programs.

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## LEGISLATION: A NEW THREAT TO THE EXOTIC ANIMAL PRACTITIONER

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### **Abstract**

Animal rights advocates recognize and are utilizing the local, state and national legislative processes to advance their positions. They are accomplishing this by crafting legislation which is often short sighted and detrimental to the animals which they purport to be protecting. An example of this is the legislative effort to ban the use of guides and tethers in elephants. Legislation of this nature was recognized as being a major deterrent and detriment to not only the on going day to day husbandry and care of these species, but also to the exotic animal practitioner, zoo or private, who is charged with the care of elephants. It would also impair much of the ongoing research effort in diagnostics, therapeutics, reproduction and other medical areas involving these species. Consequently, the American Association of Zoo Veterinarians developed a policy statement on the use of guides and tethers for elephants, with the intent of influencing American Veterinary Medical Association (AVMA) policy and future legislative deliberations. The issue was subsequently referred for study to the AVMA's Animal Welfare Committee (AWC). The AVMA's AWC, with input from all parties, addressed in considerable depth the issues surrounding the use of these aids in the management of elephants. The AWC developed a statement which the AVMA Executive Board discussed and passed as official policy in April 2008.

The AVMA Policy on "Elephant Guides and Tethers" is as follows:

Elephant guides are husbandry tools that consist of a shaft capped by one straight and one curved end. The ends are blunt and tapered, and are used to touch parts of the elephant's body as a cue to elicit specific actions or behaviors, with the handler exerting very little pressure. The ends should contact, but not tear or penetrate the skin. The AVMA condemns the use of guides to puncture, lacerate, strike or inflict harm upon an elephant.

Tethers provide a means to temporarily limit an elephant's movement for elephant or human safety and well-being. Tethers can be constructed of rope, chain, or nylon webbing, and their use and fit should not result in discomfort or skin injury. Forelimb tethers should be loose on the foot below the ankle joint, and hind limb tethers should fit snugly on the limb between the ankle and knee joints. Tether length should be sufficient to allow the elephant to easily lie down and rise. The AVMA only supports the use of tethers for the shortest time required for specific management purposes.

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## ANIMAL WELFARE POLICY AND THE ZOO/WILDLIFE VETERINARY COMMUNITY: A VIEW FROM THE TRENCHES

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### **Abstract**

Zoological medicine veterinarians can influence animal welfare policy via their daily activities or by involvement in regional, national, or international policy initiatives. Multiple options exist for addressing animal welfare topics, including working with existing veterinary organizations. One option for zoological medicine veterinarians in the USA is to work with zoological medicine veterinary associations, such as the American Association of Zoo Veterinarians (AAZV) and American Association of Wildlife Veterinarians (AAWV), in the development of policies and recommendations for other actions and activities that can be submitted to the American Veterinary Medical Association (AVMA) for review and possible adoption. However, there must be recognition that some animal welfare issues are more complex than might be initially appreciated, and caution must be exercised to ensure that policy statements, laws, rules, and various communications consider the potential for unintended consequences. Recent AAZV and AAWV position statements were the basis of new and revised AVMA policy statements, and also serve as a means of educating the public and other veterinarians about issues of concern to zoological medicine veterinarians. These are examples that illustrate the potential for zoological medicine veterinarians to influence policies and legislation that affect their daily activities, as well as educate society at large.

### **Introduction**

The 2002 the American Association of Zoo Veterinarians (AAZV) strategic plan was developed with the intent of providing strategies for members of the association to serve as advocates and resources for zoological medicine concerns.<sup>1</sup> Animal welfare is one area where the AAZV and related organizations can serve as a resource. Animal welfare is a subject that is central to the veterinarian's oath and professional responsibilities. However, animal welfare issues are often complex, and nondomestic animals present some unique challenges. Nevertheless, members of the AAZV have many opportunities in their professional activities and through their associations to raise issues for consideration and improve animal welfare. Potential avenues for influencing animal welfare policies include working within zoological medicine associations and/or the American Veterinary Medical Association (AVMA). From the perspective of representatives of the AAZV and American Association of Wildlife Veterinarians (AAWV) on the AVMA Animal Welfare Committee (AWC), it is important for members of the AAZV and AAWV to understand

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the following: 1) the basics of AVMA organizational structure and how AVMA policies are developed, 2) the complexity of the values that are incorporated into assessments of animal welfare, 3) the unintended consequences that can result when a particular animal welfare issue is addressed without considering the ‘big picture,’ and 4) recent updates to AAZV and AAWV animal welfare policy statements.

### **AVMA Organizational Structure**

The AVMA is the largest veterinary organization in North America, has a strong presence in the media, and has staff devoted to both ensuring the robustness of the Association’s scientific policy basis (Schaumburg offices) and influencing public policy (Washington D.C. for federal issues and Schaumburg for state issues). AVMA policy is often highly influential upon the development of laws and regulations in the USA, is often regarded as a source of “gold standards” for animal welfare by multiple animal interest groups, and may be used as a tool to sway public opinion. Consequently, an understanding of the AVMA organizational structure and how policies are developed is important for those wishing to influence AVMA policy. Although the AVMA may be perceived as a monolithic entity, if one wants to influence its policies, it must be remembered that it is composed of people.

Groups of people that play a role in AVMA policy development include: 1) councils, 2) committees, 3) task forces, 4) the House of Delegates (HOD), and 5) the Executive Board (EB). There are many AVMA councils, committees, and task forces that can influence AVMA policy. Committees that have AAZV or AAWV representation include the Animal Welfare Committee (AWC), Clinical Practitioners Advisory Committee, the Committee on the Human-Animal Bond, and the Committee on Environmental Issues. In addition, the American Board of Veterinary Specialties has a seat for a representative of the American College of Zoological Medicine, which includes some members of the AAZV and AAWV.

The AWC comprises representatives from multiple veterinary and veterinary-related organizations and is supported by AVMA staff with subject-matter expertise. The representatives are appointed from taxon-based (e.g., zoo and wildlife, felid, poultry) allied veterinary organizations, state veterinary medical associations, and veterinary-related interest groups (e.g., humane societies). Other individuals serve as consultants or liaisons to the AWC. The AVMA staff supporting the AWC is part of the recently created AVMA Animal Welfare Division. The AVMA staff support the AWC by coordinating the biannual meeting logistics, completing literature reviews and compiling other subject-specific background information, and by many other means. They are particularly valuable for identifying sound science on which to base policy development, which is a core philosophy of the organization. It is worth noting that the AVMA strives to support its allied veterinary groups, which include the AAZV and the AAWV.

Prior to each biannual meeting, AWC representatives are provided with a briefing book that includes references, communications, pending legislation that is of interest to the AVMA, and other information that is relevant to issues to be discussed at the meeting. Literature, documents, and opinions are also exchanged via email, telephone, or surface mail during interim periods as a part of general AWC discussion or subcommittee deliberations. A topic can be introduced to the

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AWC for discussion by an AWC representative, other AVMA entities (e.g., councils, committees, task forces, EB, HOD), an AVMA member, AVMA staff, or upon the request of outside parties. Many AVMA policy statements are based on documents developed by allied veterinary groups. All AWC policies receive close, line-by-line scrutiny from all AWC representatives, and are therefore considered from a wide range of perspectives. If the AWC passes a position statement, it is subsequently forwarded to the AVMA EB for consideration. As part of their deliberations, the EB conducts an independent review of new and revised policy statements. Background on the committee's related discussions in formulating the new or revised policy is provided in the form of a formal written recommendation and clarified as necessary by a member of the EB, who attends AWC meetings as a liaison between the two groups.

AWC representatives are nominated by their respective groups and appointed by the EB. EB members are elected by the AVMA membership within their represented district, and HOD members include representatives from each state or from allied veterinary groups that meet size and AVMA membership criteria. The AAZV and AAWV have sought to have a representative in the HOD in the past, but have not been able to meet the size and AVMA membership criteria. This may change in the future.

### **Complexity of Issues and Unintended Consequences**

Animal welfare issues sometimes appear to be straightforward, but are less so when examined more closely. This is because the issue of concern often does not exist in isolation from other considerations. In addition, personal value systems are an important part of an individual's assessment of animal welfare. For instance, whether one favors a pen, cage, or free-range housing for egg production by laying hens may depend upon how one ranks the importance of housing density, opportunities for dust baths, risk of cannibalism and injury from cagemates, risk of being predated upon, economic efficiencies, disease control, and other factors that are not directly comparable (G. Golab, unpublished) in their overall measurement of welfare.

As a consequence of the complexity of some animal welfare issues, proposals sometimes arise that are intended to address a specific concern, but that result in undesirable, unintended consequences. An example includes recent state and federal bills that are intended to prevent the slaughter of horses for food in the United States. In part, the motivation for these acts represents a societal shift in the perception of horses as livestock to a position as companion animals. As a consequence of some of these bills, there are currently no plants open in the USA for horse slaughter. This has resulted in a well-publicized increase in the export of horses to other countries, some of which have animal welfare standards that fall short of standards that were previously in effect for horse slaughter in the USA. In addition, there is concern that increased numbers of horses will be abandoned or neglected, and efforts are being made to identify means of mitigating this outcome. Whether legislation can be developed to achieve the intended goals of the original bills without undesirable, unintended consequences remains to be seen. Similar challenges exist for nondomestic animals. As an example, a proposed bill that was recently drafted for the House of Representatives was intended to curb trade in bear bile products. Although the intent of this legislation was consistent with AAZV and AAWV policy, a review of the bill by veterinarians and biologists with wildlife perspectives revealed that the original

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language of the bill may have the unintended consequence of halting many legitimate conservation, research, and diagnostic activities for bears, as well as potentially undermine existing legislation that is intended to protect bear welfare. These two examples illustrate the importance of broad perspectives and AAZV/AAWV input into animal welfare issues.

### **Recent AAZV and AAWV Policies and the AVMA**

Two recent policies recommended by the AVMA AWC for approval by the Executive Board illustrate the potential for the AAZV and AAWV to influence AVMA policy development. One concern that was raised by veterinarians with elephant veterinary care responsibilities was over legislation that was pending in various parts of the country to ban the use of guides and tethers for managing elephants. The motivation for this proposed legislation is based on concerns for the abuse of guides and tethers for elephant management, as well as the opinions of those that are opposed to the keeping of elephants in captivity. The intent of banning abuse of guides and tethers for elephants represents genuine concern for elephant welfare. However, the unintended consequences of such legislation include the potential to limit the husbandry and handling options for elephants, including reproductive management strategies such as artificial insemination and management of parturition. Consequently, the AAZV developed a policy statement supporting the appropriate use of guides and tethers for elephants. The AVMA AWC recently recommended a condensed version of this statement for consideration by the EB, and this condensed version was subsequently adopted by the EB.

The AAWV recently developed a position statement with the intent of seeking revisions to an existing AVMA policy, as well as educating other veterinarians and the public on a very complex issue. The existing AVMA policy on leghold traps simply indicated that such devices are inhumane. This statement was of concern because: 1) it was being used as a basis for banning all leghold trap use by some animal activist groups; 2) existing AVMA policy did not specifically acknowledge recently developed international standards for trap design and application;<sup>2</sup> 3) it did not recognize that such traps are needed for many important wildlife management programs, such as otter restoration and mitigation of animal damage to human enterprises; 4) it did not recognize the potential value of leghold traps for control of populations posing risks of zoonotic diseases; and 5) the existing wording had potential legal ramifications for wildlife veterinarians' licenses. Consequently, the research citations and position statement provided by the AAWV provided an important part of the justification for a new AVMA policy on foothold traps that would be more consistent with the published scientific research on foothold traps, justify the use of these devices for achieving important management objectives by natural resource agencies, and educate the public on some of the complexities of trapping as a wildlife management tool.

The above examples illustrate the importance of involvement in AAZV, AAWV, and other associations' committee activities. They also illustrate the potential for educating the public and other veterinarians via development of policy statements that are posted on the Web or communicated via other means. Consequently, a broad range of perspectives contributes to the development of balanced position and policy statements. It is also an avenue to communicate recent publications in peer-reviewed literature that can be applied to improving animal welfare.

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## **Summary**

Recent AAZV and AAWV statements have provided important background for policies recently recommended for adoption by the AVMA AWC. This demonstrates that members of the zoological medicine community can have an influence on AVMA policy and potentially have an impact on legislation and regulations in the USA that impact the daily activities of zoo and wildlife veterinarians. Consequently, for members of the zoological medicine community with specific or general animal welfare concerns, it is important to become involved in the Legislative and Animal Welfare committee of the AAZV, AAWV committees, or other organizations' committee activities. It is important for zoological medicine veterinarians to be actively engaged in dialogue with various segments of society on animal welfare issues, and where possible, provide peer-reviewed publications that can help guide development of animal welfare policies and regulations. It is also important for zoological medicine associations to effectively communicate their knowledge and concerns to the AVMA, as well as to the public.

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**CONSERVATION MEDICINE IN ACTION: THE CASE OF THE ENDANGERED ARGENTINE CROWNED EAGLE (*Harpyhaliaetus coronatus*)**

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**Abstract**

The crowned eagle (*Harpyhalieetus coronatus*) inhabits central and northern Argentina, Paraguay, Uruguay, southern Brazil and Bolivia. Internationally it is classified as endangered and is currently considered one of the most threatened raptors in Argentina. Information about its biology and natural history is limited. Like other large-sized birds of prey, crowned eagles require large territories in which to live and hunt their prey. Human persecution, reduction in the availability of prey, and loss of natural habitat are the major proposed causes for its continuing decline. Furthermore, collisions with vehicles and electrocution by power lines have been proposed as contributing factors to its endangerment. The potential role of macroparasites, microparasites and heavy metals in its decline and population dynamics has not been previously investigated. The aim of this presentation is to summarize biomedical work that is already being conducted with captive and free-ranging crowned eagles in several areas of central Argentina. Goals of this study are to: 1) collect data on baseline physiologic reference values, including hematology, serum biochemistry and plasma cholinesterase activity in captive adults and free-ranging nestling crowned eagles; 2) investigate their exposure to selected macroparasites, microparasites and lead, and 3) train argentine veterinarians, veterinary students, park rangers, biologists and conservation agents in the biomedical sampling and management of crowned eagles. Understanding the role macroparasites, microparasites and metals may play in the decline of these birds in the wild and their effect on ex-situ conservation programs may be essential for developing and maximizing effective conservation strategies for this endangered species.

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## UNRAVELING THE RELATIONSHIP BETWEEN HABITAT AND DISEASE: DO SHADE-GROWN COFFEE PLANTATIONS (“BIRD-FRIENDLY” COFFEE) POSE A DISEASE RISK FOR NEOTROPICAL BIRDS IN COSTA RICA?

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### Abstract

Habitat loss and fragmentation has reached unprecedented proportions and is now the leading cause of wildlife species extinction.<sup>1</sup> The increased “edge effect” and smaller habitat patch area that results from habitat fragmentation poses a variety of threats to biodiversity, including: 1) promoting biological impoverishment as small populations disappear, 2) creating favorable conditions for the persistence of invasive exotic species, 3) favoring the abundance of generalist species, and 4) potentially creating points of artificial aggregation or concentration of wildlife. Disease is one of the most understudied factors affecting wildlife populations. Shade-grown coffee is heavily promoted in the Neotropics as a sustainable agricultural alternative that maintains a diverse and abundant avifauna.<sup>2</sup> However, we hypothesized that the shade-grown coffee plantations in Costa Rica, because they are biologically impoverished, promote the persistence of exotic species, favor generalist species in high abundance and are small areas that artificially concentrate wild birds, might serve as disease risks for neotropical birds. We measured various parameters to estimate health, pathogen prevalence and diversity across three replicates of two habitat types (shade coffee vs. nearby forest patches). From 2005-2007, we captured 1,550 birds. Our results thus far indicate that parasite diversity is highest in birds living in shade-grown coffee and wild birds inhabiting these plantations have a higher prevalence of one directly-transmitted disease. Whereby this is unlikely to cause visible mortality, fitness trade-off from investment in immunity are likely to have ripple effects on reproductive output, which can have more subtle, yet important population effects.

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## MINIMALLY INVASIVE MOLECULAR HEALTH ANALYSIS IN ELEPHANTS

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### Abstract

This paper describes the application of a new assay platform called Stress Response Profiling (SRP) to the analysis of health status in elephants. SRP assays use a large biomarker panel as an indicator of chronically perturbed physiologic homeostasis (“chronic stress”),<sup>1,2</sup> which is a known predictor of increased morbidity, infertility and mortality rates.<sup>3-8</sup> SRP assays have a broad-based sensitivity to diverse types of stressors in multiple species of vertebrates.<sup>2</sup> A minimally invasive SRP assay is based on skin microsamples obtained using routine biopsy procedures.<sup>9</sup> The skin SRP assay was applied to captive African elephants with clinically diagnosed gastrointestinal infections and to healthy wild elephants.<sup>10</sup> The elephant health status was classified using a reference database of SR biomarker profiles corresponding to eight species of normal and stressed animals. The biomarker profiles were converted into pathway profiles indicating that the molecular mechanism of the elephant gastrointestinal infections preferentially involved responses to misfolded proteins and DNA lesions. To rapidly and economically screen samples from 70 free-ranging African elephants sampled in Northern Botswana, we used a multiplexed SRP assay called multi-SRP.<sup>1,2</sup> Statistical analysis of the multi-SRP scores showed correlations with population density, movements, and human-elephant conflict reports. In summary, this paper documents that SRP and multi-SRP assays are suitable for the elephant skin and relevant to both symptomatic diseases and asymptomatic effects of environmental and anthropogenic stressors. We anticipate that the SRP technology might have a wide range of potential applications in veterinary medicine and ecosystem conservation.

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## THROUGH THE EYE OF THE DESERT TO CAPTURE AND COLLAR WILD BACTRIAN CAMELS (*Camelus ferus*)

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### Abstract

The range of the wild Bactrian camel (*Camelus ferus*) has been reduced to only three locations world wide: two in China (Lop Nuur and Taklamakan desert) and one in Mongolia (Great Gobi Strictly Protected Area [SPA] “A”). The population is listed by IUCN as critically endangered. To better understand the movement patterns and habitat needs of wild camels in southern Mongolia several expeditions were undertaken to the Great Gobi SPA “A.” between 2005 and 2007. Using a chase method, where camels are darted from a moving jeep, 8 camels were chemically captured using a combination of etorphine-butorphanol-detomidine and tiletamine-zolazepam. In 7 of the camels darted, anesthesia and subsequent recovery was smooth and without complication and these animals were outfitted with satellite radio-collars. However, one camel died during anesthesia of undetermined cause. Extremely low ambient temperatures in the winter season and dry, hot and windy summer conditions greatly hampered fieldwork.

### Introduction

Conservation efforts often require capture and handling of animals. Wildlife capture and anesthesia is a complex operation that can cause significant injury or death. Although skill, experience and appropriate veterinary training can minimize the risk, it is necessary to evaluate whether the procedure is necessary and that the potential gains in knowledge for conservation of the species outweigh the risks.<sup>7,12</sup>

The Great Gobi Strictly Protected Area (SPA) “A” located in the southwestern part of Mongolia bordering the People’s Republic of China is one of the world’s great desert ecosystems. The extremely harsh environment has given rise to unique, particularly well-adapted species, many of which are found nowhere else in the world.<sup>24</sup> The large mammal fauna consists of several rare or globally threatened species, namely the wild Bactrian camel (*Camelus ferus*), the Gobi bear (*Ursus arctos gobiensis*), the snow leopard (*Uncia uncia*), the argali wild sheep (*Ovis ammon*) and the Asiatic wild ass (*Equus hemionus*).<sup>9,14,24</sup> Human pressures for pastures and water on the edges of the Great Gobi SPA and in its buffer zones have substantially increased since the early 1990s and are believed to have lead to significant habitat degradation in some areas.<sup>18</sup> Thus in

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June 2003 a UNDP / GEF founded project "Conservation of the Great Gobi Ecosystem and its Endangered Species" was initiated. The project aims to ensure the long-term conservation of the Great Gobi ecosystem and its umbrella species by building the capacity of the park management authority, improving participation of local communities in the management of the protected area (SPA) and supporting research and environmental monitoring activities through the development of a model conservation program using the wild Bactrian camel as an "umbrella species".<sup>18</sup>

The range of the wild Bactrian camel has been reduced to only three locations world wide: two in China (Lop Nuur and Taklamakan desert) and one in Mongolia (Great Gobi A SPA). The population is listed by IUCN as critically endangered and there remain an estimated ~600 animals in China and between 350 to 1,950 in the Great Gobi A SPA.<sup>3,4,11,14,23</sup> The large range in the population estimate is due to the large size, remoteness and harsh climate of the Great Gobi A SPA. Past population estimates often lack a detailed description of the survey methods and more recent surveys use different methods or lack documentation of search effort.<sup>2,11,13,14,17</sup> Thus, there is still significant debate concerning population numbers and trends.<sup>11</sup> The general belief is that the population is stagnating or declining due to (1) low recruitment of calves caused by wolf predation, (2) habitat deterioration, and (3) illegal hunting.<sup>10,17</sup> There are also great knowledge gaps concerning the ecology of the wild camels and data on population dynamics,<sup>8,9</sup> behaviour,<sup>9</sup> habitat use,<sup>16</sup> movement patterns,<sup>13</sup> and veterinary aspects.<sup>1</sup> Even the genetic status and the purity of the three wild Bactrian camel populations has not yet been fully resolved and preliminary results need to be treated with caution.<sup>5</sup>

## Material, Methods and Results

In order to capture camels for radio collaring we employed a chase method where the camel is darted from a moving jeep. This method has been successfully used to dart grey wolf (*Canis lupus*), Asiatic wild ass (*Equus hemionus*), Przewalski's horse (*Equus f. przewalskii*),<sup>20-22</sup> and a single wild camel and it is traditionally employed by poachers with 12-ga shotguns.<sup>1</sup> For remote dart application we used a modified high pressure CO<sub>2</sub> dart gun (Daninject JM,<sup>TM</sup> Dan-Inject ApS, 7080 Børkop, Denmark) with a short 4-cm barrel as this greatly facilitates movement in the jeep.

During the winter 2005 expedition<sup>19</sup> we used a combination of 4.4 mg etorphine (M99, C-Vet Veterinary Products, Leyland, UK); 10 mg butorphanol (Torbugesic, Fort Dodge Animal Health, Iowa, USA); 13 mg detomidine (Domosedan, Orion Corp. FI-02200 Espoo Finland) and 160 mg tiletamine-zolazepam (Tilest 500; Virbac, 06515 Carros, France) to anesthetize one male and two female camels. Because the ambient temperatures varied between -15 and -30°C during the day, all drug combinations were supplemented with propylene glycol (Sigma-Aldrich GmbH, 1120, Vienna, Austria) as an anti-freeze. Once an animal was identified, it was chased (35-40 km/h) until the jeep was able to approach within approximately 10-15 meters on a parallel track. It was then easily darted in the rump using standard pressure settings. It is essential to define a chase cut off time before the procedure is started. Our experience has shown that a chase time of 15 min is easily tolerated without a significant increase in body temperature. To ensure accuracy and reliability, only new, 3 ml darts (Dan-Inject ApS, 7080 Børkop, Denmark) were used during the captures. Once an animal was darted, we attempted to maintain visual contact, but did not

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disturb the animal before it was fully immobilized. Once the animal was recumbent, we approached on foot from behind and secured the head. Anesthesia was reversed in all cases with 200 mg naltrexone, i.v. (Trexonil, ZooPharm, Inc., Fort Collins, CO 80524, USA) and 25 mg atipamezole, i.v. (Antisedan, Orion Corp., FI-02200 Espoo, Finland).

During the summer 2007 expedition, six camels were captured using the method described above.<sup>6</sup> In five of six camels darted, anesthesia and subsequent recovery was uneventful. However, the first animal, an old bull (>15 yr) died during the capture event. This bull was easily darted as he ran much slower than the other animals in his group. It took 40 min after darting to locate the bull. The animal was in acute respiratory distress when approached. Naltrexone 250 mg, i.v., was immediately administered and the animal's respiratory rate and effort improved. However cardiac arrest occurred after 10 min and the animal could not be resuscitated. The cause of death remains unknown. Due to the lack of water and approaching sunset we could not perform a complete necropsy. The animal appeared extremely bloated and was noted to have regurgitated. We cannot exclude that it aspirated gastro-intestinal contents leading to its death. Furthermore, we speculate that this animal possibly had a pre-existing medical condition that was also responsible for the very slow pacing.

## **Discussion**

Based on previously published reports<sup>1</sup> and subsequent to our experience, we recommend a combination of 4.4 mg ethorphine, i.m., 10 mg Butorphanol, i.m. and 13 mg detomidine-HCl, i.m. for the immobilization of adult wild camels. Butorphanol is a mu-opioid receptor antagonist and through its action potentially alleviates the marked respiratory depression induced by the ethorphine at the mu-receptor and potentates the sedative effect at the kappa and sigma receptors. By the addition of 160 mg, i.m. of tiletamine-zolazepam induction to sternal recumbency is more rapid.

Etorphine was reversed with 200 mg naltrexone, i.v. and 25 mg atipamezole, i.v. Naltrexone has a longer half-life than the standard antagonist-agonist diprenorphine (Revivon, C-Vet Veterinary Products, Leyland, UK) thus eliminating renarcotization, which could leave recovering camels vulnerable to predation or injury.

Losing an animal during field anesthesia is a painful experience. With the male camel in our study, we felt that the long recovery time (and a possible pre-existing medical condition) contributed to the animal's death. Immobilizing an animal that exhibits abnormal behavior should be avoided. Decreasing the ethorphine by 1mg and omitting the Telazol, results in standing - sternal sedation which makes it easier to locate the animal in the steppe. However, this reduced dosage leads to a prolonged pacing phase in which the animal can cover a greater distance and potentially injure itself.

It is the view of these authors that the risk and cost involved in immobilizing these animals is outweighed by the need to base the wild camel management plan on sound scientific data. Some management issues are urgent and require immediate action. It is our hope that the rest of the plan will remain fluid and integrate the scientific field based knowledge as it becomes available.

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This contribution is dedicated to the memory of Gobi A ranger Mr. Chojjim - a true Gobi man.

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**FIELD APPLICATION OF RIGID ENDOSCOPY FOR DETERMINING GENDER, REPRODUCTIVE STATUS, AND DISEASE IN FREE-RANGING ENDANGERED STURGEON (*Scaphirhynchus* spp.) FROM THE MISSISSIPPI RIVER**

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**Abstract**

The shovelnose (*Scaphirhynchus platyrhynchus*) and pallid sturgeon (*Scaphirhynchus albus*) are both threatened species due to habitat loss and commercial pressures for an alternative source of cavier. Intensive research and management efforts by federal and state organizations have been underway since 1990 in an attempt to determine and stabilize free-ranging populations. One important aspect of evaluating population dynamics includes detailed life history, and in particular survivorship, recruitment, reproduction and disease. Identification of gender and determination of reproductive status has been an area of intense interest for ichthyologists studying this monomorphic species.<sup>1-4</sup>

Forty-three sturgeon were caught from the Mississippi River in Tunica, MS using baited hooks, and transferred to temporary holding nets. All fish were consecutively anesthetized using 90 mg/L buffered tricaine methanesulfonate (Tricaine-S, Western Chemical Inc., Ferndale, Washington 98248, USA) for coelioscopy using a Tele Pak and 2.7 mm telescope system (Karl Storz Veterinary Endoscopy, Goleta, California 93117, USA). Following a 3 mm stab incision in the ventral midline approximately midway between the pelvic and pectoral fins, a 2.7 mm telescope housed within a 14.5 Fr operating sheath was inserted into the coelom. Coelomic insufflation was achieved using sterile saline delivered by infusion line to one of the sheath ports. Gender and reproductive status were determined, and confirmed by gonadal biopsy. Saline was manually evacuated using external pressure, and in select females sonic transmitters were inserted before closure. All fish recovered uneventfully and were successfully released.

Coelioscopy offered unparalleled visibility of the gonads compared to previously described endoscopic and ultrasonographic procedures, and was able to consistently identify gender and accurately stage reproductive status.<sup>2,4</sup> Coelioscopy is recommended as a minimally-invasive technique for the field determination of fish gender and reproductive staging.

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## MULTI-INSTITUTIONAL COLLABORATION IN VETERINARY BASED RESEARCH IN KRUGER NATIONAL PARK: A MODEL FOR EFFECTIVE CONSERVATION PARTNERSHIPS

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### Abstract

The routine capture of various animals each year for management purposes in the Kruger National Park (KNP), South Africa, provides a remarkable opportunity for conducting ongoing research projects that will be of benefit to the management and conservation of wildlife species both in situ and those held in captive facilities including zoos. However, a limiting factor to this has been human capacity to facilitate the implementation of such projects.

In 2004, the Africa Research Consortium (ARC), a partnership of zoos within the USA, was created with the purpose of raising funds to establish a veterinary research technologist position based within KNP. This position has since been successfully realized. Accommodation for both the technologist and visiting researchers has also been built. The responsibilities of the incumbent includes the collection of biological samples during routine animal captures, and the processing, storage and distribution of these samples as required by registered research projects.

To date this collaborative effort between ARC and KNP has resulted in the successful completion of a number of projects. These include developing a novel combination of immobilizing drugs for the capture of hyaena, testing recently developed diagnostic tests for the detection of bovine tuberculosis (*Mycobacterium bovis*) infections in white rhino, developing genetic based techniques for differentiating between black and blue wildebeest, determining the genetic variation within the KNP wild dog population, and conducting prevalence surveys for bovine tuberculosis in the KNP buffalo population. Importantly, the technologist routinely facilitates the collection of biological samples from all animal species captured, amounting to approximately 700 animals annually. These samples are put into long-term storage from where they are made available to researchers as required.

Due to the initial success of this multi-institutional collaboration an increase is anticipated in the number of participants in ARC as well as the quantity and scope of research projects conducted each year.

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## **ACKNOWLEDGMENTS**

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**VETERINARY AND HUSBANDRY CHALLENGES OF THE CRITICALLY ENDANGERED MOUNTAIN PYGMY POSSUM (*Burramys parvus*) A SMALL MARSUPIAL SUBNIVEAL HIBERNATOR**

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**Abstract**

Mountain pygmy possum (*Burramys parvus*) from Mt Buller represent a separate Ecologically Sustainable Unit of a critically endangered species.<sup>1</sup> The possum is an omnivorous, subniveal hibernator.<sup>2</sup> Its body temperature will reduce to ambient during hibernation.<sup>3</sup> The ambient temperature 30 cm below the surface in boulder fields at elevations between 1400-1800 m was measured as 0.5°C for 5 mo using 18, Onset Hobo H8 data loggers (Onset 470 MacArthur Blvd Bourne, MA 02532, USA). To simulate this in captivity, refrigerated shipping containers (12 m × 2.5 m × 2.5 m) fitted with three phase 5.2 horsepower motors and reheat elements with a thermal range of -5° - 30 °C were used to house 20 individuals between 3 - 20°C.

The diet of the mountain pygmy possum in the wild is seasonal and was mimicked in captivity with emphasis on providing linoleic acid as this has been shown to effect the abdominal fat composition and hibernation duration of other mammals.<sup>4</sup> All animals were anesthetized, a whole body radiograph was taken and serum hematology and biochemistry values measured. The main ectoparasites collected were *Ornithonyssus* and *Glycyphagus* sp. Treatment of mites was successful using moxidectin (Cydectin Injectable, Fort Dodge Animal Health, Maitland Place, Baulkham Hills NSW, 2153) p.o. at 0.2 mg/kg concurrently with a substrate change and repeated after 2 wk if mites were seen. Daily urine free catch samples were used to determine the presence of oestrus. Mate choice selection trails based on the methods used in other dasyurids, were conducted to determine the order of mate introduction in this multiparous species.<sup>5</sup>

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**EFFECT OF BOMA CONFINEMENT ON HEMATOLOGIC AND BIOCHEMICAL VALUES IN FREE-RANGING WHITE RHINOCEROS (*Ceratotherium simum*) IN KRUGER NATIONAL PARK, SOUTH AFRICA**

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**Abstract**

Although African rhinoceros are captured and translocated each year, there is limited data on effects of these procedures on physiologic values in free-ranging rhinoceros.<sup>1,2</sup> The application of new diagnostic technology would permit more informed translocation decisions in the field since this information can now be obtained in some field laboratories. The primary objective of this study was to address antemortem diagnostic testing to assess the health status, provide a baseline for free-ranging rhinos, and comparison for any changes that may occur during boma confinement.

Sample collection was performed during the 2007 capture season for white rhinoceros (*Ceratotherium simum*) in Kruger National Park (KNP). Protocols were developed so the KNP veterinary technician was able to collect samples in the field. A portable chemistry analyzer (Abaxis) was used to run biochemical analyses. Hematologic results were based on estimated white blood cell counts and differential counts.

Preliminary analyses used statistical comparisons between paired samples from individuals caught in the field and resampled in the boma using the Student's t test at p=0.1. The results showed statistical higher mean white blood cell counts, relative eosinophil counts, GGT, alkaline phosphatase, phosphorus, and magnesium in animals when they were first captured. In contrast, mean values for albumin, BUN, and CPK were statistically higher after boma confinement. Implications include possible changes in immune parameters, nutritional, and hydration status while in the boma which may lead to possible health complications. Awareness of these changes may provide keys to management changes that can prevent or minimize these potential issues.

**ACKNOWLEDGMENTS**

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## HEALTH STUDIES OF TWO AVIAN SPECIES, THE FLOREANA MOCKINGBIRD AND GALAPAGOS HAWK, FACING DIFFERENT CONSERVATION CHALLENGES IN THE GALAPAGOS ISLANDS

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### Abstract

The Galapagos Islands remain the only conserved tropical archipelago in the world, with over 95% of its biodiversity intact. In fact, none of the 58 resident bird species have gone extinct. Although this may soon change as a number of species are on the brink of extinction, and disease has been confirmed as a known factor in a number of population declines. In this presentation we discuss two health studies on endemic Galapagos avian species, the Floreana mockingbird (*Mimus trifasciatus*) and the Galapagos hawk (*Buteo galapagoensis*). These two species are experiencing very different conservation challenges and exemplify how the conservation medicine approach offers a means to better understand these challenges and to develop appropriate conservation strategies for the future for these species, and other Galapagos endemics.

### Introduction

The Galapagos Islands remain the only conserved tropical archipelago in the world, with over 95% of their biodiversity intact.<sup>3</sup> In fact, none of the 58 resident bird species, of which 52% are endemic, have gone extinct; however this may soon change as a few species are on the brink of extinction, with disease a known factor in a number of population declines. Anthropogenic changes, due to the rapidly expanding resident human population (estimated at 20,000 in 2006 up from 12,000 in 1996) and tourist population (estimated at 140,000/yr in 2006 up from 60,000 in 1996) ([www.ingala.gov.ec/galapagosislands](http://www.ingala.gov.ec/galapagosislands)) are occurring in Galapagos on an unprecedented scale. Human-induced changes may be having direct and indirect impacts on the health of avifauna (and other wildlife) in Galapagos. Additionally, conservation management measures for the 97% of the archipelago which is the Galapagos National Park may themselves have impacts on the health of the avian species.

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In 2000 the WildCare Institute, Saint Louis Zoo and the University of Missouri – Saint Louis created the Center for Avian Health in the Galapagos Islands, in collaboration with the Charles Darwin Foundation and Galapagos National Park, to better understand the disease threats to the avifauna of Galapagos.<sup>5-7</sup> In this presentation we discuss two of our many projects; an avian health survey for the re-introduction of the Floreana mockingbird (*Mimus trifasciatus*) and the determination of the cause(s) of mortality in the juvenile cohort of Galapagos hawks (*Buteo galapagoensis*) on Santiago Island. These two endemic Galapagos species face very different conservation threats and necessitate different conservation medicine approaches.

### **Avian Health Survey for the Re-introduction of the Floreana Mockingbird**

The Floreana mockingbird (*Mimus trifasciatus*), with an estimated population of 136 birds in 2007, is one of the rarest bird species in the world. The potential causes of extinction for the Floreana mockingbird include loss of genetic variation, environmental (e.g., climate change), introduced species (e.g., black rats), and diseases. Although poxvirus is the pathogen of greatest concern for the Floreana mockingbird,<sup>9</sup> a number of other disease agents, including *Mycoplasma gallisepticum* and *Philornis downsi*, which are present in the archipelago, and diseases that may soon arrive (e.g., *Plasmodium relictum* and West Nile virus) are thought to cause morbidity and mortality in this species.

Having been extirpated from its native Galapagos island of Floreana over 125 yr ago, the remaining individuals reside on Floreana's two small satellite islands, Champion and Gardner-by-Floreana. It is for this reason that a collaborative effort, "The Re-introduction of the Floreana Mockingbird to its Island of Origin" was initiated.<sup>2</sup> Within this plan, a top priority for phase one is to determine disease threats associated with re-introducing this species back to Floreana.

The objectives of the health component of the re-introduction plan are to: 1) determine the prevalence and distribution of infectious and parasitic disease agents in domestic and wild birds on Floreana, 2) determine the prevalence and distribution of infectious and parasitic disease agents in the remnant Floreana mockingbird populations on the two satellite islets, and 3) make recommendations based on these data for how to best execute mockingbird re-introductions to Floreana. In summary, the health work has been designed to determine disease threats that may undermine the plan prior to any re-introductions.

In early 2008 we collected biomaterials from one half of the Floreana mockingbird population and a number of chickens and passerines on Floreana. Laboratory diagnostic testing will soon follow. Data on the prevalence and distribution of the infectious and parasitic agents within and between the different study populations (e.g., mockingbirds, chickens, and passerines) are one of the key data sets for making recommendations as we move forward with the re-introduction of Floreana mockingbirds back to Floreana.

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## Determination of the Cause(s) of Mortality of the Juvenile Cohort of Galapagos Hawks on Santiago Island

The endemic Galapagos hawk is the only hawk species in Galapagos. Currently there are breeding populations on eight of the main islands with the two largest populations (approximately 500 hawks per island) residing on Isabela and Santiago. During our long-term population studies of the hawks on Santiago, we noted a decline in the number of juvenile hawks (1-4 yr of age) on the island in a 3-yr period from 2005 (n=160) to 2007 (n=0).<sup>8</sup> Therefore, in 2008 we initiated a study to determine the cause(s) of this decline. Interestingly, the complete loss of the juvenile cohort on Santiago coincides with the completion of the largest island goat eradication campaign in the world, with approximately 100,000 goats eradicated on Santiago in the period 1996-2006.<sup>1,4</sup> We have focused our study on hypotheses of the possible cause(s) of mortality in this cohort. These hypotheses are that with the eradication campaign that resulted in the removal of 100,000 goats on the island, vegetation has recovered to a point in which non-territorial hawks can no longer hunt well, some other inadvertent response to the removal of goats (e.g., loss of a food source), or a direct negative impact from the methods used to eradicate the goats.

A short trip in early 2008 documented the presence of at least 60 non-territorial birds, using the same census techniques as in the 2005-2007 surveys. However, the vast majority of these were first-year birds produced during the 2007 breeding season. This suggests that whatever problem was associated with the disappearance of the juvenile class between 2006 and 2007 has not persisted, and thus the hypotheses of too much vegetation cover or loss of a food source are most likely incorrect. We recovered one set of dried hawk remains, containing skin, bones, and feathers. We currently are soliciting information on whether and how these remains can be informative to test other hypotheses regarding causes of death (e.g., toxins). It is good news that this unknown problem does not appear to have persisted, although we will continue to monitor this population through other seasonal changes.

Since Santiago represents approximately half of the world's population of this species, we want to do everything we can to understand the cause(s) of this sudden disappearance of an entire age class and to unravel whether the island restoration project has inadvertently affected the health of the hawks on the island, or whether this represents a shift back to a more natural population level following the removal of an important food source. These data are important to obtain before similar management programs (e.g., invasive vertebrate eradication) are used on other islands.

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## DISEASE RISK ASSESSMENT FOR REINTRODUCTIONS – ARE WE RELYING ON THE WRONG DATA?

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### Abstract

The importance of disease risk assessments and disease surveillance for reintroduction programs is universally accepted and comprehensive tools are now available to guide the process. However, the traditional approach of developing a list of diseases of concern, testing release candidates for those diseases, and making release decisions based on the test results suffers from several fundamental problems. First, most diagnostic tests are designed to detect an infectious agent (or the host response to an agent) in an animal showing appropriate clinical signs. Most diagnostic tests that are validated for the host species perform well in this situation. However, test performance declines significantly when such tests are used to screen apparently healthy animals (e.g., reintroduction candidates) for the same agent, particularly if the agent has a low prevalence in the population. This results in frequent false positives that are misinterpreted as true positives. The problem is compounded when multiple tests are performed in parallel, as typically happens with surveillance testing for reintroductions or translocations. In addition, there are few diagnostic or screening tests that have been validated for use in nondomestic species. This leads to reliance on potentially misleading test results for critical decision-making in reintroduction programs. A second problem occurs when surveillance is only conducted on the source population. In order to adequately evaluate the risk posed by the presence of an agent in the source population, one needs to know whether the agent is also present in the destination population. However, it is seldom feasible to sample sufficient numbers of animals in the field to answer this question, and the same interpretive problems with surveillance tests described above would apply. Finally, emerging disease outbreaks in domestic animals and wildlife over the past few decades have revealed that agents of highest concern may not even have been identified yet, making surveillance testing impossible.

Based on our experience with reintroduction and translocation programs for several species, we propose a different approach to disease surveillance and risk assessment. First, if all avenues of potential disease exposure are evaluated for the source and destination populations, a qualitative risk category can be assigned to each population. Movement of animals between similar risk categories reduces opportunities for inadvertent disease transmission, and reduces reliance on unreliable surveillance tests. While this is conceptually easier to apply to field translocation programs, it can also be used for reintroductions from zoos, but it may reveal that animals destined for reintroduction require greater biosecurity than zoos have traditionally provided.

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Second, we believe that the most important disease surveillance information available to reintroduction program managers is long-term necropsy data from the population, not results of screening tests performed on individual, apparently healthy, candidates for reintroduction. Comprehensive postmortem evaluations suffer from few of the interpretive problems described above, and have the advantage of providing surveillance for a wide range of agents of concern (both anticipated and unanticipated), in a broader cross-section of the population, over a broader window of time. The use of qualitative risk categories and comprehensive necropsy data, combined with judicious use of carefully selected surveillance and confirmation tests, has the greatest potential to reduce disease risk for reintroduction programs. We propose that zoos engaged in reintroduction efforts place more emphasis on population-level biosecurity and comprehensive postmortem investigations to mitigate disease risk.

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## USE OF MEDETOMIDINE IN CHEMICAL RESTRAINT PROTOCOLS FOR CAPTIVE AFRICAN RHINOCEROSSES

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### Abstract

A wide variety of drugs or drug combinations have been used for anesthesia or chemical restraint of captive and free-ranging black (*Diceros bicornis*) and white rhinoceroses (*Ceratotherium simum*).<sup>2,3,8-10</sup> Most of these anesthetic protocols utilize a potent opioid, such as etorphine, along with an alpha-2-adrenoreceptor agonist, such as xylazine or detomidine, or a neuroleptic such as azaperone or acetylpromazine.<sup>3,8,10</sup> Recently, chemical restraint protocols utilizing the mixed opioid agonist-antagonist, butorphanol, have become useful and advantageous, particularly in the white rhinoceros.<sup>2,9,10</sup> The potent, selective, and highly specific alpha-2-adrenoreceptor agonist, medetomidine, has been a useful drug to incorporate into anesthetic protocols for many wildlife species.<sup>1,4-7,11</sup> Medetomidine is a potent sedative and analgesic with anxiolytic properties and has been shown to provide good muscle relaxation with minor physiologic changes in several species.<sup>7,11</sup>

Etorphine ( $1.71 \pm 0.35$  µg/kg) was used with medetomidine ( $2.48 \pm 0.38$  µg/kg; 21 procedures) or medetomidine-midazolam ( $3.16 \pm 0.60$  µg/kg -  $20.9 \pm 2.0$  µg/kg; 13 procedures) in eight black rhinoceros for in-facility transport, electroejaculation, dental extraction, endoscopy, and other clinical procedures. Supplemental drugs used to maintain chemical restraint included ketamine, medetomidine, etorphine, and guaifenesin 5% in dextrose. Chemical restraint was reversed with naltrexone ( $119 \pm 28$  µg/kg) and atipamezole ( $14.7 \pm 3.8$  µg/kg).

Butorphanol ( $63.7 \pm 1.2$  µg/kg) was used with medetomidine ( $2.64 \pm 0.17$  µg/kg; eight procedures) in six white rhinoceros for electroejaculation, endoscopy, ophthalmic surgery, and other clinical procedures. Supplemental drugs used to maintain chemical restraint included ketamine, butorphanol, medetomidine, and guaifenesin 5% in dextrose. Chemical restraint was reversed with naltrexone ( $233 \pm 29$  µg/kg) and atipamezole ( $28.3 \pm 2.9$  µg/kg).

The addition of medetomidine to chemical restraint protocols for captive African rhinoceroses appears to improve muscle relaxation and analgesia and reduce the need for supplementation during long procedures, while maintaining clinically acceptable cardiopulmonary physiology.

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## USE OF THE ALPHA-2 AGONIST DEXMEDETOMIDINE FOR THE IMMOBILIZATION OF CHEETAHS (*Acinonyx jubatus*)

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### Abstract

Dexmedetomidine is the active enantiomer of racemic medetomidine.<sup>1,2,4</sup> Dexmedetomidine has been used extensively in human medicine and was developed as a reversible adrenergic agonist agent with potent analgesic, anxiolytic, and sedative effects.<sup>3</sup> Dexmedetomidine acts specifically and selectively on alpha-2-adrenoreceptors, more so than on alpha-1-adrenoreceptors at a ratio of 1600:1. Due to this high selectivity of alpha-2 receptors, dexmedetomidine offers more potent sedation and analgesia with less cardiovascular depression than alpha-1 receptor activation.<sup>1,2</sup>

Nineteen captive cheetahs (*Acinonyx jubatus*) at the Cheetah Conservation Fund (CCF), Namibia were immobilized for annual examination. The cheetahs received dexmedetomidine (Dx) dosages ranging from 12.8-22.5 µg/kg ( $17.4 \pm 3.02$  µg/kg) and ketamine (K) dosages of 4.2-5.9 mg/kg ( $5.01 \pm 0.56$  mg/kg) given intramuscularly. Sedation, analgesia and muscle relaxation were rated subjectively. Temperature, heart rate, respiratory rate, end-tidal CO<sub>2</sub> and indirect blood pressure were measured every 5 min for 1 hr. Blood gas analysis was done within the first 15 min after initial injection. After 1 hr, the cheetahs were placed on isoflurane by endotracheal tube. At the end of each procedure the sedative effect was reversed using a dose of atipamezole administered intramuscularly at 10 times the initial dose of dexmedetomidine

The combination of dexmedetomidine/ketamine produced a fast and smooth induction with excellent sedation level for minor procedures and transportation. Although the cardiovascular parameters were similar to those observed with medetomidine (M) and ketamine (K) combinations, the mean heart rate with the Dx/K combination was slightly higher than M/K, but the difference was not statistically significant. Two cheetahs showed marked bradycardia and few individuals experienced ventricular premature contractions as noted on the ECG. The dose of isoflurane needed for anesthesia maintenance was half the dose of isoflurane used for the maintenance of cheetahs induced with a medetomidine/ketamine combination. Recoveries were subjectively faster with the dexmedetomidine combination compared with M/K.

Dexmedetomidine, in combination with ketamine, is a safe alternative for the immobilization of captive cheetahs. Although the cardiorespiratory and clinical effects of dexmedetomidine did not differ significantly from M/K combinations, less bradycardic effects and the shorter recovery times make use of this combination more suitable than M/K combinations.

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## EFFECTS OF MULTIPLE ADRENERGIC AGONISTS ON BLOOD PRESSURE IN GREEN IGUANAS (*Iguana iguana*)

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### Abstract

Green iguanas (n=4) were instrumented with DSI Physiotel PA-C20 implantable pressure transducers to measure direct blood pressure. Medetomidine (50 µg/kg), followed 30 min later by atipamezole (350 µg/kg), was administered intravenously (i.v.) to awake iguanas to assess the effect on systemic blood pressure. In a separate experiment, hypotension was induced with isoflurane at 2.5-3%; direct blood pressure was monitored during sequential infusions of dopamine (0-40 µg/kg/min), phenylephrine (0-5 µg/kg/min) and norepinephrine (0-0.8 µg/kg/min).

No sedative or hypertensive effects were observed with medetomidine. Preanesthetic mean arterial pressure (MAP) was measured for all animals (MAP range: 65-85 mmHg). Isoflurane delivered at 2.5-3% (1.25-1.5 times the minimum alveolar concentration in green iguanas)<sup>1</sup> resulted in sustained depression of blood pressure, with MAP < 40 mmHg in all animals. An increase in systemic blood pressure was only observed with norepinephrine, which increased systemic blood pressure to preanesthetic levels or greater.

Of the adrenergic agonists commonly used in domestic mammal anesthesia,<sup>2</sup> only norepinephrine at 0.3-0.5 µg/kg/min resulted in a significant increase in blood pressure when used in hypotensive green iguanas. In addition to the clinical applications of pressor agents, understanding the effects of certain pressor agents also allows better understanding of the physiologic regulation in the iguana.<sup>3,4</sup>

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**EVALUATION OF ISOEUGENOL (AQUI-S™) FOR ANESTHESIA OF KOI CARP  
(*Cyprinus carpio*)**

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**Abstract**

Isoeugenol (Aqui-S) is a bath anesthetic agent that has potential use in both recreational and commercial fisheries.<sup>1</sup> Juvenile koi carp (n=216) were divided into nine treatment groups. Groups were exposed to solutions of isoeugenol in water at concentrations from 0 mg/L to 500 mg/L. Fish were monitored for activity, opercular rate, initial loss of equilibrium (ILE), jaw tone, handle ability, and stage 3 anesthesia (STA). Once recovered in water without anesthetic, the fish were returned to their initial holding tanks and observed for 24 hr.

Increasing concentrations of isoeugenol resulted in decreasing times to ILE and STA, all statistically different from the control and 20 mg/L groups. At a concentration of 40 mg/L, ILE occurred at  $7.21 \pm 3.51$  min, with STA at  $11.0 \pm 4.20$  min. At a concentration of 150 mg/L, ILE occurred at  $1.67 \pm 0.56$  min, with SFA at  $2.46 \pm 0.66$ . Larger fish in each group showed a trend toward a faster time to STA, but this was only statistically significant at a concentration of 80 mg/L ( $p < 0.001$ ). Twelve fish in the group exposed to 500 mg/L died within 24 hr, but no mortality was seen in other groups. Histopathologic analysis of control and 500 mg/L groups did not show lesions attributable to isoeugenol.

All fish were easily handled once at STA. All maintained opercular movement throughout anesthesia and recovery, except for those that died.

Isoeugenol is a safe and effective anesthetic for koi, causing rapid induction at concentrations between 60 – 200 mg/L. Future studies should evaluate isoeugenol for surgical procedures and anesthetic maintenance.

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## TWO NEW COMBINATIONS FOR FALLOW DEER ANESTHESIA: THIAFENTANIL, TELAZOL AND XYLAZINE OR THIAFENTANIL, KETAMINE AND XYLAZINE

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### Abstract

Captive free ranging white fallow deer (*Dama dama*; n=33) were anesthetized, of which 20 experienced standardized circumstances in a comparative study.

Group A (n=10) was administered 0.02 mg/kg thiafentanil (A3080, 10 mg/ml, ZooPharm Fort Collins, Colorado 80522, USA), 1 mg/kg xylazine (AnaSed, 100 mg/ml, Lloyd Laboratories, Shenandoah, Iowa 5160, USA) and 1 mg/kg tiletamine/zolazepam (Telazol, Fort Dodge Animal Health, Fort Dodge, Iowa 50501, USA) via remote dart. Group B (n=10) received 0.02 mg/kg thiafentanil, 1 mg/kg xylazine, and 2 mg/kg ketamine (Ketamine 200 mg/kg, ZooPharm), respectively via remote dart. All dosages were based on an approximated body weight of 60 kg.

Both combinations provided fast and smooth induction and suitable anesthesia. Initial mild respiratory depression was the most common side effect, but most animals improved unassisted within the first minutes after approach. Otherwise doxapram (Dopram-V 20 mg/ml, Fort Dodge) was administered in three cases in Group A and one animal in Group B (0.33-0.66 mg/kg i.v. or 2 mg/kg in 1000 ml LRS i.v.). Subjectively, muscle relaxation was slightly superior and of longer duration in Group A. Propofol (PropoFlo, 10 mg/ml i.v., Abbott Animal Health, N. Chicago, Illinois 60064, USA, 0.5-1 mg/kg repeatedly) was given to maintain or deepen anesthesia in three animals in Group A and two animals in Group B. Anesthesia was reversed using 1 mg/kg naltrexone (Naltrexone 50 mg/ml ZooPharm, ¼ i.v. and ¾ i.m.) and 0.1 mg/kg atipamezole (Antisedan, Pfizer Inc. New York, New York 10017, USA, ½ i.v. and ½ i.m.). Recovery was quick and uneventful in all animals. Animals in both groups regained full consciousness in less than 3 min. These combinations provided predictable, stable and safe anesthesia for fallow deer, which has proven difficult in the past.<sup>1-7</sup>

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## CURRENT UNDERSTANDING OF ANALGESIC EFFICACY AND ASSOCIATED SIDE EFFECTS IN REPTILES

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### Abstract

While our current understanding of pain and analgesia in reptiles is somewhat limited, data from our laboratory support the following conclusions:

1. Several reptile species respond consistently to application of a noxious thermal stimulus by withdrawing a hindlimb or tail.<sup>1,2</sup>
2. Mu-opioid receptor agonists (e.g., morphine) provide analgesia in red-eared slider turtles and bearded dragons, while kappa- (e.g., butorphanol) and delta-opioid receptor agonists do not.<sup>1,2</sup> However, both morphine and butorphanol cause moderate to marked respiratory depression in red-eared slider turtles.<sup>1</sup>
3. Corn snakes were variable in their responses to noxious thermal stimuli, but did demonstrate an increased latency to tail withdrawal after a high dosage of butorphanol was administered.<sup>2</sup>

Studies involving opioid analgesic efficacy and respiratory side effect are ongoing in our laboratory. The objectives are to: 1) determine the effects of multiple analgesic drugs on nociceptive behaviors in adult, red-eared slider turtles (*Trachemys scripta*), using a thermal hind limb withdrawal latency test; and 2) evaluate effects of these same analgesic drugs on respiration. Infrared heat stimuli were applied to the plantar surface of turtle hindlimbs, and thermal withdrawal latencies were measured before and after subcutaneous administration of physiologic saline, buprenorphine (1.0 mg/kg), tramadol (10 and 25 mg/kg), meperidine (50 mg/kg), and methadone (1.0, 3.0 and 5.0 mg/kg). Thermal withdrawal latencies following drug administration were similar in turtles receiving saline or buprenorphine. However, hind limb thermal withdrawal latencies increased in turtles after administration of tramadol, methadone, and meperidine. All of these analgesic drugs caused respiratory depression.

### ACKNOWLEDGMENTS

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## FIELD ANESTHESIA OF RING-TAILED LEMURS (*Lemur catta*) USING TELAZOL®, MEDETOMIDINE, AND BUTORPHANOL

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### Abstract

Ring-tailed lemurs (*Lemur catta*) have been studied in the wild in Madagascar for many years.<sup>1-7</sup> In Beza Mahafaly Special Reserve, a long-term research project focuses on studying the dental wear patterns of ring-tailed lemurs and relating these findings to health, ecology, behavior, and evolution.<sup>1,4,5</sup> Although tiletamine-zolazepam (Telazol®) has been commonly used to anesthetize free-ranging lemurs,<sup>2,7</sup> we have found that this combination alone provides insufficient sedation and muscle relaxation for consistent, high-quality dental impressions. This investigation evaluated Telazol® induction in ring-tailed lemurs and compared supplementation with medetomidine or medetomidine-butorphanol.

Lemurs (n=48) were anesthetized with Telazol® administered via Daninject® blow dart. Twenty minutes later, they were supplemented via hand-injection with either medetomidine (0.04 mg/kg; n=22) or medetomidine-butorphanol (0.04 mg/kg and 0.1 mg/kg; n=23), followed by additional doses as necessary; three lemurs received other supplemental agents.

Young lemurs (1-3 yr old) were 4 times more likely to need multiple darts for induction than mature adults ( $\geq 4$  yr old), even though the initial dose of Telazol® received by young lemurs ( $18 \pm 7$  mg/kg) was significantly higher than the initial dose administered to mature adults ( $12 \pm 5$  mg/kg). Both supplementation protocols provided good muscle relaxation and sedation for all procedures; full dental impressions were completed for all individuals. Physiologic values were generally stable, although low oxygen saturation values and low body temperatures sometimes occurred. Additional supplements were needed for procedures  $> 90$  min. Further refinements in these protocols are needed to increase induction reliability, particularly in young lemurs.

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## USE OF LUTEINIZING HORMONE ELISAs IN BREEDING ELEPHANTS

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### **Abstract**

Successful artificial insemination (AI) of elephants depends heavily on determining the unique luteinizing hormone (LH) surges that occur during the follicular phase of the elephant's estrous cycle. Natural breeding of elephants also can benefit from a rapid and accurate determination of the two LH surges found in elephants. There are three ELISAs available for determining the LH surge; two are commercially-available assays and one is a laboratory in-house assay. Each vary in their cost, time to complete the assay, and ease of performing the procedures. Detection of the initial non-ovulatory peak in luteinizing hormone (LH1) is best accomplished by use of an in-house LH assay, or use of the LH assay available from Dr. Nancy Dahl (UC-Davis, Davis, CA 95616 USA), both of which are quantitative assays for detection of LH. For cow-side use during estrus, the qualitative ELISA Witness<sup>®</sup> LH Ovulation Timing Test Kit (Symbiotics Corporation, Kansas City, MO 64163 USA) detects LH in elephants within 20 min. This assay requires a minimum of laboratory precision to detect the ovulatory LH peak (LH2).

### **Introduction**

Elephants are the only species known to exhibit a double LH peak during a single estrous cycle.<sup>2,4</sup> Increased success of artificial insemination in elephants occurred partly in response to the ability to detect the LH1 surge about 21 days prior to the ovulatory LH2 surge that occurs at the end of a two to three day estrus.<sup>1</sup> The first reports regarding detection of the double LH surges were performed in laboratories using custom ELISA technology that require exacting procedures and two days to complete the quantitative assays.<sup>2,4</sup> A semi-quantitative elephant LH ELISA that can be performed in the field in about 2.5 hr was developed at UC-Davis.<sup>3</sup> A qualitative LH assay was developed for use in dogs and cats that uses a latex strip ELISA. The time for development of the test is 20 min and detects a LH surge greater than 1 ng/ml using serum.

Elephants have LH1 and LH2 surges in the 4-16 ng/ml range,<sup>2,4</sup> well within the detectable range for all of the assays described. The detection of the LH1 peak usually is from daily samples submitted weekly; this allows some efficiency of assay resources and provides at least a two-wk notice of LH2. However, accurate and timely detection of LH2 is needed at least daily and at times twice daily during estrus. The use of an LH assay which can be performed 'cow-side' and accurately detect LH2 is essential for successful AI and can be helpful in determining estrus status for natural breeding. The Witness<sup>®</sup> LH Ovulation Timing Test Kit from Symbiotics was

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developed for use in dogs and cats, but is effective in other species, including elephants, and meets these requirements.

## Discussion

Detection of LH1 provides information for predicting the LH2 surge and performance of assays that require more laboratory time and precision are useful since detection of LH1 is not as time-sensitive as LH2 detection. Both of the quantitative assays have unique advantages. An in-house assay can be set up, but requires greater preparation time, precision of laboratory procedures is more demanding, often takes two days to perform, and is more susceptible to environmental variables. The assay developed by UC-Davis costs about \$5.00 per well, takes about 2.5 hr to perform and is more stable. However, for quantitative results the overhead costs of the standard curve requires about 16 wells (\$90), plus two wells for each unknown sample. The UC-Davis assay can be set up as a qualitative test with high and low controls and no standard curve. This requires from three to six wells for a single sample. The Witness® LH Ovulation Timing Test Kit has a control built into each test strip and costs about \$25.00 per sample. Because ‘cow-side’ testing possible using the Witness® LH Ovulation Timing Test Kit, I recommend its use for detection of LH2, although the UC-Davis Elephant ELISA is competitively priced and can be performed in a nearby temporary laboratory. Because timing is critical in detecting LH2 and performing subsequent AI, I recommend using the Witness® LH Ovulation Timing Test Kit at the time of estrus, preceded by either one of the other assays for detecting LH1, depending on availability of laboratory labor and equipment.

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## REGULATION OF IRON BALANCE IN FOUR SPECIES OF RHINOCEROSSES

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### Abstract

All rhinoceros species are endangered by encroachment and poaching in their natural habitats. In captivity, the health and longevity of African black (*Diceros bicornis*) and Sumatran (*Dicerorhinus sumatrensis*) species are further threatened by progressive development of pathologically extreme iron overloads.<sup>2,4,5</sup> By contrast, African white (*Ceratotherium simum*) and Asian greater one-horned (Indian) (*Rhinoceros unicornis*) species exhibit no evidence of iron-storage disorder in captivity.<sup>2,4,5</sup> This distinct clinical disparity between browser and grazer rhinoceros species might be explained by evolutionary differences in the mechanisms by which they assimilate dietary iron and/or regulate its subsequent balance.<sup>3</sup>

Recent advances in studies of iron homeostasis have identified molecular mechanisms involved in the pathogenesis of human iron disorders. Since these regulatory pathways are highly conserved in mammals, those in rhinoceroses are likely to be similar. The phenotype of iron loading in black rhinoceroses is characterized by extensive iron deposition in various organs, and by serum transferrin saturations and ferritin concentrations that are markedly elevated over those of free-ranging black rhinos or captive white rhinos.<sup>4,5</sup> This disorder resembles certain forms of human hereditary hemochromatoses, which are now known to be caused either by deficiency of the key iron regulatory hormone hepcidin, or by resistance to hepcidin. Hepcidin controls iron homeostasis by blocking the absorption of iron from the diet, the release of iron from macrophages recycling old red blood cells and the mobilization of iron from stores in the liver. Hepcidin acts by causing the internalization and degradation of its receptor, ferroportin, the sole channel for egress of intracellular iron into plasma from enterocytes, hepatocytes, macrophages, and placental trophoblast.<sup>3</sup> In humans, hepcidin deficiency or resistance to hepcidin causes excessive iron absorption and deposition of iron in the liver and other tissues. Hepcidin *deficiency* results either from autosomal recessive mutations in the hepcidin gene itself or in genes encoding hepcidin regulators: hemojuvelin, transferrin receptor 2 (TfR2), and the hemochromatosis gene, *HFE*. Hepcidin *resistance* results from autosomal dominant mutations in the hepcidin receptor, ferroportin.

Our experimental approach compared regulatory gene sequences from both affected and unaffected rhinoceros species to search for possible underlying molecular defect(s). Previously, no differences were found in the sequences of rhinoceros HFE genes between affected and unaffected species,<sup>1</sup> but sequences for other iron related genes in rhinoceroses were not known. In this study, DNA was extracted from peripheral blood samples from all four available rhino

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species, and genes encoding hepcidin, ferroportin, hemojuvelin, TfR2 and HFE were cloned and analyzed by PCR amplification based on conserved regions of related species (horse and dog).

Over half of the DNA sequences of these five genes have now been determined without identifying a specific cause for the apparent disparity in iron homeostasis among these four species. The sequence encoding the rhinoceros hepcidin was found to be identical between the black and white rhinos. We synthesized the rhinoceros hepcidin peptide and showed that it was active in an *in vitro* bioassay. However, the responsiveness of rhinoceros ferroportin to hepcidin modulation, as well as the level of expression of rhinoceros hepcidin, is still to be determined. Assays to measure hepcidin concentration in rhinoceros urine and sera, similar to methods used for human hepcidin, remain under development.

#### ACKNOWLEDGMENTS

We thank Alan Waring and Piotr Ruchala for synthesizing and purifying rhino hepcidin. We are especially grateful to the zoo veterinarians, keepers and staff who have provided rhino blood, serum and tissue samples, including those at the San Diego Zoo and Wild Animal Park, the Los Angeles Zoo and Fossil Rim Wildlife Center.

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**DAILY CLINICAL EXAMINATIONS IN A HERD OF CAPTIVE ASIAN ELEPHANTS (*Elephas maximus*) WHERE ENDOTHELIOTOPHIC ELEPHANT HERPES VIRUS HAS OCCURED**

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**Abstract**

The captive population of Asian elephants (*Elephas maximus*) is not self-sustaining.<sup>2</sup> Poor reproduction and high juvenile mortality are key factors in the decreasing population. Infection with endotheliotropic elephant herpes virus (EEHV) is one of the major causes of death in the captive population, and has resulted in the loss of at least 40 captive animals.<sup>1</sup> EEHV has been responsible for the peracute death of two juvenile males at Zurich Zoo, Switzerland.

Mortality due to peracute infection with EEHV mainly is seen in juveniles. Early detection of characteristic clinical signs of EEHV and immediate initiation of therapy are of crucial importance due to its rapid progression. Based on past fatal EEHV experiences, Zurich Zoo modified its daily clinical health monitoring program to increase staff awareness of EEHV infection. Examinations have been incorporated into the daily routine and include daily evaluation of behaviour, appetite, colour of mucosal membranes and the measurement of body temperature; these examinations are performed by keepers.

In our experiences, characteristic signs of acute EEHV infection are lethargy, anorexia, mild colic, and cyanosis of the mucosal membranes. Results of temperature measurements have shown that best estimations of body temperature are done by measurement of the temperature in the centre of a fecal ball 5-9 min after defecation. Mean values of 36.5°C ( $\pm$  0.2°C SD) are within published reference values, although adult elephants have shown significantly lower body temperature than juveniles. Establishment of individual reference values for each elephant is essential to detect unusual temperature peaks that may indicate possible EEHV viremia.

The present study has shown that daily health examinations increase the awareness of keepers for early signs of EEHV infection (e.g., peaks in body temperature and cyanotic mucosal membranes).

**ACKNOWLEDGMENTS**

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**RETROSPECTIVE USE OF MULTIANTIAGEN PRINT IMMUNOASSAY (MAPIA) TO DOCUMENT SUCCESSFUL TREATMENT OF A BLACK RHINOCEROS (*Diceros bicornis*) FOR MYCOBACTERIOSIS**

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**Abstract**

In late 1992 a 40-yr-old male black rhinoceros (*Diceros bicornis*) was found to be positive on tuberculin skin test using 0.1cc PPD and ELISA positive for mycobacteriosis. An attempt to collect a sample for culture via gastric lavage was not successful. This mycobacterial testing was performed after the rhinoceros's mate was found to be skin test positive, ELISA positive and culture positive for *Mycobacterium tuberculosis* via gastric lavage.

Both rhinoceros were treated for mycobacteriosis. The female was treated for 10 mo before she died suddenly and was found to be culture positive for *M. tuberculosis* on necropsy.<sup>2</sup> Treatment for the male rhinoceros initially utilized isoniazid (INH) alone at a dose of 8.0 mg/kg s.i.d. Five months later, rifampin was added at a dose of 12.5 mg/kg s.i.d. After serum rifampin levels were determined to be lower than therapeutic levels for the female, the dose of rifampin was increased to 37.6 mg/kg s.i.d.; both drugs were continued for a total treatment period of 41 mo. Drugs were purchased in raw form from CIBA, and were given daily by sprinkling them directly on pelleted diet. There was a 4-mo period (May 1994-September 1994) when treatment with rifampin was interrupted while waiting for drug delivery.

During treatment and for a 5-yr period afterward, a total of ten anesthetic procedures were performed to collect bronchoalveolar and gastric lavage samples for mycobacterial culture, acid-fast staining and PCR. Samples were sent to three different laboratories for culture. No samples were found to be positive for *M. tuberculosis* and the rhinoceros never demonstrated any clinical signs consistent with mycobacteriosis.

In 2001, the male black rhinoceros was euthanatized at the age of 49 due to discomfort caused by chronic arthritis. Necropsy confirmed that there was severe osteoarthritis. There were no gross or microscopic lesions consistent with mycobacteriosis.

In recent years the MAPIA (Multiple Antigen Print Immunoassay) has been developed to assist in the diagnosis of mycobacteriosis in elephants and other wildlife species.<sup>3,4</sup> The same MAPIA antigen profile used in elephants was used to do a retrospective analysis of samples from both the

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female and male rhinoceros. The results show a positive band for the ESAT 6 / CFP 10 antigen in both rhinoceros after diagnosis. In the case of the male this band faded after over a year of therapy. In elephants the ESAT 6 band has been the most useful indicator of both infection and therapeutic success.<sup>4</sup> Overall, the results suggest that the male rhinoceros was infected with *M. tuberculosis* and was successfully treated and cleared of the infection. It is considered unlikely that the mycobacteriosis responsible for the positive test reactions were MOTT (Mycobacterium other than tuberculosis), as MOTT have been found not to evoke a response using either RT or MAPIA.<sup>1</sup>

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## EVALUATION OF ACUTE PHASE PROTEINS FOR DIAGNOSIS OF INFLAMMATION IN ASIAN ELEPHANTS (*Elephas maximus*)

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### Abstract

In many domestic species, routine hematology assays are useful diagnostic tools to diagnose inflammatory conditions. Unlike other species, these hematologic tests apparently are insensitive indicators of inflammation in elephants.<sup>1</sup> We studied a novel group of blood proteins, called acute phase proteins, which increase during inflammatory conditions, for their usefulness in diagnosing elephants with inflammatory diseases. Although these proteins currently are useful in humans and domestic animals, each species has a different set of important proteins that must be individually investigated.<sup>2</sup> We tested several acute phase proteins (C-reactive protein, alpha-1 glycoprotein, alpha-1 antitrypsin, serum amyloid A, haptoglobin, fibrinogen, ceruloplasmin, and albumin) as well as complete blood counts, chemistry panels, serum protein electrophoresis, and 3-D gel electrophoresis to determine their usefulness for diagnosing different types of inflammatory conditions in Asian elephants (*Elephas maximus*). Animals with inflammatory conditions were classified as those individuals with known illnesses such as mycobacteriosis, arthritis, nail bed abscesses, and malignant tumors. Control animals were those animals that were suspected to not have any inflammation and be healthy at the time of testing as determined by physical examination and obtaining a thorough medical history.

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## INTEGRATED PARASITE MANAGEMENT IN A HERD OF CAPTIVE GIRAFFE (*Giraffa camelopardalis*)

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### Abstract

With the death of two adult female giraffe attributed to haemonchosis, endoparasitism has been identified as a significant problem in the semi-free ranging captive giraffe herd at Lion Country Safari in south Florida. Lack of a winter freeze and inability to rotate pastures contribute to pasture contamination with *Haemonchus contortus* larvae. In addition, larval development assays have shown varying resistance to three groups of anthelmintics (benzimidazoles, macrocyclic lactones and cholinergic agonists), making deworming less effective.<sup>1</sup>

Intensive monthly monitoring of individual giraffes' fecal egg counts interpreted in light of pregnancy or other health status allows for deworming only when necessary. This helps establish a refugia population of endoparasites, which will be more susceptible to dewormers in the future.<sup>1,3</sup> In addition, voluntary blood draws using operant conditioning are possible in certain giraffe. Haemonchosis can be better characterized by correlating packed cell volume (PCV) and serum protein (SP) with fecal egg counts. Judicious use of dewormers based on accurate weights alternated with copper oxide wire particles (COWP, Copasure®, Animax Ltd., Columbus, OH 43215 USA) also has helped assuage the problem.<sup>2</sup>

Environmental control is another important aspect of the integrated parasite management program. Pasture decontamination methods such as vacuuming fecal pellets, grazing pastures with non-susceptible (non-ruminant) species such as zebra (*Equus burchelli*), killing grass in certain areas, and removing giraffe shedding high numbers of eggs from pasture help reduce field contamination.<sup>1</sup> Excess grazing behavior was recognized as a risk factor for infection with *H. contortus*. Thus, behavior modification through the use of browse feeders has helped reduce grazing and subsequent parasite load.

Potential future methods of control of *H. contortus* include offering giraffe a high condensed tannin legume such as Sericea lespedeza hay and feeding them nematophagous fungi.<sup>1</sup> Although *H. contortus* will never be eradicated, our goal is to manage the *H. contortus* worm population much as we manage the rest of our herds.

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## USE OF ORAL GABAPENTIN TO AID HEALING OF A PERIPARTURIENT PELVIC FRACTURE IN A COMMON ZEBRA (*Equus burchelli*)

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### Abstract

An 11-yr old, primiparous Burchell's Zebra (*Equus burchelli burchelli*) gave birth to a full-term stillborn fetus. Left-sided vulvar edema and a non-weight-bearing (grade V/V) lameness on the left rear leg with knuckling was noted post-partum. The zebra was placed on stall rest and given oral phenylbutazone and omeprazole. Over the following month the animal starting placing slightly more weight on the affected leg (grade IV/V), but the opposite leg became severely dropped at the fetlock due to increased weight-bearing. Gabapentin (2.5 mg/kg b.i.d. p.o.; Glenmark Pharmaceuticals Inc., Mahwah, New Jersey, 07430 USA) was administered from day 30 to 90 for added pain relief and mild sedative effects to decrease excessive movement. Gradual improvement of the lameness was observed during the 4 mo after the injury. Six months after the initial injury anesthesia was performed, and a healed, comminuted fracture of the left ischium continuing into the acetabulum was diagnosed via rectal and transcutaneous ultrasonographic examination.

Both the analgesic and sedative effects of gabapentin are considered to have contributed to the successful management and outcome of this case. Gabapentin initially was used as an anticonvulsant, but has been shown also to be effective for the management of neuropathic and chronic pain in humans; few side effects are noted.<sup>4</sup> Its use has been infrequently reported in the equine and zoological medicine literature.<sup>1-3</sup> This drug has great potential as an adjunctive pain management tool for non-domestic animals.

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**RECENT ADVANCES IN THE MOLECULAR EPIDEMIOLOGY,  
ETIOPATHOGENESIS, DIAGNOSIS, AND TREATMENT OF AVIAN  
MYCOBACTERIOSIS**

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**Abstract**

Avian mycobacteriosis is a common disease in pet, avicultural, and zoo birds. In contrast to mycobacterial diseases of humans and livestock, little attention has been paid to the avian disease, and there is little new information available on these infections. A recent development in this field includes the finding that several species of mycobacteria other than *Mycobacterium avium* appear to commonly cause disease in birds. Information is lacking concerning the role different *Mycobacterium* species play in the development of the avian disease, as well as the importance of a host's genetic makeup and immune response. Studies on the predictive value of tissue sampling, the sensitivity and specificity of different diagnostic techniques, and the efficacy of multi-drug therapy in naturally infected birds have been rarely investigated. In this study of *Mycobacterium avium* subsp. *avium* infection in ring-necked doves (*Streptopelia risoria*), differences in prevalence of infection, number of organs affected, and susceptibility to disease were explained by genetic differences associated with a phenotypic characteristic, color morph. Diagnosis of mycobacteriosis in these doves was greatly improved by examining different organs and by performing a combination of culture, PCR, and acid fast staining. However daily treatment with a combination of azithromycin, ethambutol, and rifampin for 6 mo did not produce a cure.

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**AN OUTBREAK OF AVIAN MYCOBACTERIOSIS CAUSED BY *Mycobacterium intracellulare* IN LITTLE BLUE PENGUINS (*Eudyptula minor*)**

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**Abstract**

*Mycobacterium intracellulare* (MIT) was diagnosed post mortem by culture and histopathology in seven birds from a flock of little blue penguins (*Eudyptula minor*) at the Henry Doorly Zoo (HDZ) representing 20% of the population deaths. Clinical signs in affected birds included respiratory distress with chronic debilitation. On exam, plaques were noted in the larynx, trachea and soft tissue of the caudal oropharynx.

A treatment protocol of Rifampin (Sandoz Inc., Princeton, New Jersey, 08540, USA; 15 mg/kg p.o. s.i.d.), Ethambutol (Pantheon Inc., Toronto, Ontario, Canada M3B 1Y5; 15 mg/kg p.o. s.i.d.) and Clarithromycin (Abbott Laboratories, North Chicago, Illinois, 60064, USA; 10 mg/kg p.o. s.i.d.) was initiated in 2004. Gastric washes, fecal samples and throat plaques were obtained ante mortem on five birds and tested by culture and polymerase chain reaction (PCR). MIT was detected on gastric wash and throat plaque PCR. Antimicrobial susceptibilities indicated the organism was sensitive to the regimen. Three more birds died during treatment. After the seventh bird died; a susceptibility performed in 2007 indicated the MIT was resistant to most antibiotics tested including Rifampin and Ethambutol. One more death occurred. Treatment was changed to Minocycline (Ranbaxy Pharmaceuticals Inc., Jacksonville, Florida, 33216, USA; 10 mg/kg p.o. b.i.d.) and Clarithromycin (10 mg/kg p.o. s.i.d.).

Clinical signs and visualization of plaques have not recently been noted. The proposed mechanism of transmission is exposure to wild birds but the etiology has not been determined. Avian mycobacteriosis caused by MIT has not previously been reported in this species.

**ACKNOWLEDGMENTS**

The authors would like to thank the laboratory staff at the University of Nebraska Medical Center and the veterinary technicians and the aquarium bird keeper staff at Omaha's Henry Doorly Zoo for their participation and support in this case study.

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## TREATMENT OF SQUAMOUS CELL CARCINOMA IN A GREAT INDIAN HORNBILL (*Buceros bicornis*)

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### Abstract

Annual skull radiographic screening of a 38-yr-old female great Indian hornbill (*Buceros bicornis*) identified a small lesion in the rhinotheca rostral to the casque/beak interface. Excisional biopsy confirmed squamous cell carcinoma (SCC), with no invasion of tumor into the casque. Radiographs were repeated every 3 mo. Treatment with oral meloxicam was initiated for its potential effect against carcinoma.<sup>2</sup>

Nine months after initial diagnosis radiographs revealed a 5-cm diameter mass in the rostral casque. The casque was nearly completely resected and the floor of the casque space was aggressively debrided. Histopathologic examination confirmed SCC. Topical treatment was begun using a previously described drug combination, OLCAT-005, to inhibit angiogenesis in cutaneous neoplasms, thereby inducing regression or suppressing tumor regrowth.<sup>1,3,4</sup> Therapy consisted of equal parts by weight of imiquimod 5 %, tretinoin 0.1 %, calcipotriene 0.005 %, diclofenac 3 %, and hydrocortisone valerate 0.2 %, mixed together and applied locally to the site three times a wk. Meloxicam was discontinued.

Biopsy was negative after 5 mo, and treatment was discontinued. The tumor remained in remission for 5 mo until recurrent SCC was discovered by biopsy. Surgical debridement and topical therapy was repeated using the protocol above. At the time of submission of this abstract, > 2 yr since initial diagnosis and 15 mo since initiation of treatment, the animal continues to receive topical antiangiogenesis treatment.

The authors conclude that early detection via annual skull radiographs, aggressive resection and debridement, and adjunct antiangiogenic treatment can produce long-term survival in management of SCC in great Indian hornbills.

### ACKNOWLEDGMENTS

The authors gratefully acknowledge the advice and support of Dr. Kathryn Gamble, Coraciiformes TAG veterinary advisor. Treatment of this bird could not have been done without the excellent assistance of the veterinary technicians, animal curators, and bird keepers of Zoo New England.

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**PERINATAL MORTALITY IN A COLONY OF CAPTIVE HORNED GUAN (*Oreophasis derbianus*)**

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**Abstract**

This paper describes the clinicopathologic findings of 13 cases of embryonic (10/13) or neonatal (3/13) mortality in a colony of captive horned guan (*Oreophasis derbianus*) at Africam Safari Zoo in Puebla, México, from 2002 to 2008.

Five embryos presented with malformations of the yolk sac with entrapment of the right hindlimb (4/5) or both hindlimbs and neck (1/5). One affected embryo had unilateral renal hypoplasia. There were five cases of embryonic malposition, which was considered lethal in four embryos. Histologically all processed lungs (two embryos with yolk sac entrapment and two with malposition) had aspiration of amniotic fluid. Mineralization of yolk sac, kidney, and/or proventriculus was observed in five embryos examined microscopically. Two neonates had necrosuppurative omphalitis with bacteria; one of which also had bacterial myocarditis, bronchopneumonia, and osteomyelitis of the skull. A third neonate had bacterial pneumonia. *Pseudomonas aeruginosa* was isolated from all neonates.

During 2002 and 2003, when yolk entrapment was observed, eggs were artificially incubated using Humidaire 20 and 21 models at 99.5 ° F (37.5 ° C). Humidity was maintained at 45 – 60 % to achieve a 15 % weight loss. Eggs were placed on their sides and automatically turned 24 times a day. In subsequent seasons eggs were incubated by domestic turkeys, and viable chicks were obtained. Weight loss during the incubation under the turkeys was determined to be 17.1 ± 2.6 % (n=25). Artificial incubation was attempted again in 2006 and 2007, with a Grumbach incubator at 99.0 ° F (37.2 ° C) with humidity adjusted to obtain 17 % weight loss. A total of 29 embryos were successfully hatched during the study period.

This is the first description of embryonic and neonatal mortality in endangered horned guan. The embryonic malpositions are attributed to inappropriate egg rotation and positioning.<sup>1</sup> Rough handling or improper turning can contribute to yolk sac entrapment, which is more common in artificially incubated eggs.<sup>2</sup> The mineralization may have been due to inappropriate incubation temperatures, dehydration, or tissue degeneration due to hypoxia associated with malpositions. Yolk sac entrapments were no longer observed in this colony after artificial incubation was changed to incubation using domestic turkeys. The neonatal bacterial infections that were observed were part of two *Pseudomonas* spp. outbreaks also involving scarlet macaw and golden eagle chicks in the avian nursery unit.

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**INVESTIGATION OF AN INFECTIOUS DISEASE OUTBREAK IN BUDGERIGARS  
(*Melopsittacus undulatus*)**

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**Abstract**

In spring 2007 a new aviary for budgerigars (*Melopsittacus undulatus*) was opened at the Henry Doorly Zoo (Nebraska, USA), and high mortality was observed soon thereafter. Most of the cases were due to trauma, but some animals presented at necropsy with emaciation and hepatosplenomegaly. PCR performed on liver and spleen was positive for *Chlamydophila psittaci* in two of three birds tested, and histologic findings in two additional birds were supportive of avian chlamydiosis. The aviary was subsequently closed to the public and a 45-day treatment regimen with doxycycline (Doxycycline hyclate, Mutual Pharmaceutical Co., Philadelphia, Pennsylvania 19124, USA) was initiated at 300 mg/kg seed. No further cases of splenomegaly or hepatomegaly were observed after the first day of treatment. In addition five animals with signs of disease (cachexia, inability to fly) were separated from the main aviary, placed in an indoor cage, and treated with doxycycline at the previously mentioned dose; all animals regained their weight and ability to fly within 45 days. Further investigation of animals that died during the outbreak with emaciation and hepatic and splenic enlargement revealed severe necrosis of the spleen and liver suggestive of reovirus infection, which was supported with PCR analysis from paraffin-embedded tissue. The outbreak did not affect cockatiels (*Nymphicus hollandicus*) or blue quails (*Coturnix chinensis*) kept in the same aviary. It is believed that the group of budgerigars added to the collection soon before the opening of the aviary may have introduced reovirus and *Chlamydophila* into the collection.

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**DISCOVERY OF NEW, PATHOGENIC PROTOZOANS CULTURED FROM FREE-RANGING MOCKINGBIRDS (*Mimus polyglottos*), HOUSE FINCHES (*Carpodacus mexicanus*), AND CORVIDS: GENOTYPING, PREVALENCE, AND CLINICOPATHOLOGIC CHARACTERISTICS**

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**Abstract**

This study documents newly discovered trichomonads associated with morbidity and mortality in free-ranging house finches (*Carpodacus mexicanus*), mockingbirds (*Mimus polyglottos*), and corvids [Western scrub jay (*Aphelocoma californica*), American crow (*Corvus brachyrhynchos*), common raven (*Corvus corax*)] in northern California. Sensitivity and specificity of wet mount screening in house finches were > 95 % (via comparison to InPouch TF<sup>TM</sup> culture kits, BioMed Diagnostics, White City, Oregon 97503 USA). Prevalence of infection on admission to a wildlife hospital ranged between 0.34 % – 6.25 %. Clinicopathologic findings and response to treatment were highly variable between species. Less than 30 % of infected individuals displayed clinical signs that increased suspicion of trichomonad infection. These signs included sticky material on head or beak, caseous oral lesions, and ocular lesions in house finches; caseous sinusitis and conjunctivitis or neurologic signs in mockingbirds; and ocular and oral lesions in corvids. Case fatality ratio was > 80 % in house finches, scrub jays, and crows, 37.5 % in mockingbirds, and 0 % in ravens. Trichomonads were not detected in any surviving house finches (n=3) or corvids (n=3) after treatment with carnidazole (20 – 30 mg/kg p.o. s.i.d. for 5-10 days; Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado 80522). Identical treatment resolved clinical signs but did not clear trichomonad organisms from mockingbirds. DNA sequencing found that the house finch and corvid organism was closely related to *Trichomonas gallinae*, while the mockingbird organism was genetically distinct from all other sequenced trichomonads. Wet mounts are a reliable and inexpensive method of screening for trichomonad infection in these species.

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## EMERGENCE AND SPREAD OF USUTU VIRUS IN CENTRAL EUROPE ON THE BASIS OF THE OUTBREAK IN SWITZERLAND

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### Abstract

In late summer 2006 considerable mortality in wild and captive passeriformes and strigiformes was observed around the Zurich Zoo, Switzerland. All animals were found in a range of 2 km.<sup>2</sup> Observed clinical signs involved depression, ruffled plumage, incoordination, and dropping to the ground with seizures and death within minutes. At necropsy the nutritional status was generally moderate to poor in wild birds and poor to good in captive animals. Necropsy showed marked splenomegaly, mild hepatomegaly, and pulmonary hyperemia in most animals. Histopathologic lesions were very discrete and consisted mainly of neuronal necrosis, leucocytolysis in and around the brain blood vessels, and miliary liver necrosis. The diagnosis of Usutu virus (USUV) infection was confirmed with immunohistochemistry (IHC) and reverse transcription polymerase chain reaction (RT-PCR). Partial nucleotide sequence comparisons revealed > 99 % identity between the viruses that emerged in Zurich in 2006, in Vienna in 2001, and in Budapest in 2005. In 2007 significantly lower mortality was observed, but USUV infection was confirmed for the first time beyond Zurich city limits.

Usutu virus is a poorly recognized, mosquito-borne flavivirus, which was first isolated from *Culex naevei* in South Africa in 1959.<sup>6</sup> When USUV emerged for the first time outside Africa, it caused fatalities in warm-blooded hosts such as blackbirds (*Turdus merula*) and great grey owls (*Strix nebulosa*) in the region around Vienna between 2001 and 2005.<sup>2,4,5</sup> More recent cases have been reported in Italy<sup>3</sup> and Hungary.<sup>1</sup>

### ACKNOWLEDGMENTS

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**RECENT ADVANCES IN IMMUNOLOGY AND VACCINOLOGY OF CAPTIVE  
NONDOMESTIC SPECIES**

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**Abstract**

This “Master Class in Immunology” will focus on advances in our understanding of immunology as applied to captive populations of nondomestic animals. We will discuss topics such as the impact of genetic diversity on immunologic function, new approaches to vaccination against infectious diseases and other conditions, and current information on the efficacy and safety of adjuvanted vaccines. We will conclude with a group activity to identify critical areas for future research.

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## **WOUND HEALING IN ELEPHANTS**

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### **Abstract**

#### **Basic Wound Healing**

A wound into the subcutaneous tissue follows sequential stages of healing, namely inflammation, débridement, proliferation, epithelization and contraction (scarring). Elephant wounds go through the same sequences if allowed to do so. Basic mammalian wound healing involves the epidermis, germinal epithelium, dermis and subcutaneous tissue. In the elephant foot the epidermis becomes the cornified sole, pad or nail, which is produced by the germinal epithelium. The dermis becomes the corium (vascularized fibrous tissue connecting the cornified shell to the digits).

The healing process may take weeks, months and even years. Particular emphasis will be given to anatomy as it relates to foot infections, basic principles of wound healing in mammals as applied to elephants, predisposing factors and factors that inhibit wound healing.

#### **Predisposing Factors of Foot Infections**

Genetics (conformation defects), malnutrition (rickets), abnormal behavior (stereotypy, pawing, resting with pressure on a specific area of the foot, excessive pressure to compensate for pain in another limb), degenerative joint disease, poor sanitation, no variation in the enclosure substrate, and minimal opportunity to exercise are such factors.

#### **Cardinal Rules Governing Wound Management, Specifically Foot Infections**

1. Elephants should be trained to allow foot inspection of all feet on a daily basis.
2. Minimize or eliminate predisposing factors.
3. Remove all necrotic material, dirt, feces, urine and debris from the wound cavity.
4. Obtain adequate drainage for an exudate to exit the cavity.
5. Prevent recontamination of the clean wound either by packing the wound cavity with disinfectant soaked gauze or by applying a protective boot.

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6. Avoid over-zealous trimming once the cavity has been thoroughly cleansed of necrotic tissue and debris and adequate drainage obtained. Avoid trimming back the germinal epithelium as it attempts to cover the granulation tissue.

7. Foot soaks may be used judiciously to accomplish specific purposes. Avoid any solution that may be caustic to the delicate granulation tissue or germinal epithelium. Do not use concentrations higher than recommended.

8. The presence of excessive granulation tissue is likely an indication of the continued presence of debris or foreign material in the wound cavity.

### **Inhibition of Wound Healing**

Factors that act locally include lack of vascularity, denervation, infection, necrotic tissue, hematoma, foreign bodies, mechanical stress, drugs (disinfectants) and trauma (continued débridement). Factors acting systemically include malnutrition, anemia, systemic infection, compromised immune system, temperature at the wound site and age of the elephant.

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## THE ZOOLOGICAL INFORMATION MANAGEMENT SYSTEM

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### **Abstract**

The Zoological Information Management System (ZIMS) is a pioneering effort to provide the zoo and aquarium community with record keeping software that delivers an integrated specimen (or group) record on the web, in real-time. It is the largest effort of its kind ever undertaken across the global community and it will be a critical component of veterinary care for zoo and aquarium collections.

This class will provide an update on the status of ZIMS. In addition, characteristics and concepts of the ZIMS-Health section will be shared, data standard terminology (MedARKS-related and novel) in selected areas will be reviewed, and some steps to ease transition to this exciting new system will be provided.

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## SUCCESSFUL CAESAREAN DELIVERY AND EARLY REINTRODUCTION OF A SUMATRAN ORANGUTAN (*Pongo abelii*)

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### Abstract

A 20-yr-old nulliparous Sumatran orangutan (*Pongo abelii*) delivered a full-term stillborn infant in 2005. A second pregnancy was confirmed by urine beta-hCG on 27 June 2007. A birth plan was established to address a potentially complicated pregnancy. Serosanguinous fluid was observed on bedding by keepers on the morning of 13 December 2007. When parturition failed to progress within 4 hr, the female was induced by hand and dart injection with ketamine and transported to the UMN CVM for C-section. A male neonate was delivered by a ventral midline approach. The neonate experienced cardiac and respiratory depression within 10 min of delivery, necessitating intubation and aggressive resuscitation. The mother was returned to the zoo for recovery while the neonate was moved to the critical care unit of the UMN CVM. Successful recovery of the neonate involved intensive monitoring with antibiotic, oxygen, intravenous fluid, and nutritional support.

The neonate was able to maintain stable blood gases and glucose levels without intravenous support at 4 days after delivery and was moved to the zoo where a reintroduction program was initiated. The mother was allowed progressive amounts of protected contact with the neonate. At 10 days after delivery, the infant was considered strong enough and the mother was showing the necessary maternal interest for introduction. Maternal lactation was stimulated by oral metoclopramide in preparation for reintroduction. Successful reintroduction occurred on the 12<sup>th</sup> day after delivery. This is the ninth reported orangutan Caesarean delivery and the earliest recorded reintroduction by 8.5 mo (Lori Perkins, pers. comm., 1/9/08, International Orangutan Studbook).

### ACKNOWLEDGMENTS

The authors would like to acknowledge Mark Bergeron and Grace Doolittle for advice on initial infant care and lactation management based on experience in human medicine, and Lori Perkins and Carol Sodaro for advice on historical care of dystocia and reintroduction in Orangutans. The authors also extend thanks to Jessica Nyholm, Jessica Swartout, Daniel deAlmeida, Justine Lee, Danielle Babski, Heather Loehrer, Vickie Skala, Kelly Tart, Theresa Schrachta, Rachel Rylander, Megan Kunau, and all of the keepers of Como Zoo who played critical roles in the successful delivery, care and reintroduction of Jaya, the infant Orangutan.

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**EVALUATION OF A *Mycobacterium tuberculosis* RAPID LATERAL-FLOW TEST (RT) FOR ASSESSMENT OF NON-SPECIFIC TUBERCULIN RESPONSES IN SILVERED LEAF MONKEYS (*Trachypithecus cristatus ultima*)**

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**Abstract**

Intradermal tuberculin testing is widely used for non-human primate *Mycobacterium tuberculosis* screening. The most commonly used tuberculin for screening, mammalian old tuberculin (MOT), shows high cross-reactivity due to its composition of poorly defined mycobacterial antigens.<sup>2</sup> Frequent non-specific intradermal eyelid tuberculin responses have been observed in silvered leaf monkeys (*Trachypithecus cristatus ultima*) with confirmed negative status on ancillary testing at the Wildlife Conservation Society's Bronx Zoo. A review of all individuals tested over a 10-yr period (1998-2008) showed a 51.6% (16/31) period prevalence of non-specific intradermal eyelid tuberculin responses.

A rapid lateral flow test approved for use in *Macaca* spp. (PrimaTB STAT-PAK Assay®, Chembio Diagnostic Systems, Inc., Medford, NY 11763 USA) was employed to assess its utility for evaluation of non-specific tuberculin responses in langurs. This test utilizes a membrane based antibody detection method for detection of antibodies against *M. tuberculosis* and *M. bovis* and is a host species-independent test. The test also did not cross-react with *M. avium* or *M. kansasii* inoculated rhesus macaques (*Macaca mulatta*).<sup>1</sup>

Serum or plasma samples from 16 silvered leaf monkeys (11 with non-specific tuberculin responses and five with negative tuberculin tests) were assessed. All suspect tuberculin responders received comparative tuberculin testing (either eyelid or chest) with avian old tuberculin or balanced PPD-avian and balanced PPD-bovis and the responses to these antigens indicated that they were non-specific responders. Six animals (four non-specific responders and two negative) died of unrelated causes and gross necropsy and histopathology demonstrated no evidence of granulomatous inflammation. Results of RT tests are listed in Table 1. These results suggest this assay may be a useful screening test to evaluate non-specific tuberculin responses in silvered leaf monkeys.

**ACKNOWLEDGMENTS**

The authors thank Chembio Diagnostic Systems, Inc. and Centaur, Inc. for donation of RT test kits for use in the study. We additionally thank the efforts of the Wildlife Conservation Society's Global Health Programs staff, especially Krysten Marchese, for their assistance and the Columbus Zoo and Omaha's Henry Doorly Zoo for contribution of additional information.

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**Table 1.** Intradermal eyelid tuberculin test and rapid lateral flow test (RT) results for silvered leaf monkeys (*Trachypithecus cristatus ultima*) at the Wildlife Conservation Society’s Bronx Zoo.

Intradermal Eyelid Tuberculin Test Status	Number of Animals	Number RT Negative	Number RT Positive	Number RT Equivocal
Non-specific tuberculin response	11	10	0	1
Negative tuberculin response	5	4	0	1

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## MANAGEMENT OF A UTERINE LEIOMYOMA IN A WESTERN LOWLAND GORILLA (*Gorilla gorilla gorilla*)

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### Abstract

A 46-yr-old female western lowland gorilla (*Gorilla gorilla gorilla*) presented for metrorrhagia. Vaginal and rectal ultrasonographic examination of the reproductive tract under anesthesia revealed a uterine mass consistent with a leiomyoma (fibroid). Hormonal therapy was initiated to reduce the mass but was unsuccessful. Over the next several months, vaginal bleeding continued and appeared to increase in volume and frequency with a resulting non-regenerative anemia. Uterine hydrothermal ablation was performed in an effort to alleviate some of this animal's clinical signs.<sup>3</sup> The procedure was executed successfully in this gorilla with an excellent recovery, cessation of vaginal bleeding and a resolution of the anemia; however, vaginal bleeding recurred within 1 mo. Uterine fibroid embolization (UFE) was subsequently performed and the animal had no complications from the procedure.<sup>4</sup> Immediately following the procedure, vaginal bleeding stopped. At a 4-mo recheck, the uterine mass had a significant reduction in size and the animal continued to be amenorrheic. Uterine tumors have been described in great apes and have been associated with serious disease.<sup>1,2,5</sup> UFE appears to be a minimally invasive technique that can potentially halt ongoing menorrhagia and can be an effective alternative to treating uterine leiomyomas without surgical intervention.

### ACKNOWLEDGMENTS

Special thanks to the Brookfield Zoo's great ape staff, Dr. Michael Zinnaman, MD (Loyola University of Chicago, Maywood, IL), the technical staff at Adventist Health Systems (La Grange, IL), Jennifer Langan DVM DACZM, Tom Meehan, DVM, Mark Warneke and Kimberly Schmidt, DVM. Very special thanks to Boston Scientific (Chris Barron and Matthew Wyatt Fischer) and the General Electric Company (Sam D'Amico, Heiner Fuchs, Javier Guizar, Katie Bohling) for their generous collaborations in providing equipment, supplies and technical expertise.

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**DORSAL LAMINECTOMY FOR REMOVAL OF A CAVERNOUS ANGIOMA IN AN  
EMPEROR TAMARIN (*Saguinus imperator*)**

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**Abstract**

A 4-yr-old, 750-g, female emperor tamarin (*Saguinus imperator*) was presented for a routine health exam under anesthesia. The following day, the tamarin appeared weak in the rear limbs. Three days after the exam, the tamarin was re-examined due to signs of urinary retention and tail and bilateral rear limb weakness. The urine retention and rear limb weakness improved over the next 2 wk with antibiotic and anti-inflammatory treatment, but the tail remained hypotonic. Six months later, the tamarin developed left leg weakness. There was no improvement in limb weakness during one wk of antibiotic and steroid therapy. A magnetic resonance image revealed a space-occupying mass in the spinal canal at L3-4. During the next five days, the left leg weakness progressed and right leg weakness developed. A dorsal laminectomy was performed to remove a cavernous angioma and a blood clot. Over the following 5 mo, the tamarin regained full use of the right rear limb but continued to have residual left rear limb dysfunction. The tail was partially amputated due to repetitive trauma secondary to lost function. One year later, the tamarin has adapted well to the residual left rear limb dysfunction and partial tail amputation.

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**DIAGNOSIS, REHABILITATION AND FUNCTION OF A CLUBFOOT (*Talipes equinovarus*) VARIANT IN A HAND-REARED ORANGUTAN**

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**Abstract**

Upon evaluation of a 4-wk old Bornean orangutan (*Pongo pygmaeus pygmaeus*) born at Cheyenne Mountain Zoo to a primiparous female who was being hand reared due to maternal neglect, bilateral hyperflexed (plantar flexed) feet were noted. There was no active motion of the ankle, only minimal passive motion (10° range) was present. The peroneal muscles were overstretched but the Achilles tendon and posterior tibialis muscle lacked flexibility. An EMG was completed to evaluate muscle conductivity and prognosis, and found to be normal. In comparison to the clinical presentation of the human clubfoot where three consistent variations to the normal anatomy are observed (talonavicular subluxation, calcaneal equinovarus and metatarsus adductus), only the metatarsus adductus was absent in the orangutan. Radiographically, there was also parallelism of the talus and calcaneus in the orangutan as seen in the human condition. Thus, physical examination and radiographs performed correlated to what is seen and found in the human talipes equinovarus. To our knowledge, this medical condition has not been reported in orangutans.

Our goal with this infant was to obtain active plantigrade locomotion with grasping ability as soon as possible in order to allow reintroduction with conspecific. Currently, there are many methods of treatment to make clubfeet functional. In humans, the approach is a conservative method, as was chosen with the orangutan, though surgery was an option if this method did not achieve full function. Conservative treatment consisted of physical therapy (massage, stretching and strengthening of muscles, mobilization of joints involved, functional electrical stimulation) and graduated active splinting. Once functional range was reached, active muscle function was encouraged using an adaptive climbing apparatus. After 5 mo of 24-hr care and excellent caregiver compliance with the rehabilitation program, the goals of fully functional feet were attained.

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## SURGICAL CORRECTION OF AN ARTERIOVENOUS FISTULA IN A RING-TAILED LEMUR (*Lemur catta*)

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### Abstract

A 10-yr-old ovariohysterectomized female ring-tailed lemur with prior history of repeated examinations for perivulvar dermatitis, laceration repair, and suspected pneumonia presented for tachypnea. A right femoral arteriovenous fistula was diagnosed by the presence of a palpable thrill and continuous machinery murmur over the inguinal area, positive Branham's sign, and suggestive ultrasonographic findings. Cardiac enlargement and mild pleural and abdominal effusion consistent with congestive heart failure were identified on radiographs and echocardiography. This animal was maintained on furosemide (0.5 mg/kg p.o. s.i.d.) until surgical ligation of the fistula was performed. Postoperative recheck examination confirmed successful closure of the fistula with resolution of the signs of heart failure.

Arteriovenous fistulas are abnormal connections between an artery and a vein which bypass the capillary bed. Acquired arteriovenous fistulas may result from invasive cardiovascular procedures, vascular trauma, or erosion of an arterial aneurysm.<sup>1,2</sup> Large arteriovenous fistulas may result in an increase in cardiac output with consequent cardiomegaly and high output heart failure.<sup>1</sup>

This lemur's high flow arteriovenous fistula with secondary heart failure is suspected to have resulted from prior femoral venipuncture based on the history and the anatomic location. To the authors' knowledge, this is the first report of an arteriovenous fistula in a lemur. Surgical correction of a suspected iatrogenic arteriovenous fistula in a macaque has been previously reported.<sup>3</sup> Arteriovenous fistula formation should be considered as a potential complication of venipuncture in primates and as a treatable cause of congestive heart failure.

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**SUCCESSFUL TRANSABDOMINAL SUBXIPHOID PERICARDIOSTOMY TO RELIEVE CHRONIC PERICARDIAL EFFUSION IN A 46-YEAR-OLD FEMALE SUMATRAN ORANGUTAN (*Pongo abelli*)**

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**Abstract**

During a preventive medicine examination in July 2006, a 46-yr-old female Sumatran orangutan (*Pongo abelli*) was diagnosed with pericardial effusion and dilated cardiomyopathy. Clinical signs were not apparent for the next 6 mo. In January 2007, pericardiocentesis was performed, but provided only short-term relief. The pericardial effusion continued to accumulate over the next 4 mo to a point that intervention was again required. In May 2007, a pericardial-peritoneal window was created using a transabdominal subxiphoid pericardiosotomy technique, providing immediate and long-term relief. Eight months later the orangutan was euthanatized due to worsening heart failure and no pericardial effusion was present.

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**SURGICAL AMPUTATION OF A DIGIT AND VACUUM ASSISTED CLOSURE (V.A.C.) MANAGEMENT IN A CASE OF OSTEOMYELITIS AND WOUND CARE IN AN EASTERN BLACK RHINOCEROS (*Diceros bicornis michaeli*)**

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**Abstract**

A 14-yr-old, female, eastern black rhinoceros (*Diceros bicornis michaeli*) presented with progressive osteomyelitis in her left hind lateral toe. Beta hemolytic *Streptococcus* was isolated. The animal was treated with multiple systemic antibiotics, and local wound cleansing. Repeated debridements and trimmings were performed for 5 mo prior to electing amputation. The toe was surgically amputated at the level of P1/P2. Analgesia was diffused into the wound via a catheter and elastomeric pump (Mila International, Erlanger, KY 41018 USA). The open amputation site was covered with adherent drapes and a negative pressure wound therapy device applied (V.A.C.® Kinetic Concepts Incorporated, Grand Rapids, MI 49544). The V.A.C. machine was attached to the animal for 72 hr. Three months later this animal developed a deep leg ulcer on the lateral aspect of the right hind limb, at the level of the stifle. Methicillin resistant Beta hemolytic *Streptococcus* was isolated. The wound was managed by initial daily lavage, followed by one mo of V.A.C., with 72 hr between dressing changes. Our clinical impression is that this therapy expedited the formation of healthy granulation tissue and that overall healing was accelerated. The animal tolerated the machine and bandage changes well.

V.A.C. therapy has been used successfully in humans, dogs, turtles, a tortoise, and a juvenile tiger.<sup>1-5</sup> The use of this therapy also appears to facilitate wound healing in slow-healing wounds in black rhinoceros.

**ACKNOWLEDGMENTS**

The authors would like to thank Kinetic Concepts Incorporated for donation of machines and dressings and help with technical support, with an extra thank you to Vickie Wardlaw, R.N. for all of her help. The authors would also like to thank all the Potter Park Zoo staff for their assistance in this case. Additionally, we would like to thank Dr. Michele Miller for her consultation and assistance. Thanks also to: Ron Blum, the Lansing Fire Department Station 5, Dr. Paul Coe, and the Michigan State University College of Veterinary Medicine veterinarians, technicians, and students for their extensive help in this case.

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## USE OF A NOVEL PROSTHESIS FOR TREATMENT OF LAMENESS IN A WHOOPING CRANE

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### **Abstract**

A 14-mo-old male whooping crane (*Grus americana*) presented for acute onset, non-weight-bearing lameness of the right leg, holding the limb caudally directed. No fractures or luxations were identified, although the stifle had reduced range of motion on extension. After no response to a six day course of non-steroidal anti-inflammatory drug therapy (meloxicam), an exploratory surgery on the stifle was performed by a veterinary surgeon. Muscle and tendon were found constricted around a distal segment of the fibula which had healed in an abnormal orientation from a previous fracture. This segment was excised, permitting stifle extension and normal orientation of the leg, but with knuckling-over of the foot.

The foot was splinted to encourage extension of the toes to facilitate weight-bearing. Unfortunately, splinting the digits in extension conflicted with the leg's normal biomechanical movements (hock flexion coincident with flexion of the toes). To permit normal range-of-motion and maintain the foot with the toes extended, a flexible prosthetic "boot" was fabricated from fiberglass mesh and silicone. This prosthesis enveloped digit 3 extending proximally to the distal tarso-metatarsus. It permitted normal flexion of the toes as the hock flexed, yet maintained that digit in extension to allow for weight-bearing. The bird adjusted to the "boot" well and demonstrated near normal gait shortly after application. The device was removed after 12 wk and normal use of the limb has maintained to date.

This novel prosthesis allowed near normal function of the foot. It can serve as both a support during convalescence after injury or as a permanent "prosthesis" should function not return.

### **ACKNOWLEDGMENTS**

The authors wish to thank Dr. Tom McNicholas for his surgical expertise, Mike Lincoln for his fabricating skills on the "boot" and the Bird Division staff for their daily care and monitoring of the crane during its treatment and recovery.

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## BRAIN NATRIURETIC PEPTIDE AS A NOVEL DIAGNOSTIC AND PROGNOSTIC INDICATOR OF CARDIAC DISEASE IN GORILLAS: TWO CASE REPORTS

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### Abstract

Cardiac disease is a leading cause of death in captive adult western lowland gorillas (*Gorilla gorilla gorilla*).<sup>4</sup> Recent evidence suggests that affected gorillas may present acutely, or may remain subclinical for extended periods. The progression of two cases of gorilla cardiac failure – one acute and one chronic – are compared and contrasted by examining serial cardiac ultrasound examinations over 10 yr in conjunction with a novel blood test, brain natriuretic peptide (BNP).

In humans, BNP is a sensitive and noninvasive method of diagnosing congestive heart failure, monitoring response to treatment, and determining prognosis.<sup>3</sup> This peptide is secreted by the ventricles in response to myocardial stretching, and levels are significantly increased in patients with cardiac disease.<sup>1</sup> Brain natriuretic peptide appears to be a more sensitive test for diagnosing certain types of cardiac failure than measuring left ventricular ejection fraction alone.<sup>2,3</sup> In humans a BNP level of >100 pg/ml diagnoses congestive heart failure with 90% sensitivity, 76% specificity, and 83% predictive accuracy.<sup>1</sup>

In a preliminary study to determine the value of BNP as a diagnostic test and prognostic indicator for cardiac disease in gorillas, banked blood from the two adult male gorillas at the Smithsonian National Zoological Park was analyzed for BNP levels and compared with ultrasound examinations taken at the same time. The retrospective analysis of BNP levels in these two cases suggests that BNP may be a novel and effective method of diagnosing and monitoring cardiac disease in gorillas.

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## VETERINARY INTERVENTIONAL RADIOLOGY: WHAT WE CAN DO FOR YOU

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### **Abstract**

Interventional radiology (IR) involves the use of contemporary imaging modalities such as fluoroscopy to selectively access different structures in order to deliver materials for therapeutic purposes. For example, interventional radiology permits us to use minimally invasive techniques to stop hemorrhage, embolize the blood supply to tumors or vascular malformations, deliver medications (such as chemotherapy) locally and regionally when system therapy is not working or results in inadequate local concentrations, stent open malignant obstructions of the respiratory, gastrointestinal, cardiovascular, and urogenital systems, etc.

These techniques are replacing more invasive procedures in both human and veterinary medicine in order to reduce morbidity and mortality rates in our patients. In addition, these procedures offer alternatives when conventional therapies have failed, are declined, or are not indicated due to various other co-morbidities. The very small incisions and ability to use natural orifices with interventional radiology procedures can be particularly useful in animals that traumatize their incisions or in environments in which wound complications or the animal patients themselves would be difficult to manage. These techniques could have broad therapeutic potential in zoo and exotic animals of all shapes and sizes.

Case examples (with particular emphasis on techniques that have already been performed in ferrets, horses, goats, pigs, and fish) using a body systems approach and describing the equipment (guidewires, catheters, sheaths, and stents) used are presented.

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## INTERVENTIONAL ENDOSCOPY (IE) AND INTERVENTIONAL RADIOLOGY (IR) IN ZOO ANIMAL PATIENTS: A NEW WAY OF “LOOKING” AT THINGS

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### **Abstract**

Interventional techniques such as interventional endoscopy (IE) and interventional radiology (IR) utilize fluoroscopy with endoscopy to gain access into any part of the body minimally invasively. This novel approach provides the clinician with an option where traditional surgery is either not ideal or potentially contraindicated. These techniques could potentially benefit zoo and wildlife patients as exemplified in two cases with on the urinary tract problems.

An adult female African lion (*Panthera leo*) was presented with persistent frank hematuria for at least 1.5 yr. Bloodwork, urine culture, ultrasound and CT imaging were normal. The patient was anesthetized and a urethrocystoscopy was performed. Frank hematuria was noted jetting from the left ureteral orifice, suggesting renal hematuria as a cause of her condition. The right ureter had normal yellow colored urine. The lion subsequently had a ureteronephroscopy performed and her renal pelvis was evaluated in a retrograde fashion through her ureterovesicular orifice. A lesion was found in her renal pelvis and was cauterized using Bugbee (Valley Lab) cautery through the ureteroscope. Post-procedure recovery was uneventful and the hematuria resolved.

A 15-yr-old male neutered spectacled langur monkey (*Presbytis obscura*) presented with stranguria. On radiographs the patient had bladder and urethral stones. An emergency cystotomy was performed, but a stone remained in the urethra and therefore the animal continued with dysuria. A flexible urethroscope was used to identify the urethrolith, a Hol:YAG laser was used to perform lithotripsy, and a stone basket was used to remove the fragments. A cystourethrogram was performed to confirm patency of the urethra. The monkey recovered uneventfully.

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## SCINTIGRAPHIC MEASUREMENT OF RENAL PERFUSION IN BOTTLENOSE DOLPHINS (*Tursiops truncatus*) WITH RENAL CALCULI

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### Abstract

Scintigraphic measurement of renal perfusion using 99m-Technetium mercaptoacetyltriglycine-3 (99mTc-MAG3) was performed on nine bottlenose dolphins (*Tursiops truncatus*) in a case-control study. Animals were transported on foam pads in covered trucks to the University of California San Diego Medical Center. Upon arrival, an intramuscular injection of 0.07 – 0.09 mg/kg midazolam was given in the epaxial muscle cranio-lateral to the dorsal fin for the purpose of sedation. Fifteen minutes after the injection, the animals were transferred by stretcher to a specially engineered table and positioned over a gamma scintillation camera. A 30mCi 99mTc-MAG3 injection was made into the caudal peduncle periarterial venous rete. Serial images of the kidneys were captured for a total scan time of 30 min. Animals were transported back to the home institution and, if needed, midazolam was reversed with 0.005 mg/kg of flumazenil i.v. The flow and parenchymal phases of the renograms were analyzed by a board-certified radiologist to determine relative renal perfusion, comparing left and right kidneys within each animal. Results showed dolphins with ultrasound evidence of >5 renal calculi and at least one dilated urinary collecting duct were significantly more likely to have a greater difference in renal perfusion between left and right kidneys compared to dolphins with < 5 calculi and undetectable urinary collecting ducts (P=0.02). Although the total number of renal calculi per animal was not a significant predictor of renal perfusion (P=0.48), a dilated urinary collecting duct was a reliable predictor of reduced relative renal perfusion (P=0.02).

### ACKNOWLEDGMENTS

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## ADVANCED TECHNOLOGIES AND METHODOLOGIES IN DIAGNOSIS AND TREATMENT OF ENDODONTIC DISEASE IN CALIFORNIA SEA LIONS

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### Abstract

Four California sea lions (*Zalophus californianus*) with advanced dental disease were diagnosed under voluntary control using new technologies including the Nomad (Aribex, Inc., 744 South 400 East, Orem, UT 84097 USA) handheld portable x-ray machine and EDR5 (Eklin Medical systems, Inc., 532 Weddell Drive, Sunnyvale, CA 94089 USA) digital radiography. In one sea lion under general anesthesia, CT was utilized to further characterize the endodontic and periodontal lesions. Restoration of incisor, canine, and post-canine dentition was performed under anesthesia with new methods that included a customized endodontic condensing instrument, performing YSGG Laser (Waterlase MD, Biolase Technology, Inc., 4 Cromwell, Irvine, CA 92618 USA) mediated sterilization of the root canal, filling apical osseous and endodontic defects with Consil (Nutramax Laboratories, Inc., 2208 Lakeside Boulevard, Edgewood, MD 21040 USA) synthetic particulate bone graft material, and sealing and filling the interior of the endodontic canal with EndoREZ (Ultradent Products, Inc., 505 W. 10200 S., South Jordan, UT 84095 USA). The coronal portion of the tusk was restored with Herculite XRV (Kerr Corporation, 1717 West Collins, Orange, CA 92867 USA) hybrid dental resin composite material that was polymerized with a plasma arc Demetron<sup>f</sup> curing light unit. In two animals, follow-up radiographs taken at 3 and 9 mo after completion of restoration, showed continued healing and successful repair of the diseased dentition. With these new instruments and techniques, we were able to successfully diagnosis and restore California sea lion teeth that would have typically been extracted in the past.

### ACKNOWLEDGMENTS

We thank our colleagues at the Navy Marine Mammal Program: Cynthia Smith, Stephen Cassle, Nate Daughenbaugh, and Sam Ridgway for their assistance with treatment and care of the animals. The U.S. Army Animal Care Specialists and Trainers were crucial in the implementation and execution of the procedures.

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## CHARACTERIZATION OF THE REPRODUCTIVE CYCLE IN FEMALE VEILED CHAMELEONS (*Chamaeleo calyptratus*)

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### Abstract

Preovulatory follicular stasis, or dystocia, is a significant cause of disease in female reptiles that can result in the death of the animal, or in loss of reproductive potential following surgical intervention. The aim of this study is to characterize the hormonal patterns associated with the reproductive cycle in female veiled chameleons to gain a better understanding of the basic reproductive biology of this species.

Fecal samples from 29 female veiled chameleons were analyzed for estrogen, progesterone, and testosterone over three reproductive cycles. Hormone patterns were correlated with changes in skin colour and behaviour indicative of distinct cycle stages. A subset of animals also had ovarian activity assessed by MRI every 2 wk.

Enzyme immuno-assays (EIA) for estrogen (E2), progesterone (P4), and testosterone have been validated for fecal analysis in veiled chameleons. Preliminary results for estrogen on three control animals revealed baseline levels between 500 and 1000 ng/g dry feces and significant peaks reaching 4000 ng/g and lasting 3-4 wk. Current results suggest correlations between estrogen levels, skin color changes, and ovarian activity.

Minimal work has been done to date using fecal hormone analysis in reptiles,<sup>1,2</sup> and it is believed that this is the first study to validate the use of EIA for fecal reproductive hormones in a reptile species. This non-invasive monitoring technique will be a valuable tool for the proper assessment and manipulation of reproductive function in the veiled chameleon and potentially other reptile species.

### ACKNOWLEDGMENTS

The authors thank the Toronto Zoo Foundation, the Endangered Species Fund, and the Pet Trust for funding this research. Special thanks go out to Jane Sykes and Matheson Boulevard Veterinary Services for providing the MRI imaging and Paula Mackie for assistance with the endocrine analyses. The authors also wish to acknowledge the support of Dale DeNardo, Rebecca Spindler, and Andrew Lentini.

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## USE OF ANTIBIOTIC INTRAVENOUS REGIONAL PERFUSION FOR DISTAL LIMB INFECTIONS

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### Abstract

Distal limb bacterial infections can be life-threatening to captive non-domestic mammalian species. As frequent and long-term topical or systemic options may not be feasible for those individuals that require chemical immobilization for handling and treatment, intravenous regional perfusion may be useful. This route has the ability to attain higher concentrations of antimicrobial drugs at the site of infection<sup>3</sup>, thus maximizing the effects of each immobilization and treatment. It is a successful technique in human<sup>1</sup> and equine medicine,<sup>3,4</sup> and limited use has been reported in other non-domestic species.<sup>2,5</sup> This technique is easily performed, well-tolerated, does not require specialized equipment, and can be adjusted to suit different management limitations. It has been observed to resolve infections even where surgical debridement and systemic antibiotic treatment has failed.

A 10-yr-old male swamp wallaby (*Wallabia bicolor*) presented for progressive lameness and a swollen right pes of 3 wk duration. Radiographs confirmed chronic osteomyelitis of the fourth pedal digit, and surgical amputation was performed. An aerobic culture revealed a mixed population of bacteria, including *Streptococcus* spp., *Staphylococcus* spp., *Escherichia coli*, and *Corynebacterium* spp. Despite systemic antibiotics (metronidazole 420 mg i.v. b.i.d. for 2 days then azithromycin 125 mg p.o. s.i.d. for 2 days), swelling was increased and purulent material present at the site of amputation two days after surgery. Five events of intravenous regional perfusion were performed. The affected limb was wrapped tightly with gauze proximally to the stifle and a tourniquet was placed at the stifle. The bandage was then removed and imipenem (500 mg) was infused into a catheter in the lateral saphenous vein. The tourniquet was removed after 10 min. Three weeks following i.v. regional perfusion the wallaby was fully weight-bearing on the affected limb. It remained free of clinical signs for the subsequent 2 yr.

A 12-yr-old male lesser kudu (*Tragelaphus imberbis*) presented for swelling over the left rear pastern. He was immobilized and physical exam confirmed a severe fluctuant swelling over the left rear pastern, extending to the fetlock. A large volume of purulent material was drained from the swelling which cultured *Morganella morganii*, *Clostridium bifermentans*, and *Fusobacterium necrophorum*. The joint was lavaged with diluted povidone-iodine. Despite systemic treatment with penicillin G benzathine/procaine (1,620,000 IU s.c.), amikacin sulfate (700 mg i.v.), long-acting ceftiofur (600 mg s.c.), and oral sulfamethoxazole/trimethoprim (960 mg b.i.d. for 14 days), initial improvement was followed by progression to sloughed skin over

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and extensive involvement of the pastern joint. Intravenous regional perfusion therapy was initiated 10 days after presentation. A tourniquet was applied at the hock, and ampicillin/sublactam (1800 mg) was infused into a catheter in the lateral saphenous vein. After 10 min, the tourniquet was removed for 5 min. The procedure was then repeated using enrofloxacin (600 mg). The treatment was repeated 14, 16, 18, 32, 38 days after initial presentation, with enrofloxacin being excluded from the protocol at the 32- and 38-day treatments due to phlebitis. Marked improvement of swelling and lameness was noted by day 16. Examinations at 59 days and 8 mo following initial presentation revealed no visible evidence of infection.

#### **ACKNOWLEDGMENTS**

The authors thank Dr. J. Farese, Dr. J. Siegal-Willott, D. Peck, C. Teare, A. Whitaker, and the staff of the UF-VMTH Zoological Medicine Service, White Oak Conservation Center, and St. Augustine Alligator Farm.

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**“CYCLIC” REGIMEN OF LOW-DOSE DOXYCYCLINE TO TREAT PERIODONTAL DISEASE IN A CHACOAN PECCARY (*Catagonus wagneri*), RED PANDAS (*Ailurus fulgens*), AND BAT-EARED FOXES (*Otocyon megalotis megalotis*)**

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**Abstract**

Doxycycline at sub-antimicrobial doses has effectively treated human and non-human primate periodontal disease.<sup>1-3</sup> Oral bacteria can damage periodontal tissue, but it is the host immune response that triggers the inflammatory cascade leading to periodontal disease. Inflammation causes ongoing periodontal ligament loss and bone resorption eventually leading to tooth loss. Low-dose doxycycline inhibits this inflammatory cascade without inducing microbial resistance.<sup>4</sup> This regimen is generally well-accepted by the animal and inexpensive.

A “cyclic” low-dose doxycycline regimen was used to treat periodontal disease in three widely different species: a chacoan peccary (*Catagonus wagneri*), three red pandas (*Ailurus fulgens*) and two bat-eared foxes (*Otocyon megalotis megalotis*). Each animal had received standard dental cleanings without periodontitis resolution. Low-dose doxycycline (0.3 mg/kg p.o. b.i.d.) was then administered, in a pulsatile manner (30 days on, 30 days off; 1-8 cycles). Improvement following the low-dose doxycycline regimen included decreased or resolved clinical signs of periodontitis, gingival pocket formation and tartar accumulation (Table1).

The experiences with these animals and multiple non-human primate species have demonstrated that it is essential to perform a detailed dental examination, including periodontal pocket assessment, prophylaxis, and systemic antibiotics when indicated. After the teeth are cleaned and initial infection treated, the low-dose doxycycline will slow or stop the progression of periodontal disease. It is important to stop the dental disease early before substantial gum retraction and bone resorption occur; hence, early intervention and repeat oral examinations as needed are justified.

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**Table 1.** Number of cycles used to resolve periodontal disease.

Species	Number of Cycles	Current Status
Bat eared fox	2	Alive
Bat eared Fox	1	Died with resolution of periodontal disease
Peccary	8	Periodontitis resolved <sup>a</sup>
Red panda	7	Periodontitis resolved <sup>a</sup>
Red panda	5	Periodontitis resolved <sup>a</sup>
Red panda	7	Periodontitis resolved <sup>a</sup>

<sup>a</sup>Ongoing cycles as part of preventive medicine plan.

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## GLUCOCORTICOID USE IN AVIAN SPECIES: A SURVEY OF VETERINARY PRACTITIONERS

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### Abstract

Glucocorticoids have immunomodulating, analgesic, and anti-inflammatory effects in some avian species.<sup>1,2</sup> However in birds, immunosuppression and systemic life threatening fungal infection can occur secondary to prolonged glucocorticoid administration.<sup>3</sup> The incidence of adverse steroid related events based on steroid administration to birds has not been compared and differential species susceptibility has rarely been examined.<sup>4,5</sup> This survey provides a baseline for investigation of appropriate glucocorticoid administration in avian species.

Veterinarian attendees of the 2007 Association of Avian Veterinarians (AAV) Annual Conference and Expo and the American Association of Zoo Veterinarians (AAZV) Annual Conference were surveyed on site at their respective meetings. Survey responses were collected confidentially; personal information was only used in summary format in the final results. Surveys (n=431) were collected with a response rate of 58%.

Within the year preceding the meetings, 54% of veterinarians surveyed had administered exogenous glucocorticoids to an avian patient; 51% of veterinarians surveyed reported positive outcome in these cases while 25% reported negative case outcomes. The majority of respondents (40%) had administered glucocorticoids to only 1-10 avian patients within the last year. The predominant indication for administration of glucocorticoids to the avian patient was trauma (39%) followed by shock (32%). The most common route of glucocorticoid administration in the avian patient was intramuscular (46%). Common steroid preparations used included dexamethasone and dexamethasone s.p. (27 and 28% respectively). The findings of this survey support the hypothesis that glucocorticoid administration in avian species is not uncommon and may have beneficial effects in the avian patient.

### ACKNOWLEDGMENTS

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## A NEW APPLICATION FOR ENDOTHELIAL INHIBITORS: INTRALESIONAL THERAPY

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### Abstract

A 38-yr-old Southern white rhinoceros (*Ceratotherium simum simum*) developed a focal conjunctival lesion on the left sclera that grew into a proliferative mass (2 cm diameter) over 8 wk. Through positive reinforcement training, topical proparacaine was utilized to biopsy the mass. Histopathology consisted of polypoid hyperplastic conjunctivitis with considerable dysplasia. Treatment with bevacizumab (Avastin® 25 mg/ml, New England Compounding Center, Framingham, MA 01702, USA; 2.5 mg intralesionally with 30-ga needle once) resulted in rapid shrinkage and complete resolution within 7 wk.

A 20-yr-old Pacific walrus (*Odobenus rosmarus divergens*) developed a focal, rapidly growing gingival mass (1.5 cm diameter) lateral to the most rostral right mandibular cheek tooth. Biopsies obtained using training and topical lidocaine revealed ulcerated granulation tissue with gingivitis. Treatment with enrofloxacin (50 mg intralesionally) and topical Betadine and gentamicin resolved the gingivitis but the mass remained. Treatment with bevacizumab (5 mg intralesionally) produced minimal effect; an additional two doses (5 mg) were administered into two areas of the mass and repeated at 2 wk. Although complete resolution was not achieved, the mass was reduced to less than 0.5 cm in diameter.

Bevacizumab, an anti-angiogenic compound, prevents neovascularization via inhibition of vascular endothelial growth factor. It is approved to treat metastatic or non-resectable colorectal, pulmonary, and mammary neoplasia and used in ophthalmology to treat neovascular macular degeneration.

In both cases, partial to complete resolution was achieved with no deleterious effects. Intralesional bevacizumab should be considered as a potential treatment for non-resectable or recurring masses.

### ACKNOWLEDGMENTS

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**ANALGESIC EFFECTS OF MELOXICAM FOR EXPERIMENTALLY INDUCED ARTHRITIC PAIN IN HISPANIOLAN PARROTS (*Amazona ventralis*)**

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**Abstract**

A partial cross-over study was performed to determine the analgesic efficacy of i.m. meloxicam in Hispaniolan parrots (*Amazona ventralis*) (n=16) using sodium urate monoarthritis as a model of tonic pain. Four dosages (0.05, 0.1, 0.5, and 1.0 mg/kg) of meloxicam i.m. and a saline control were evaluated using two quantification methods of lameness: 1) simultaneous weight-bearing of affected and unaffected limbs and 2) period of time bird was able to walk on a motorized perch that rotates with step-wise increase in rotations per minute every 10 sec.

Following administration of 8% sodium urate solution (0.1 ml) into the tarso-metatarsal joint of the bird's unbanded leg, each bird received saline (0.03 ml) or meloxicam 6 hr following the intra-articular injection, then every 12 hr until lameness resolved. This treatment interval was designed to mimic the treatment of a chronic inflammatory condition. The parrots were observed at 0, 4, 6, 8, 12, 18, 26, 30, 32, and 38 hr post induction of arthritis. The testing was repeated on each bird using a different treatment, either the saline control or a different dosage of meloxicam. Each bird received a minimum 2 wk interval between testing periods. The highest dose of meloxicam evaluated (1.0 mg/kg i.m. every 12 hr) provided statistically significant improvement in weight-bearing and walking function than the lower doses when compared over all time points in the study. These findings indicated that intramuscular injection of 1.0 mg/kg meloxicam every 12 hr has a positive clinical effect on relieving arthritic pain in the parrot.

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***Lawsonia intracellularis* AS A POSSIBLE CAUSE OF A FIBRINONECROTIC ENTEROCOLITIS IN FREE RANGING RING-TAILED LEMURS (*Lemur catta*) ON ST. CATHERINES ISLAND, GEORGIA**

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**Abstract**

The free-ranging ring-tailed lemur (*Lemur catta*) program on St. Catherines Island has provided researchers an excellent opportunity to conduct behavioral research for over 2 decades. Transmission of infectious disease from native wildlife to this lemur population has been previously documented with raccoons (*Procyon lotor*), white tailed deer (*Odocoileus virginianus*), gray squirrels (*Sciurus carolinensis*) and feral pigs (*Sus scrofa*) posing the biggest threats.<sup>1-3</sup>

An acute fibrinonecrotic enterocolitis has been documented in 14 ring-tailed lemurs over the last decade with 10 of the cases occurring over the last 2 yr. Younger lemurs were more commonly affected with ages ranging from 4 to 15 mo. One 10-yr-old male undergoing an introduction to a new troop develop signs and died from the disease. The onset of clinical signs was acute and included hypothermia, anorexia, lethargy, abdominal pain, and vomiting. Physical examination usually revealed a mid-abdominal mass. Gas distended bowel loops were the most common radiographic finding. Clinical pathology abnormalities included leukocytosis, monocytosis, elevated packed cell volume, hypoproteinemia, hypoalbuminemia, hyperkalemia, and hyponatremia. Feces and tissue submitted for *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* culture were negative in all cases. Toxin assays for *Clostridium difficile* and *Clostridium perfringens* were negative. *Lawsonia intracellularis* is the suspected etiology of this condition based on positive polymerase chain reaction and immunohistochemistry staining of feces and tissue from several lemurs, feral pigs and raccoons. Successful treatment depends on early intervention with fluid therapy, azithromycin, metronidazole, and analgesics. Oral vaccination with a product developed for domestic swine has had promising results.

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**AN OUTBREAK OF HUMAN INFLUENZA VIRUS IN GIANT ANTEATERS  
(*Myrmecophaga tridactyla*)**

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**Abstract**

In February 2007, an outbreak of respiratory disease occurred in a group of giant anteaters (*Myrmecophaga tridactyla*) housed at the Nashville Zoo. Over a period of several days all 11 adult animals with an age range of 1.75 to 6.5 yr were affected, while the one nursing neonate appeared unaffected. Clinical signs included nasal discharge and congestion, lethargy, and inappetance with severity ranging from mild to severe.

Diagnostics included nasal swabs submitted to the Clinical Virology laboratory at the University of Tennessee College of Veterinary Medicine. The samples were prepared and inoculated onto cell cultures with cytopathic effect noted on first passage. Characterization of the virus isolate was consistent with influenza virus of the Orthomyxoviridae. Further testing identified the pathogen as influenza A H1N1, a human subtype. This was the first known occurrence of influenza documented in giant anteaters.

These anteaters were housed in an off-exhibit facility with no exposure to the public, other collection animals nor native wildlife. Despite increased vigilance, no other collection animals were noted to have respiratory disease during this period. Many zookeepers, including the anteater keepers, were ill with respiratory disease preceding and during this outbreak in the anteater colony. It was concluded that the keepers were the most likely source of the virus affecting the anteater colony.

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**INVESTIGATION INTO BOVINE VIRAL DIARRHEA (BVD) IN A GROUP OF SCIMITAR-HORNED ORYX (*Oryx dammah*): DIAGNOSIS OF A TRANSIENT INFECTION WITH BOVINE VIRAL DIARRHEA VIRUS 1 STRAIN SINGER ARG**

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**Abstract**

Bovine viral diarrhea (BVD) is a contagious disease of cattle and other ruminants caused by an RNA virus of the genus *Pestivirus*.<sup>1,2</sup> Domestic cattle are considered the reservoir for BVD. The virus is shed in feces and nasopharyngeal secretions and infection occurs through ingestion or potentially inhalation. In domestic cattle, BVD most often causes subclinical infections including pyrexia and leukopenia with an appropriate immune response, although severe infections and herd outbreaks are common.<sup>1</sup>

A Scimitar-horned oryx calf with chronic diarrhea and suspected lack of passive transfer (LPT) was diagnosed with BVD-1, strain Singer-Arg through whole blood and fecal polymerase chain reaction (PCR). Successful therapy included two plasma transfusions and supportive treatment. A second calf was positive through PCR on whole blood but was asymptomatic. Fecal PCR for BVD in this animal was negative. Complete evaluation of the entire herd of Scimitar-horned oryx, consisting of whole blood PCR, fecal PCR and immunohistochemistry of pinna tissue specimens were negative. Additionally, banked sera samples for BVD from Common eland (*Taurotragus oryx*); new additions to the herd, were negative.

It is difficult to assess the role, if any, of BVD in this population but it seems likely that at least a transient infection was present in the group, and may be detectable during calving. In this particular calf, a lack of maternal antibodies may have predisposed to clinical infection with BVD. Continued surveillance for BVD virus in exotic hoofstock, especially those species considered for repatriation programs, is warranted.

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## ZOOS AS DISEASE SENTINELS: PILOTING AN AVIAN INFLUENZA SURVEILLANCE SYSTEM IN ZOOLOGICAL INSTITUTIONS

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### Abstract

Though the United States has been fortunate in thus far avoiding highly pathogenic avian influenza (AI), the risk remains that it could find its way to our shores. With the continued gap in knowledge on the full range of species susceptibility to AI, it is difficult to know with certainty the degree of risk posed by this disease to collection animals at zoological institutions. What is known is that interactions between wild birds and collection animals are a common occurrence at many zoos, and that zoological institutions are ideal sentinels for the detection of zoonotic diseases, like avian influenza, for a variety of reasons.

On behalf of the Association of Zoos and Aquariums (AZA) and with the support of the United States Department of Agriculture (USDA), Lincoln Park Zoo (LPZ) is launching a Zoonotic Emerging Disease Surveillance Center that will use zoological institutions across the country as disease sentinels to monitor for avian influenza virus. The system includes free sample diagnostics for participating institutions and access to real-time test results from a centralized, confidential database. This presentation will describe the surveillance system and its importance as a novel form of surveillance. Additionally, information obtained from the pilot test of the surveillance system regarding the role of avian influenza in captive populations is discussed.

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## ***Salmonella* AND *Campylobacter* spp. IN CAPTIVE HOOFSTOCK AND CARNIVORES: A SURVEY OF CARRIERS AND ENVIRONMENTAL SOURCES**

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### **Abstract**

*Salmonella* and *Campylobacter* spp. are highly zoonotic emerging pathogens capable of persistence within asymptomatic animal hosts and survival in water and fecal matter for long periods of time.<sup>1</sup> Clinical implications of infection in animals may include acute severe gastroenteritis, abortion, and a chronic decrease in fecundity, which can pose a significant threat to endangered populations in captivity.<sup>2,3</sup> The purpose of this project was to determine the role of housing, environment, or local wildlife on the persistence of *Salmonella* and *Campylobacter* spp. in fecal matter, food and water sources of non-domestic species. This study examined seasonal and clinical, fecal and environmental prevalence in semi-free ranging and indoor-housed groups of hoofstock (n=223), cranes (n=6), carnivores (n=14) and wildlife (n=15) species inhabiting the same areas at The Wilds over a year. Culture and identification utilized conventional approaches. Antimicrobial susceptibility was tested using Kirby-Bauer disc diffusion, following CLSI standards. *Salmonella* was found in fecal samples from 9.5% of animals housed indoors over winter and 1% of environmental samples from enclosures with culture-positive animals. *Campylobacter* spp. was found in 66% of animals housed indoors over winter, 47% of environmental samples, and 70% of animals in large yards with hutch shelters. Wildlife species which frequented areas with culture-positive managed species also cultured positive for both pathogens. Genotypic analysis was undertaken to identify epidemiologic relationships among bacterial strains. The findings underscore the high prevalence of these important zoonotic pathogens which could be of clinical significance to imperiled populations in captivity as well as to staff and visitors in zoological parks.

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**TEETERING ON THE BRINK: A MASSIVE MORTALITY EPISODE IN THE CAPTIVE MASKED BOBWHITE QUAIL (*Colinus virginianus ridgwayi*)**

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**Abstract**

Masked bobwhite quail (*Colinus virginianus ridgwayi*) are a subspecies of quail originally described in 1884 by H. Brown in Sasabe, Sonora. Overgrazing by cattle, introduction of non-native invasive grass species, and drought made these quail increasingly rare. By 1900 they had disappeared from Arizona. Attempted translocations and reintroductions to Arizona and New Mexico in the 1930's failed. By 1950 the subspecies was considered extinct.

The birds were re-discovered in Rancho El Carrizo, Sonora in 1964. The Endangered Species Act of 1968 included the masked bobwhite quail. Sixty birds were captured in Sonora in 1968 and sent to Patuxent Wildlife Research Center for captive breeding. The entire captive flock was moved to the Buenos Aires National Refuge in 1996. Masked bobwhite quail have been bred in captivity for reintroduction for the past 25 yr. Up to 2000 birds were produced for release each year since the program's inception. Over 21,000 birds have been released over time on the Refuge alone. A total of 30,000 birds have been released into the Altar valley of Arizona. To date, no effective wild population has been established. Current efforts are underway to locate more birds and/or proper habitat in northern Mexico.<sup>1</sup>

A disease outbreak in the Spring of 2007 reduced the flock from 680 birds to less than 300 in 3 wk time. Amyloidosis, quail disease (*Clostridium*-associated enteritis) and inclusion body-associated salpingitis were the most important histologic findings. The remaining quail were bred during a shortened season and produced a total of 600 birds that have replenished the flock. Many of the recently identified genetic founders died during the outbreak. The genetic effect of the mortality is not known.

Only five to seven calling birds were detected in the wild in Sonora, Mexico during 2007 – down from several hundred estimated only a few years ago. After decades of failed reintroductions in Arizona, the need to re-direct efforts south of the border has become evident. For this reason, zoos are being asked to receive and manage parts of the captive flock. Suitable habitat needs to be identified and protected in Mexico. The current captive flock may represent the only hope for this highly endangered species.

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## PREVALENCE OF *Ranavirus* INFECTION IN EASTERN BOX TURTLES IN EAST TENNESSEE

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### Abstract

Declines in eastern box turtles (*Terrapene carolina carolina*) have been reported from numerous locales throughout their range,<sup>5,6</sup> but the role of disease is unknown. Recently, mortality events in both amphibians and chelonians have implicated *Ranavirus* as the causative pathogen.<sup>4,7</sup> Regionally, ranaviral outbreaks have been reported in the southeast US in chelonians and amphibians.<sup>1,4-7</sup>

In 2007, whole blood samples were obtained from 40 wild eastern box turtles from a stable, well-studied population in east Tennessee. No evidence of *Ranavirus* infection was found using PCR. Forty-one injured or sick free-ranging eastern box turtles presented to the Veterinary Teaching Hospital at the University of Tennessee by good Samaritans and were also sampled. Whole blood, oral swabs, and/or necropsy tissues from (for those which died; n=10), were tested by PCR and two animals were positive for *Ranavirus*. Histopathologic lesions consistent with *Ranavirus* infection were noted in only one PCR-negative animal. The overall prevalence, based on PCR and histopathology, was 10% (3/30). In the three positive cases, one was presented with clinical signs consistent with upper respiratory tract disease, one for trauma with no evidence of upper respiratory tract disease, and the third was a 5-g neonate with no clinical signs. The presence of this virus in a neonate may indicate vertical transmission. Further research is needed on the ecology of this disease in free-ranging turtles to establish the specific mechanisms and pathogenesis of disease in order to determine the effect on populations of threatened chelonians.

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## PATTERNS OF INFECTION OR EXPOSURE TO MARINE-ORIGIN *Brucella* IN NORTH AMERICA

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### Abstract

Using novel diagnostic techniques for detection of infection with, or exposure to, marine-origin *Brucella*, including indirect and competitive ELISA, real-time PCR, bacteriologic culture and immunohistochemical staining, >6,000 blood or tissue samples have been tested. These samples represent >4,250 individuals, including wild and captive cetacean, pinniped, and marine carnivore species from Atlantic, Pacific and Arctic regions of North America. In preliminary results, seropositivity by cELISA averages 41% in cetaceans, 42% in pinnipeds, 10% in sea otters and 12% in manatees, but there is significant variation between species and populations in different geographic regions. In >900 animals tested, culture- or PCR-confirmed cases occur uncommonly (<5%) in both cetaceans and pinnipeds. In pinnipeds, predictive value of a positive serologic test to indicate active *Brucella*-associated disease is low, but the predictive value of a negative serologic result is high; these data reflect high seroprevalence and low rates of active disease, as well as a few cases of active disease without seroconversion. Immunohistochemical staining of positive cases suggests a carrier state in pinnipeds, with little evidence of inflammatory reactions to resident bacteria in many cases; however, *Brucella*-associated abortion has recently been found in one population of California sea lions. PCR detection of *Brucella* in feces and lungworms of pinnipeds suggests fecal-oral or dietary exposure as potential routes of transmission, in addition to transplacental infection. Our data highlight widespread distribution of *Brucella* spp. infections in marine mammals, and the necessity of using multiple testing techniques to determine effects of these pathogenic bacteria on marine mammal populations.

### ACKNOWLEDGMENTS

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***Francisella tularensis* TYPE B INFECTION AND PREVALENCE OF *F. tularensis* IN FREE-RANGING BEAVERS (*Castor canadensis*)**

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**Abstract**

Tularemia is an infectious zoonotic disease caused by one of several subspecies of the bacterium *Francisella tularensis*. In the spring of 2007, the National Park Service discovered an unusually high number of deceased free-ranging beavers (*Castor canadensis*) on a lake within Voyageurs National Park, Minnesota. The autolyzed carcasses of five beavers were recovered and submitted to the University of Minnesota Veterinary Diagnostic Laboratory for necropsy. Tularemia caused by *F. tularensis* subsp. *holarctica* (type B) was diagnosed in all five animals. The diagnosis was based on post mortem findings of pyogranulomatous splenitis and hepatitis, molecular detection (polymerase chain reaction from spleen samples), and antigen detection (direct fluorescent antigen detection assay from spleen samples). Attempts to isolate *F. tularensis* from spleen samples using sheep blood agar with cysteine and chocolate agar were unsuccessful, possibly due to the advanced autolysis of the tissues. In the fall of 2007, beavers in the region were captured (and released) for collection of fecal and serum samples. Fecal samples obtained from 94 beavers were negative for *F. tularensis* by culture. Serum samples collected from 22 beavers were negative for *F. tularensis*-specific antibodies by microagglutination testing at the Centers for Disease Control. Future studies aimed at increasing the understanding of the impact of tularemia on the beaver population in this region are planned. Individuals working with free-ranging beavers should be mindful of the potential for exposure to *F. tularensis*.

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## THE TEXAS OCELOT: 17 YEARS OF HEALTH ASSESSMENT

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### Abstract

Currently, in the United States the ocelot *Leopardus pardalis*, an endangered species, is found only in south Texas. The ocelot is linked to dense thornscrub habitat with  $\geq 95\%$  canopy cover; this habitat is reduced to  $< 5\%$  of the Lower Rio Grande Valley.<sup>2</sup> The only known breeding population of ocelots on public land survives at the Laguna Atascosa National Wildlife Refuge. Since 1982 this population of ocelots has been monitored in accordance with the USFWS Ocelot Recovery Plan. Genetic analyses indicate this population has been isolated since before monitoring began and is experiencing genetic diversity loss.<sup>1</sup> This survey is establishing baseline disease spectrums in preparation for translocation of ocelots from other populations to address genetic depression.

Since 1991, 52 animals have been captured for 101 blood collection events. These animals were tested for a variety of infectious diseases; the majority was negative for titers to *Leptospira interrogans* serovars pomona, hardjo, icterohemorrhagiae, and canicola, nine demonstrated titers to the grippotyphosa serovar. All samples showed low titers to *Toxoplasma gondii*; 100% (n=83) were negative for titers to *Coccidioides immitis*; 100% (n=67) were negative for titers to *Histoplasma capsulatum*; 100% (n=75) were negative for feline leukemia virus; 97% (n=64) were negative for *Herpesvirus*; 100% were negative for *Coronavirus*; 98% (n=91) were negative on the *Dirofilaria immitis* antigen test; 100% were negative for microfilaria. 89% (n=38) were negative for hemoparasites, whereby there were three instances of *Hepatozoon*, two *Cytauxzoon* and one *Hemobartonella* observed. Forty-five percent of samples showed gastrointestinal parasites in irregular frequencies and types.

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## IT'S THE FOAM: A NOVEL HARMFUL ALGAL BLOOM THAT PRODUCED A SURFACTANT WETTING AGENT AFFECTING MARINE BIRDS

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### Abstract

In November 2007, a series of beaching events involving more than 500 marine birds of various species occurred within Monterey Bay over approximately 3 wk. This event was variously blamed on the aerial spraying of a pheromone product for light brown apple moth (LBAM), on a spill of non-petroleum oil, and/or a massive red tide event that occurred in Monterey Bay during the same time period. Affected birds presented with wet feathers and were weak and hypothermic, some had a linseed oil-like smell. Scant, slimy pale yellow-green fluid could be wiped off the feathers of affected birds on presentation, but it dried out with time. The birds beached in three distinct pulses that corresponded temporally with the presence of a large dinoflagellate bloom just offshore. Upon washing, rehydration, warming, and nutritional supplementation, many affected birds recovered, suggesting that the product was minimally toxic or nontoxic. The event was ultimately traced to the dominant dinoflagellate in the red tide, *Akashiwo sanguine*. During breakdown and augmented by wave action, this organism elicited large quantities of a protein dimer of the same composition and molecular weight as protein recovered from feathers of affected birds, but was not present on unaffected birds. Further, surface foam and scum that was present on the water near the dinoflagellate blooms during this event, when spread on normal feathers, acted as an effective wetting agent. Dinoflagellate blooms have been previously linked to coral bleaching, due to the formation of a proteinaceous material similar to that found in the 2007 event. This is the first report of an ostensibly nontoxic, but harmful algal bloom (HAB) caused by a dinoflagellate surfactant protein impacting marine birds. Although the protein was relatively easy to clean off the birds using modified oil spill procedures, the event occurred just following seasonal migration of many species from the arctic. It effected about 10% of northern fulmars (*Fulmarus glacialis*) arriving in Monterey Bay; of which approximately half died. Significant mortality in surf scoters (*Melanitta perspicillata*) and Clark's (*Aechmophorus clarkii*) and western grebe (*Aechmophorus occidentalis*) added to large numbers of these species killed by the concurrent Cosco Busan oil spill.

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## PROSIMIAN TAXON ADVISORY GROUP UPDATE

*Randall Junge, DVM, Dipl ACZM<sup>1</sup> \* and Cathy Williams, DVM<sup>2</sup>*

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### Abstract

Prosimian Taxon Advisory Group (TAG) veterinary advisors are Randy Junge, St. Louis Zoo (TAG advisor); Cathy Williams, Duke Lemur Center (Eulemurs, nocturnal lemurs, and sifaka); Roberta Wallace, Milwaukee County Zoo (ringtailed lemurs); Meg Sutherland-Smith, San Diego Zoo (lorises); and Ilse Stalis, Dan Diego Zoo (pathology). The postmortem protocol has been updated and is available on the AAZV website.

### Hemosiderosis and Iron Overload

Recent projects have contributed to clarifying iron storage disease in prosimian species. Historically and anecdotally this syndrome is thought to be important for lemurs in captivity. It has been suggested that high dietary levels of iron combined with low levels of iron binding compounds in captive diets result in excess iron absorption and storage in tissues. A recent survey documented hemosiderosis (iron pigment in tissues) in 32% of lemur postmortem reports; however hemochromatosis (actual pathology associated with iron deposition) was rare. In a subsequent study that measured iron accumulation in liver tissue of lemur species, ruffed lemurs (*Varecia rubra*) had highest levels, ring-tailed lemurs (*Lemur catta*) lowest levels, and black lemurs (*Eulemur macaco*) intermediate levels. This suggests that there may be species differences in the propensity to develop hemosiderosis. Iron metabolites in serum, specifically ferritin and the calculated transferrin saturation value, may have some value in evaluating iron status in lemurs ante-mortem however correlation of these tests with hepatic iron content showed marked variability within and between taxa. Individual variation between samples was also marked. Thus the reliability of using these tests to evaluate iron status in individual animals remains uncertain. The development of species – specific reference ranges and performing repeat sampling for individuals may improve the diagnostic value of these tests.

### Anesthetic Complications in Giant Mouse Lemurs (*Mirza*)

Very little information is available regarding appropriate anesthetic drugs and doses for lemurs, however, common anesthetics used in other primate species works well in most lemur species. A major exception is the use of isoflurane anesthesia in giant mouse lemurs (*Mirza coquereli*). There are several anecdotal reports of respiratory arrest resulting in death in this species when individuals were mask induced with isoflurane. The mechanism is not known and data is not currently available on the safety of other inhalant anesthetics such as sevoflurane. Until further information is available inhalant anesthetics should only be used in *Mirza sp.* with extreme caution.

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## **Diabetes Survey**

In attempts to better understand the incidence of diabetes in lemurs in North American zoos, a preliminary survey is being developed for distribution to veterinarians working with lemurs in zoo settings. The goal is to collect preliminary information concerning numbers, species, diagnostic approaches, and treatment used for lemurs suspected of having diabetes.

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**SPECIES SURVIVAL PLAN VETERINARY UPDATE: CALIFORNIA CONDOR**  
*(Gymnogyps californianus)*

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**Abstract**

The California condor continues to thrive and succeed in captivity, but challenges to the success of the re-introduction program exist due to the highly degraded nature of the environment they are released into.

Lead poisoning is the number one medical problem of released California condors with regards to morbidity and mortality, and therefore the most serious threat to the recovery of the species in the wild. A major event occurred in the California condor program in California in the last year relating to lead poisoning in released birds in the state. In the fall and winter of 2007, after years of testimony and requests, the California Fish and Game Commission approved a ban of lead ammunition in the condor's range in California. This came immediately after a bill to do the same, AB 821, by Assemblyman Pedro Nava, D-Santa Barbara, was signed into law by Governor Arnold Schwarzenegger. The Fish and Game Commission extended the restrictions in the bill, which was less inclusive, to include all rifle and pistol ammunition within condor country (a V-shaped swath of land comprising approximately one-fifth of the state) including hunting of nongame animals such as wild pigs, coyotes and rodents. At this writing, as we wait for the ban to begin on July 1, seven birds were hospitalized at the Los Angeles Zoo for treatment for lead poisoning, including a several-month-old wild-fledged chick and its mother.

A strong veterinary program made a vital contribution to the success of this ban finally passing. Testimony by the Species Survival Plan (SSP) veterinarian (also the field veterinary coordinator) presented scientific data analyzed in conjunction with a well-respected toxicologist involved in the banning of DDT, Robert Risebrough, and the head pathologist and head of condor pathology at the Zoological Society of San Diego, Bruce Rideout. Statistics were presented regarding mortality in California, proposed mortality rates if birds did not continue to receive invasive screening and intervention with chelation therapy provided by the field personnel and the Los Angeles Zoo. These were coupled with concerns backed by scientific human and veterinary medical data for the long-term implications of chronic lead exposure in adults and offspring now being hatched in the wild. (Figure 1)

Chicks hatching in the wild in Southern California have suffered due to the ingestion of small pieces of trash (termed microtrash) fed to them by their parents. Due to the intensive involvement by veterinary and condor staff at the Los Angeles Zoo (impossible without the support of the USFWS), an aggressive pro-active monitoring and intervention plan is succeeding in decreasing mortality of these birds.

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## ACKNOWLEDGMENTS

Keeping these birds flying in the wild would be impossible without the tremendous dedication of all condor biologists and volunteers, and the condor and veterinary staffs of the Los Angeles Zoo, San Diego Zoological Society, the Phoenix Zoo, and the Oregon Zoo. We all aim for the same goal: A day when our intervention is no longer needed.

**Figure 1.** Ventura County Star 8/28/2007 “Commissioners near bullet ban.”

### Veterinarian tells of lead poisoning in state condors

By Timm Herdt

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SACRAMENTO — California Fish and Game commissioners signaled Monday they are leaning toward enacting a ban on lead ammunition in deer-hunting zones within the range of the 70 condors now flying free in the state.

At the close of a daylong hearing, commissioners asked that the formal public comment period on the proposed ban be opened quickly so they can consider enacting the ban at their Nov. 1 meeting.

“I’d like to see us take the lead on this,” said Vice President Cindy Gustafson. “I’m very concerned that if, as a commission, we don’t address this issue, others will address it for us.”

Conservationists and condor researchers are seeking to bar the use of lead ammunition, arguing lead poisoning is the leading threat to the condors’ survival in the wild. Bullet fragments in the flesh of wild animals that have been shot to death and then consumed by condors are the chief source of contamination.

“I haven’t heard a credible challenge to the scientific evidence,” Gustafson said.

Two other commissioners on the five-member panel said they concurred with Gustafson’s remarks.

Moorpark College Professor Cynthia Stringfield, a veterinarian who advises the federal Condor Recovery Program, presented a comprehensive history of the rate of lead poisoning among condors since they were re-released into the wild.

She estimated as many as 12 of the 51 wild condor deaths over that period were the result of lead contamination. Without the intensive trapping, testing and treatment of condors, she said, as many as 23 would have died from lead.

“To me, the case is clear,”

Stringfield testified. “Lead is killing these endangered birds, sometimes quickly, sometimes slowly.”

The population of California condors dwindled to 22 in 1982, and the last of the birds was taken from the wild. A successful captive breeding program allowed biologists to re-release some birds beginning in 1992.

There were no incidents of lead poisoning during the first five years after their release, Stringfield said. The problem arose after the birds began to expand their range and forage on their own, and it has become increasingly acute.

Through 2005, there had been seven deaths from either confirmed or likely cases of lead poisoning, she said. That number has doubled in just the past two years with the most recent fatality recorded July 31 in a condor that had been brought to the Los Angeles Zoo for treatment.

Commissioner R. Judd Hanna praised Stringfield’s report as “the best presentation putting things together” the commission has heard over the four years it has been considering the issue.

“They wanted more science, and they’re getting more science,” Stringfield said in an interview after her presentation.

But an alliance of gun and hunting groups has called the proposed ban on lead bullets “draconian.” In a letter to the commission last month, the group said the proposal “reflects a hidden agenda by some to ban all hunting in California.”

Commissioners were told nontoxic copper ammunition is now widely available for use in the rifles most commonly used by deer hunters, at a price only marginally higher than traditional lead bullets.

Legislation to enact such a ban is pending in the state Senate, but officials from the Department of Fish and Game assert if such action is to be taken, it should be in the form of regulations adopted by the commission.

Assemblyman Pedro Nava, D-Santa Barbara, the author of AB 821, has said he intends to continue to push for legislation because the commission has been too slow to respond.

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**A BRIEF UPDATE ON THE MEDICAL MANAGEMENT OF THE GEOFFROY'S MARMOSET (*Callithrix geoffroyi*)**

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**Abstract**

During the calendar year 2007 the Association of Zoos and Aquariums (AZA)-managed population of Geoffroy's marmoset (*Callithrix geoffroyi*) consisted of 100 animals (40.57.3). Nine pairs produced 29 offspring, of which 15 were viable and parent-reared; the remaining 14 were stillborn or did not survive. Eighteen total deaths (10.4.4) were reported. Among institutions responding to AZA Veterinary Advisory Group Survey, there were no apparent patterns of morbidity or mortality during the survey period.

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## MANED WOLF (*Chrysocyon brachyurus*) SPECIES SURVIVAL PLAN MEDICAL UPDATE

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### Abstract

The maned wolf (*Chrysocyon brachyurus*) is an endangered, omnivorous canid native to South America. This species has been kept in captivity in the United States for more than 30 yr and has a documented history of diarrhea, unthriftiness (hair loss, poor coat condition), low reproduction, and cystinuria. Despite advances in the knowledge of cystinuria in maned wolves, three animals have died over the past 5 yr due to complications from cystine urolithiasis. In addition to genetics, nutrition is a main factor contributing to this disease.<sup>2</sup> A nutritional survey was completed in the fall of 2006 to determine what diets are currently fed to captive maned wolves. To complement this survey, the Maned Wolf Species Survival Plan© (MWSSP) is currently seeking funding to perform serum nutritional assays on captive maned wolves. In addition, research is being conducted to determine whether nutrition is playing a role in the poor rate of reproduction observed over the past 3+ yr.

Naïve maned wolves may be sensitive to modified live canine vaccines (parvo and canine distemper viruses) and have been known to contract disease after vaccination.<sup>1,5</sup> As of 2006, there is no commercially available killed canine parvovirus vaccine in the US. For the past several years, maned wolf pups have been vaccinated with the killed feline parvovirus product (Fel-O-Vax® PCT, Ft Dodge Laboratories, Ft. Dodge, IA 50501 USA), and titers have been followed (Table 1). Once the parvo titers were measurable and/or the pups reached ~6 mo of age, the modified live canine vaccine was given (i.e., Duramune® Max Pv, Ft Dodge Laboratories, Ft. Dodge, IA 50501 USA). No adverse effects have been noted.

With the advent of a recombinant canarypox-vectored canine distemper vaccine (PURE VAX™ Ferret Distemper vaccine, Merial, Athens, GA 30601 USA), the MWSSP has recommended this vaccine for use in maned wolves. No adverse reactions have been reported. Serologic titers in response to vaccination have been documented in maned wolves and are considered to be protective based on previous studies in other species.<sup>3</sup>

Conservation of the maned wolf in situ is another priority of the MWSSP, which is currently helping to support several international field projects. The Maned Wolf Conservation Project in

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Serra da Canastra National Park, Brazil involves monitoring radio-collared maned wolves, analysis of fecal cortisol metabolites and periodic, hands-on assessment of health and reproduction. Results obtained during the past 3 yr show that human development significantly affects hematologic values and adrenal activity of free-ranging maned wolves. Wolves living on farmed land have higher fecal cortisol metabolites than those living around park borders and inside the park. Pending funding, it is hoped that serum nutritional analysis from the wild maned wolves can be compared to the parameters observed in captive individuals. A similar maned wolf health project in Noël Kempff Mercado National Park, Bolivia is currently looking at disease transmission from domestic canids, and co-investigators have recently published a serosurvey of maned wolves.<sup>4</sup>

Contraception is necessary for certain captive maned wolves. GnRH agonists, such as deslorelin, are recommended by the AZA Wildlife Contraception Center as the safest reversible contraceptive method. In addition, the MWSSP reproductive advisor is investigating the use of deslorelin in estrus induction in maned wolves for artificial insemination.

#### **ACKNOWLEDGMENTS**

The authors would like to thank all institutions holding maned wolves and especially the Fossil Rim Wildlife Center for the information regarding canine distemper titers.

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**Table 1.** Maned wolf pup parvovirus vaccination (vax) schedule and titers 2006.

SB #	Date of Birth	Age	Date of Vax	Type of Vax	Parvo Titer (IgG)
2844	1-Dec-05	9 wk	9-Feb-06	Fel-o-Vax	NA
		12 wk	2-Mar-06	Fel-o-Vax	NA
		15 wk	23-Mar-06	Fel-o-Vax	1.100
		18+ wk	17-Apr-06	Fel-o-Vax	0
		21 wk	9-May-06	Duramune	1:25
		35 wk	22-Aug-06		1.1600
2845	1-Dec-05	9 wk	9-Feb-06	Fel-o-Vax	NA
		12 wk	2-Mar-06	Fel-o-Vax	NA
		15 wk	23-Mar-06	Fel-o-Vax	0
		18+ wk	17-Apr-06	Fel-o-Vax	0
		21 wk	9-May-06	Duramune	1.1600
		35 wk	22-Aug-06		1.3200
2846	1-Dec-05	9 wk	9-Feb-06	Fel-o-Vax	NA
		12 wk	2-Mar-06	Fel-o-Vax	NA
		15 wk	23-Mar-06	Fel-o-Vax	0
		18+ wk	17-Apr-06	Fel-o-Vax	0
		21 wk	9-May-06	Duramune	0
		35 wk	22-Aug-06		1.1600

**Table 2.** Maned wolf pup canine distemper vaccination (vax) schedule and titers 2007.

SB#	Date of Birth	Age	Date of Vax <sup>a</sup>	CDV Titer
T0710	22-Feb-07	6 wk	6-Apr	NA
		9 wk	27-Apr	NA
		12 wk	18-May	NA
		16 wk	14-Jun	1:128
		19 wk	5-Jul	1:256
T0711	22-Feb-07	6 wk	6-Apr	NA
		9 wk	27-Apr	NA
		12 wk	18-May	NA
		16 wk	14-Jun	1:128
		19 wk	5-Jul	1:256

<sup>a</sup>Pups were vaccinated with Merial's PURE VAX™ Ferret Distemper vaccine.

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**SPECIES SURVIVAL PLAN VETERINARY ADVISOR REPORT FOR 2007-2008:  
VIRGIN ISLANDS BOA (*Epicrates monensis granti*) AND MONA BOA (*Epicrates  
monensis monensis*)**

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**Abstract**

The Mona boa (*Epicrates monensis monensis*) is a species of Caribbean boa listed as threatened, and is found only on one small island, Isla Mona, also off the coast of Puerto Rico. There has been a captive breeding plan in place for this subspecies since 1990, but the Species Survival Plan (SSP) focus for the population has been management in the wild.<sup>6</sup> The Toledo Zoo holds a captive assurance population of 16 Mona boas (8.8 animals).

The Virgin Islands boa (*Epicrates monensis granti*) is an endangered subspecies of the Mona boa, endemic to a constellation of small islands off the coasts of Puerto Rico and the U.S. and British Virgin Islands. There has been a captive breeding and reintroduction program in place for this subspecies since 1985.<sup>4,5</sup> Two successful reintroductions have been carried out- one to the U.S. Virgin Islands and one to a satellite Island of Puerto Rico. Ten-year evaluations of these reintroductions have recently been completed. There are currently 42 Virgin Islands boas in captivity (14 male, 26 female, 2 undetermined) housed at 7 different institutions. The Toledo Zoo holds 32 of the 42 captive specimens.

Review of medical records of both subspecies from January 2007 through April 2008 revealed two cases of bullous spectaculopathy/ subspectacular abscessation, and one case of cloacitis.<sup>1-3</sup> Etiologies were undetermined. Stomatitis was associated with one of the two cases of spectaculopathy. One of the cases of subspectacular abscessation was ultimately euthanatized after extensive treatment, and necrotizing histiocytic dacryoadenitis was found on histopathologic examination. One other VI boa death was reported during this period. The histopathologic diagnosis for this animal was generalized septicemia of undetermined etiology.

Current health recommendations for these species include annual physical exam and biannual fecal examinations for parasites. Preshipment/quarantine testing should include physical exam, fecal exam for parasites (ideally three negative fecals during the quarantine period), fecal culture, blood work, including CBC and chemistries (minimally calcium, phosphorus, uric acid, AST levels), whole body radiographs, and paramyxovirus testing (Hemagglutination Inhibition (HI), paired samples) if there has been exposure potential. The recommended quarantine period is minimally 90 days. Testing and quarantine protocols of animals designated for release programs are being reviewed. Blood collection has proven to be somewhat challenging for this species in the authors experience due to the snake's small size. Blood has been successfully collected using standard tail vein and cardiac puncture techniques. There has been one reported death in this species resulting from complications after cardiac puncture for routine blood collection. Caution

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should be used when attempting this technique and general anesthesia (isoflurane) should be considered to aid in the collection process.

A retrospective review of medical records for the captive population of Mona/VI boas is underway to identify any disease trends. Health care recommendations may be adjusted once the review is completed. There are no reported infectious disease issues present in the captive population at the time of this writing.

Active research projects include a long-term population study of the Mona boa on Isla Mona by the Toledo Zoo, and periodic evaluations of the status of the reintroduced populations in Puerto Rico and the U.S. Virgin Islands. A joint study of the University of Nevada at Las Vegas, the Departamento Recursos Naturales y Ambientales de Puerto Rico, and the Toledo Zoo is examining the phylogenetic relationships among the disjunct populations of Virgin Islands boas using gene sequencing.

Information from the field reveals that despite recommendations from both the Virgin Islands Division of Wildlife and the Departamento de Recursos Naturales de Puerto Rico, it appears that the U.S. Fish and Wildlife Service will attempt to downlist the Virgin Islands boa to "Threatened" from "Endangered" (Pierce, pers. comm.).

Other field reports indicate that the attempt to eradicate rats from Congo Cay, USVI (prime boa habitat and a potential reintroduction site) by the U.S. Department of Agriculture was unsuccessful. Rat eradication on another cay in the USVI is being considered. A rapid evaluation of the wild boas at Río Grande, Puerto Rico in November 06 showed continued deterioration of habitat as development continues to surround the locality (unpublished observation).

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## AN UPDATE ON THE CUBAN CROCODILE (*Crocodylus rhombifer*) AND THE SPECIES SURVIVAL PLAN

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### **Abstract**

The Cuban crocodile (*Crocodylus rhombifer*) is one of the worlds most endangered crocodylians and wild populations remain in only two small freshwater marsh habitats in Cuba. Loss of habitat, human encroachment, poaching for skin and meat, and ecological competition with two other crocodylian species are threatening the long term viability of this species. Hybridization with one of those competitors, the American crocodile (*Crocodylus acutus*), remains the most serious problem threatening the genetic integrity of the species. The Cuban Crocodile Species Survival Plan was formed in 1993 and has focused its efforts on captive management, *in-situ* education, and select field studies. Recently, a morbidity and mortality review on the *ex-situ* captive population has been conducted and at the time of this writing, the first Husbandry Manual for the species is near completion.

The political relationship between Cuba and the United States has been an obstacle to efficient communication, importation of new animals, and more frequent and comprehensive *in-situ* activities. In the short and long term, recruitment of additional holding space dedicated for Cuban crocodiles in AZA accredited zoological parks is vital.

### **Current Wild Population and Endangered Status**

The Cuban crocodile (*Crocodylus rhombifer*) is classified as endangered (IUCN Red List) and has one of the smallest natural geographic ranges (<500 sq km) for a megavertebate predator. The species inhabits freshwater marshes and is currently endemic to Cuba. There are currently two remaining wild populations, one limited to central freshwater portions of the Zapata Peninsula and a second reintroduced population tentatively established in the eastern portion of the Lanier Swamp on Isla de la Juventud.

Historically, the species was more widespread throughout Cuba and the Western Caribbean. Natural climate changes (i.e., interglacial period warming leading to rising sea levels) during the Holocene period lead to a progressive decline in the Caribbean freshwater marsh ecosystem.<sup>5</sup> Human encroachment and poaching for hides and meat has also been responsible for a decline in both the Cuban crocodile range and overall population. Cuban crocodiles were extirpated from Isla de la Juventud before captive-raised animals were reintroduced between 1993-1995. Despite government protection, poaching continues to be a significant pressure on both of the current populations due to inadequate protection and enforcement (Roberto Soberón, pers. comm.).

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In addition, Cuban crocodiles currently share their habitat with two other crocodylian species that historically were not present. In the 1950's, spectacled caiman (*Caiman crocodilus*) were introduced on Isla de la Juventud. By the early 1990's Cuban biologists estimated a well established population of over 40,000 animals in the region (Roberto Soberón, pers. comm.). The presence of this adaptable and invasive species has placed added pressure on the Cuban crocodile due to resource competition. More significantly, both remaining Cuban crocodile populations share their habitat with the American crocodile (*Crocodylus acutus*), another highly adaptable and successful generalist. Historically, the American crocodile occupied brackish or marine regions along Cuba's coast allowing Cuban crocodiles to act as the keystone species within interior freshwater swamps. However, man-made canals were constructed in the 1800's beginning from the coastline into the interior of the Zapata Peninsula. These canals were utilized for deforestation and to facilitate industrial and agricultural developments,<sup>5</sup> and served as an immigration route for the American crocodile to penetrate the interior of the freshwater swamp. Today, American crocodiles cohabitate with Cuban crocodiles in Zapata, as well as on Isla de la Juventud, and unlike with spectacled caiman, these *Crocodylus* species will hybridize and produce fertile offspring. These hybrid crocodiles, identified phenotypically, range in abundance depending on the locality sampled. Hybridization with the American crocodile remains the most deleterious factor threatening the genetic integrity and existence of the Cuban crocodile.

### **The Species Survival Plan: Past and Present**

The Cuban Crocodile Species Survival Plan (SSP) was formed in 1993 with the goals of helping to insure the long term survival of the species in nature as well as to establish a self-sustaining *ex-situ* captive population. The current SSP-managed captive population consists of only 32 individuals (6.23.3) housed at 10 institutions, ranging in age from 4-50<sup>+</sup> yr. The 2008 Regional Collection Plan calls for a target population size of 75 animals. Since inception of the SSP, 17 Cuban crocodiles have hatched at two institutions.

The following represents a timeline of significant SSP activities:

1993 – The Cuban Crocodile SSP was formed.

1996 – The first SSP Master Planning meeting was held in Toledo, Ohio. *In-situ* field work was conducted monitoring the reintroduced population of Cuban crocodiles within Lanier Swamp on Isla de la Juventud. This field study was the first contact between an AZA-accredited zoo and Cuba in regards to Cuban crocodile conservation. An Education Liaison joined the SSP.

1997 – Further *in-situ* field studies were conducted monitoring the Lanier Swamp population of Cuban crocodiles. At the same time, an attempt was made to raise public awareness about the reintroduction of Cuban crocodiles to Lanier Swamp and foster development of a conservation ethic towards this endemic species. A community festival was planned to promote the Cuban crocodile as a strong patriotic symbol unique to Cuba's natural heritage.

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1999 – Further *in-situ* field studies were conducted in Lanier Swamp. An SSP Master Planning meeting was held in Louisville, Kentucky. The SSP hosted Roberto Soberón, Cuba's national coordinator of crocodile programs, at the meeting.

2000 – Further *in-situ* field studies were conducted in Lanier Swamp. The Cuban crocodile festival that was planned in 1997 was implemented and held in Mellá, a small community that borders Lanier Swamp on Isla de la Juventud. In addition, a Populations and Habitat Viability Assessment (PHVA) workshop was held in Varadero, Cuba, in conjunction with the World Conservation Union's (IUCN) Crocodile Specialist Group meeting. This workshop provided tools to evaluate and integrate the interaction of biological, physical, and social factors on the Cuban crocodile population.

2001 – The SSP hosted Damarys Lopez, a Cuban biologist that conducts studies on all three crocodylian species on Isla de la Juventud, at the Louisville Zoo.

2002-2003 – The SSP collaborated with the Wildlife Conservation Society/Bronx Zoo and the Havana Zoo in Cuba in offering a series of workshops that offered environmental education materials and training to local educators focusing on wetlands and the Cuban crocodile.

2003 – Veterinary Advisors joined the SSP. The SSP hosted Dr. Elsie Pérez, then Director of the Havana Zoo and Head of the Cuban Association of Zoos and Aquariums, at the Louisville Zoo.

2003-2005 – A survey was conducted collecting medical records and pathology data from all zoos and related facilities that house, or have housed, Cuban crocodiles. Data collected, including morbidity and mortality information, were compiled.

2004 – An SSP Master Planning meeting was held in Louisville, Kentucky

2007 – An SSP Master Planning meeting was conducted via electronic mail.

Coming Soon – The first comprehensive Husbandry Manual for the Cuban crocodile.

### **Veterinary Issues**

With proper husbandry, the Cuban crocodile is extremely hardy and long-lived in captivity and few disease problems have been reported. Most health problems appear to be due to conspecific trauma or husbandry issues (i.e., nutritional diseases, access to coin foreign bodies, exposure to suboptimal temperatures).

Nutritional diseases have been observed. Rickets in the form of general osteopenia, scoliosis, long bone bowing, lameness, and pathologic fractures can occur in young hatchlings that are not fed a diet that meets calcium, phosphorus, and vitamin D<sub>3</sub> needs. Feeding hatchlings whole vertebrate prey or a balanced commercial carnivore diet as early as possible, as opposed to unfortified insects, is desirable. Hypovitaminosis E leading to steatitis has been documented in young Cuban crocodiles fed exclusively fish.<sup>4</sup> Animals fed unsupplemented, thawed frozen fish

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are particularly susceptible to hypovitaminosis E. If fish comprise a significant component of the diet, supplementation with vitamin E and thiamin is advised.

Zinc toxicosis secondary to coin ingestion has been seen in at least two captive Cuban crocodiles at two different institutions. Signs included anorexia, weight loss, and depression, and gastric biopsies from one of the animals revealed gastritis and mucosal necrosis. Both cases were treated with coin removal and chelation therapy. Due to the potential for zinc toxicosis from coin ingestion, Cuban crocodiles should be housed in a manner that eliminates, or at least minimizes this risk (i.e., glass or mesh separating the crocodiles from the public). High blood lead levels have also been documented in at least two Cuban crocodiles, although it appears that crocodilians have a tolerance for high lead burdens. In one of those cases, the elevated blood lead level was secondary to feeding urban feral pigeons.<sup>1</sup>

Dental issues have also been reported. Malocclusion has been observed in some captive Cuban crocodiles where the teeth from one arcade appear to cause gingival trauma and secondary infection to the opposing arcade. It is unclear if high temperature incubation, chronic lack of ultraviolet light exposure, and/or lack of physiologic jaw loading (i.e., eating soft or small food items) during the more rapid growing years play any role in this observation. A captive adult female Cuban crocodile was euthanized due to severe, chronic, maxillary and mandibular osteomyelitis. This crocodile had a history of chronic gingivitis and periodontitis suggesting that the bone infection may have been an extension of dental disease.

A review of medical records suggests that endoparasitism does not appear to be a significant problem in captive Cuban crocodiles. Due to this apparent low incidence, routine deworming is likely unnecessary. The Cuban Crocodile SSP does not recommend the use of avermectin anthelmintics (i.e., ivermectin and its relatives) in this species due to reports of toxicity in crocodilians.<sup>2,3</sup>

A pathology review was conducted and trauma due to conspecific aggression is the leading cause of morbidity and mortality in this species. Unlike Nile crocodiles (*Crocodylus niloticus*) or American alligators (*Alligator mississippiensis*), Cuban crocodiles are less tolerant of conspecifics. Puncture wounds, lacerations, lameness, cloacal trauma, facial/jaw fractures, and secondary infections/abscesses have all been reported by holding institutions. Animals under constant social stress can also suffer from secondary hypoglycemia. Individual housing is recommended for this species except in very young hatchlings or in bonded pairs.

Additional post mortem diagnoses revealed through the pathology survey included bacterial sepsis, pneumonia, visceral and articular gout, coelomitis, myopathy, dystocia, hepatitis, myocarditis, nephritis, and disseminated sarcoma.

## **The Future**

The political relationship between Cuba and the United States has been an obstacle to efficient communication, importation of new animals, and more frequent and comprehensive *in-situ* activities. It is hoped that in the future, perhaps this relationship will change allowing for a freer

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exchange of information, educational materials, animals, and biomaterials. In time, the Cuban Crocodile SSP is interested in the importation of additional founder stock from Cuba, continued monitoring of the two wild populations, significant expansion of *in-situ* education elements, molecular investigation of the hybridization problem, and a comprehensive health assessment of the free-ranging population.

Although the Cuban crocodile faces an extremely challenging reality in nature, this species may be an ideal animal to manage long term in captivity. The species is relatively easy to propagate, is hardy and long-lived, and individuals remain reproductively viable for decades after reaching sexual maturity. Long generation times are ideal for the long-term maintenance of gene diversity. Additionally, population growth can be managed by selective incubation of eggs, and gender of offspring can be potentially selected for via careful manipulation and monitoring of incubation temperatures. From an exhibition standpoint, Cuban crocodiles are vividly colored, medium sized, living “dinosaurs,” and can facilitate the communication of a compelling conservation story to the public. They are intelligent, food motivated, impressive jumpers, and very trainable; a combination of qualities that offers great potential for utilizing them in public demonstrations. The Cuban Crocodile SSP is very interested in recruiting additional AZA accredited partners to help grow the captive population. Please contact the SSP Coordinator, Bill McMahan if interested (502-238-5351, bill.mcmahan@louisvillezoo.gov).

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**GUAM RAIL (*Gallirallus owstoni*) RECOVERY PROGRAM AND SPECIES SURVIVAL PLAN: IN SITU POPULATION HEALTH STATUS AND MEDICAL HUSBANDRY FOR PRE-RELEASE CANDIDATES**

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**Abstract**

The contribution of Association of Zoos and Aquariums (AZA) institutions to the in situ Guam Rail Recovery Program has been exceptional. Transfer of birds from the mainland captive population to the captive population on Guam requires careful medical evaluation of each individual bird prior to shipment. Medical husbandry of these individuals can dictate whether birds are suitable for participation in the repatriation program due to governmental and Species Survival Plan (SSP) guidelines.

**ACKNOWLEDGMENTS**

The authors thank all AZA institutions that currently or historically have held Guam rails. All institutions have made significant contributions to these programs. Financial support for health assessments for the recovery program were provided by Disney's © Animal Programs Conservation Action Strategy and Captive Conservation Programs.

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## WHITE WING WOOD DUCK (*Cairina scutulata*) SPECIES SURVIVAL PLAN UPDATE

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### **Abstract**

The white wing wood duck (WWWD) Species Survival Plan (SSP) focus is split between problems of captive population and working to support wild population conservation.

We have previously reported on the problems of the captive population. Currently the U.S. SSP population comprises approximately 70 ducks. The majority are 3 yr of age and younger as the population sustains upwards of 85-95% losses from mycobacterial disease in certain large collections. In addition, the SSP population exhibits a genetic diversity of only 30%, since all SSP individuals derive from four or five ducks imported into England in the late 1960's.

Our work over the past 7 yr has focused on environmental factors significantly contributing to the mycobacterial losses. We are currently addressing these issues in SSP recommendations and in environmental changes at the major holding site of the SSP population. Currently all SSP WWWD's are privately, non-AZA owned. In addition to the primary holding site, the Akron Zoo, in partnership with Hiram College, has opened a Waterfowl Conservation Center for the study and propagation of WWWD's. We operate the facility under quarantine-like conditions, incubator raise the ducklings and control environmental parameters to minimize mycobacterial exposure. Our goal is to produce future generations free of early mycobacteria exposure and raise them past a possible major risk age to allow an alternative source of breeding-aged individuals for SSP institutions. We are hopeful to establish one or two secondary controlled holding/breeding institutions in the future as well.

In addition to partnering for disease controlled propagation, Hiram College is also participating with us to continue research into the specie's apparent mycobacterial susceptibility. Dr. Jodi Moderelli of Hiram College is developing a metabolomics project investigating mico-lipids as biologic markers for early disease (mycobacteria) recognition in the WWWD.

In situ SSP supported conservation of the specie has been minimal in the past, represented by a single year Malaysian nest box project in 2001. No full-scale surveys of wild populations have been attempted. This past year we have begun to increase SSP involvement with WWWD conservation. With the help of the WWT and the WCS, we determined that our best area to focus our current conservation efforts is in Cambodia. The WCS already has a very good general conservation initiative in place in the Northern Plains and protected forest on the Thailand/Laos border. A concept paper was produced for survey and possible nest protection program. The first phase was undertaken in Feb. 2008. We surveyed several areas of the protected forest and had 10 sightings probably constituting three to four pairs of WWWD's. Interviews with local people and rangers also revealed other sightings and good potential

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habitats. We are now funding three WWWD projects in Cambodia. Project 1 is to build dry season ponds in protected areas to allow better security and survival of ducks. Project 2 involves protecting a portion of a lake commonly used by WWWD's. This project involves cooperative efforts with a local village which utilizes the lake for fishing. In addition to reducing usage of a portion of the lake, a viewing blind is also being constructed to encourage ecotourism and its resultant financial support of the local village that is cooperating on the project. Project 3 involves moving a bridge and relocating a road in the protected forest to move traffic away from a known WWWD feeding pond. Longer term projects will include nest protection programs and regional education plans.

We are hopeful that a stable enduring population of WWWD's can be secured both in the captive population as well as in isolated areas in the wild

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**ASTROVIRAL DIARRHEA IN A GROUP OF CAPTIVE CHEETAH (*Acinonyx jubatus*):  
DESCRIPTION OF A NOVEL VIRAL PATHOGEN**

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**Abstract**

Five young adult and two adult cheetahs presented with lethargy, anorexia, watery diarrhea, and regurgitation over an 11-day period. Fecal samples were submitted for electron microscopy and culture. Electron microscopy results revealed all animals to be positive for an astrovirus, and no significant bacterial pathogens were identified on fecal cultures. All animals were monitored and treated with bismuth subsalicylate tablets (524 mg p.o. b.i.d. for 5 days), and recovered without additional intervention.

The astrovirus was confirmed and sequenced using consensus astroviral PCR. Phylogenetic analysis was performed on both the RNA dependent RNA polymerase (RdRp) and the capsid protein.

Astroviruses are small RNA viruses that are difficult to distinguish by electron microscopy from picornaviruses and caliciviruses. In mammals, they typically cause diarrhea. Astroviral diarrhea differs from other viral diarrheas in that the capsid interacts with apical enterocyte membranes, causing a secretory diarrhea without leaving much of a histologic lesion.<sup>1,2</sup> This is the first report we are aware of documenting an astrovirus outbreak in cheetah.

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## FORELIMB AMPUTATION IN AN OCELOT (*Leopardus pardalis*)

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### Abstract

A 3-yr-old female ocelot (*Leopardus pardalis*), weighing 6.2 kg, was assessed for a severe avulsion of the right forelimb at the level of the proximal third of the radius. The trauma was inflicted by a jaguar (*Panthera onca*) through the enclosure's separation fences during the night.

In the morning, the animal was very calm, most likely in pain and shock and it was easily caught in a net and immobilized with tiletamine-zolazepam 5.3 mg/kg i.m. (Zoletil 50<sup>®</sup>, Virbac Portugal, Almeirim, Portugal) via hand-syringe. Shortly after, 0.35 mg of morphine s.c. (Morfina 1% Braun<sup>®</sup>, B.Braun Medical, Queluz, Portugal) was administered and the animal was maintained on isoflurane 1% (Isoflo<sup>®</sup>, Veterinaria Esteve, Esteve Farma, Carnaxide, Portugal) during the whole procedure. After 1 hr 30 min fentanyl 0.02 mg i.v. (Fentanil B.Braun<sup>®</sup> 0.1 mg, B.Braun Medical, Queluz, Portugal) was administered to improve anesthesia.

It was decided to amputate the forelimb by removing the scapula (forelimb amputation with scapulectomy). In order to preserve the skin, the incision line started over the scapular spine in distal direction up to the edge of the remaining skin, and the subcutaneous tissue was undermined. After the amputation the remaining skin was removed. The thoracodorsal and axillary artery and vein were separately ligated and divided using a 4-0 multifilament non-absorbable suture (silk; Silkan, B.Braun) and the muscle and subcutaneous tissue using a 3-0 monofilament absorbable suture (polidioxanone; PDS, Ethicon) with a cruciate pattern for the former and simple interrupted for the last. The skin was closed with a horizontal mattress using 2-0 multifilament non-absorbable suture (silk; Silkan, B.Braun). The ocelot was medicated with morphine 0.17 mg s.c. (Morfina 1% Braun<sup>®</sup>, B.Braun Medical, Queluz, Portugal) just before the end of the surgery, cephalosporin 90 mg s.c. (Ceporex Vet 18%<sup>®</sup>, Schering-Plough Animal Health, Schering-Plough Veterinária, Cacém, Portugal) and meloxicam 1.5 mg p.o. (Metacam<sup>®</sup>, Boehringer Ingelheim, Labiana Life Sciences, Spain). Post-surgically the animal was kept isolated and the suture area covered with a modified Robert Jones bandage. The suture was taken off on the 10<sup>th</sup> day after surgery and the recovery was uneventful as was the re-integration with the male in the enclosure. The animal is perfectly healthy and adapted to its new condition.

Scapular removal is often preferred because more distal procedures produce less aesthetically pleasing results once the potential for unsightly muscular atrophy around the scapular spine and acromial prominence is eliminated.<sup>1,2</sup> Removal of the scapula may render the chest wall more susceptible to blunt trauma.<sup>3</sup> Due to the well developed muscles in this species the shoulder disarticulation is more difficult and the prominent scapula spine is prone to being exposed due to

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post-surgically atrophy of the remaining muscles. In the non-domestic felines scapulectomy is more adequate and can be a reasonable surgery option.

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## A NOVEL ADENOVIRUS IN A COLLECTION OF WILD-CAUGHT DUSKY SMOOTH-HOUNDS (*Mustelus canis*)

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### Abstract

Seven juveniles from a group of wild-caught dusky smooth-hounds developed inappetance and multifocal irregular skin lesions while in quarantine. All of these individuals died over a 3-mo time period (December 2006 – March 2007) despite medical and supportive therapy. All sharks had evidence of ulcerative dermatitis with intralesional ciliated protozoans and septicemia on histopathology. One of these juveniles had hypertrophy and hyperplasia of the oral mucosal epithelium associated with the formation of large intranuclear basophilic inclusions. A second shark had diffuse marked subacute branchitis with intraepithelial intranuclear inclusions. Electron microscopy performed on gill extracted from paraffin block revealed that the intranuclear inclusions contained hexagonal viral particles 60-65 nm in diameter consistent with an adenovirus.

Postmortem tissues from five adult sharks that expired between May and August 2007 were submitted for viral culture and isolation. A replicating agent isolated from the tissues induced cytopathic effect on primary shark embryo cells and was transferable in cell culture. No viral particles were observed in cell culture supernatant samples submitted for electron microscopy. A sample of the cell culture monolayer was submitted for electron microscopy. In addition, a sample of the cell extract and supernatant was submitted for PCR using an adenovirus primer set. Results of these ancillary tests are still pending.

Adenovirus infections are rare in fish. Documented cases have been reported in Atlantic cod (*Gadus morhua*),<sup>3</sup> dab (*Limanda limanda*),<sup>1</sup> and white sturgeon (*Acipenser transmontanus*).<sup>2,4</sup> To the authors' knowledge, this is the first confirmed report of an adenovirus infection in an elasmobranch.

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**WELFARE AND CONSERVATION IMPLICATIONS OF PATHOLOGIC FINDINGS IN A BREEDING COLONY OF WATER VOLES (*Arvicola amphibius*)**

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**Abstract**

The water vole (*Arvicola amphibius*) is Britain's rarest native mammal and, in the wild, has an average life expectancy of <1 yr, with few individuals surviving their second winter. During 2006 and 2007, over 300 water voles were housed and managed at Chester Zoo as part of breed and release/relocation programs. This poster summarizes the findings of 51 post-mortem examinations (approximately 50% of all reported deaths) that were performed during this period.

The most frequent post-mortem diagnoses were dental disease, respiratory disease and neoplasia. These accounted for >40% of the post-mortems, with a mean age of almost 2 yr. Chronic, degenerative/progressive diseases are of welfare concern as the animals have often undergone a period of disease and deterioration before they are diagnosed. Successful, curative treatment of these diseases can be difficult, if not impossible. Thus to improve the welfare of captive populations of water voles we need to consider if there are improvements in diet and/or husbandry that may prevent the onset of some of these diseases. If not, then euthanasia at the end of their "natural" lifespan should be considered as a management option for captive populations of water voles.

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***Mycobacterium genavense* IN A BLACK-FOOTED PENGUIN (*Spheniscus demersus*)**

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**Abstract**

A 19-yr-old female black-footed penguin (*Spheniscus demersus*) presented for labored breathing and anorexia. Radiographs revealed two soft-tissue density lesions in the left lung fields and fluid in the right. Aspergillosis was suspected. It was given 100 ml Normosol<sup>®</sup>-R (Hospira, Lake Forest, Illinois, 60045) subcutaneously and nebulized with 1 mg/ml amphotericin B (X-Gen, Northport, New York, 11768) for 15 min, and placed on oxygen. The penguin died during the night. Necropsy revealed multiple granulomas in the left lung field and the right hemicoelomic area was filled with milky fluid and a 4 cm mass. Large numbers of acid fast positive, rod-shaped bacteria were noted in histopathologic preparations of the lung mass, liver, kidney, and spleen indicating mycobacteriosis as the primary disease. *Mycobacterium genavense* DNA was detected in the fixed paraffin-embedded tissues by polymerase chain reaction (PCR) using primers specific for *M. genavense*.<sup>7,8</sup> *Mycobacterium avium* complex was not detected by PCR techniques.<sup>2</sup> *Mycobacterium genavense* is one of the common causes of mycobacteriosis in avians.<sup>1,3-6</sup> This is the first report of *M. genavense* in a black-footed penguin.

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**INDIVIDUAL IDENTIFICATION OF BOREAL TOADS (*Bufo boreas boreas*) BY BELLY PIGMENTATION PATTERNS AND THE EFFECT OF DIFFERENT UV SPECTRUMS ON GROWTH RATE**

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**Abstract**

Individual identification of animals is vital in captivity as well in the wild. Individuals must be identified for breeding programs, research, monitoring, and surveying. Being able to use unique characteristics of an animal for identification can be a reliable and permanent method of identifying the animal. Microchips are not a reliable, long-term method of identifying boreal toads so another method is necessary for breeding programs, release and surveys. Boreal toads have an intricate pigmentation pattern on their abdomen that could be used as a type of fingerprint.

Although the Native Aquatic Species Restoration Facility uses photographs to identify adult toads successfully, it was unknown if these patterns would remain the same from the time toads metamorphosed to adulthood. To determine this, forty toadlets from the same cohort were placed into two identical tanks except that one tank utilized a Bulbs Plus<sup>®</sup> (Colorado Springs, CO, 80909, USA) ultraviolet bulb and the second utilized a ZooMed<sup>®</sup> (San Luis Obispo, CA, 93401, USA) ultraviolet bulb. Photographs were taken of their abdomens periodically for comparison and length and weight was recorded for each toad to determine growth rates.

The growth rates between the tanks were nearly identical in average weight and length of the toads in each tank. The effect of the different ultraviolet spectrums cannot be determined. There was, however, 100% identification capability. Boreal toads belly pigmentation patterns, especially distinct marks at the throat, are very unique and provide a reliable means of individual identification of toads.

**ACKNOWLEDGMENTS**

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## LONG-TERM CATHETERS FOR PHOCIDS UNDERGOING REHABILITATION

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### **Abstract**

While the epidural vertebral vein of phocids is readily accessible, repeated needle insertions to provide i.v. medications to or to obtain blood samples from debilitated animals can be locally damaging. These concerns can be mitigated by using long-term catheters but the depth of this vein and the surrounding anatomy make it challenging to set and maintain standard, peripheral over-the-needle catheters. A technique developed for obtaining serial research blood samples from seals can be used to safely set a 15 cm catheter in rehabilitating phocids. All supplies can be assembled separately or are available in kits (Long Term Catheter, Guidewire Style, Mila International, Inc., Florence, KY, 41042, USA). Unless the animal is moribund, sedation in combination with physical restraint may be required to keep the animal sufficiently still during the procedure. The area over lumbar vertebrae is shaved, prepped, and injected with a local anesthetic. A 16 ga over-the-needle catheter of appropriate length for the patient is used to initially gain access to the vein. Following removal of the needle, a 'J' tip guidewire is slowly fed into the vein and the initial catheter is replaced with a 16 ga × 15 cm indwelling catheter. After removal of the wire, an extension set or cap can be attached to the catheter hub which should be sutured in place. The site can be further protected with a neoprene patch glued to surrounding fur. This set up has been maintained successfully for several days in rehabilitating phocids at the Alaska SeaLife Center.

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**COMPARISON OF THE FECAL PATHOGENS OF WILD CAUGHT, CAPTIVE, AND STRANDED NORTHERN SEA OTTERS (*Enhydra lutris kenyoni*)**

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**Abstract**

Since around 1985, southwest Alaskan northern sea otters have declined catastrophically, exceeding 90% in some areas. In 2002, a sea otter screening program showed a leading cause of death in beach cast otters from Homer, south-central Alaska, was infectious disease with a large percentage of otters shedding *Streptococcus bovis / equinus* complex spp. (SBE). Having a large proportion of animals die of a single infectious disease condition is unusual. To better understand this disease, samples from presumed healthy wild caught and captive northern sea otters were screened for fecal pathogens. Of 43 wild caught animals, one was culture positive for SBE, two for *Campylobacter*, six for *Clostridium*, four for *Vibrio*, and one for *Pleisomonas*. None of 16 captive animals were culture positive for SBE, two were positive for *Campylobacter*, one for *Clostridium*, three for *Vibrio*, and none for *Pleisomonas*. No wild caught or captive animal grew *Salmonella* or pathogenic *E. coli*. Fecal pathogens were cultured from 11 (25.6%) wild animals and 5 (33.3%) captive animals; samples from 3 wild animals and 1 captive animal grew two pathogens. All otters appeared healthy on exam and had no signs of illness despite presence of bacteria which causes gastrointestinal illness in humans. The high percentage of SBE in stranded animals compared to its virtual absence in apparently healthy animals underscores the unusual nature of the mortality event. Additionally, the frequent identification of organisms that can cause illness in humans, emphasizes the importance of proper hygiene practices for personnel working with these animals.

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**HORMONAL LEVELS OF SEX STEROID HORMONES (ESTRADIOL AND PROGESTERONE) RELATED TO REPRODUCTIVE BEHAVIOR IN FEMALE OSTRICHES (*Struthio camelus*) RAISED IN BRAZIL**

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**Abstract**

There is a lack of basic reproductive endocrine information for ostriches, though they are raised commercially in many countries, The aim of this work was to study the serum hormone levels of progesterone (P4) and estradiol (E2), in 20 2-yr-old female ostriches during different phases of the reproductive behavior. The behavioral reproductive phases evaluated were determined by the frequency of reproductive displays such as beating wings, swinging head and sitting position. Weekly blood samples were collected from all animals and the serum was stored in freezer at -30° C. The hormones were assayed with commercial kits (Progesterone™ DPC Coat-a-Count, Los Angeles, CA 90045-6900, USA and Estradiol™ DSL, Webster, TX, 77598-4217, USA).

The results showed differences ( $p < 0.0001$ ) between the mean values of both hormones of NSB group (P4:  $0.21 \pm 0.18$  ng/ml and E2:  $91.32 \pm 39.41$  pg/ml), when compared with the mean of all other groups with some degree of sexual behavior (P4:  $0.42 \pm 0.04$  ng/ml and E2:  $161.61 \pm 95.00$  pg/ml).

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## ULCERATIVE AND GRANULOMATOUS STOMATITIS IN A FEMALE PUMA (*Puma concolor*)

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### Abstract

A 12-yr-old, 27-kg female puma (*Puma concolor*) was reported with a depressed appetite, which gradually worsened over a 2-wk period to total anorexia. Upon immobilization and medical examination a protruding, cauliflower-like mass was discovered at the base of the tongue as well as an ulcerative lesion over one third of the hard palate. Biopsies were taken from the tongue and the hard palate. Histopathology confirmed the presence of ulcerative stomatitis of the hard palate and granulomatous lesions on the base of the tongue. Serologic testing showed a titre of 1:640 for feline herpes virus (FHV) by indirect fluorescent antibody test (IFA) and 1:80 for feline calicivirus (FCV) (IFA). Chronic stomatitis has been associated with FHV and FCV infections but the development of lesions is thought to be multifactorial.<sup>1</sup> Treatment consisted of the long-acting corticosteroid Dexafort<sup>®</sup>, 1.5 ml (dexamethasone sodium phosphate (1 mg/ml) and dexamethasone phenylpropionat (2 mg/ml), Intervet, Kempton Park, 1619, South Africa) and ranitidine HCL 100 mg (Zantac<sup>®</sup>, Aspen Pharmacare, Sandton, 2052, South Africa). The animal received three treatments of Dexafort and Zantac 10 days apart. Two days after the first treatment, the puma started eating a diet of ground beef and the following day she resumed her normal diet of beef on the bone. A follow-up examination 3 mo later showed weight gain and lesions in the mouth and on the tongue had not progressed. Serology revealed an increased titre of 1:640 for FCV (IFA) and no change in the FHV titre.

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## BIO-MIMICRY STUDIES OF AQUATIC SPECIES AND THE DEVELOPMENT OF SOLAR HYDROGEN ELECTRIC AUTONOMOUS UNDER WATER VEHICLES AND AUTONOMOUS AIRBORNE VEHICLES (sHe<sup>-</sup>UAVs&sHe<sup>-</sup>AAVs)

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### Abstract

We have developed an un-manned solar hydrogen electric (UAV or drone) air sampling air ship and a drone solar hydrogen electric water sampling boat. The air craft was designed from data derived from the fluid dynamics of the manta ray (*Manta birostris*) and bio-mimicry studies of cartilaginous and teleostean fishes. The aircraft has a wing span of 6m and a head to tail length of 4 m. It utilizes Mylar balloons with a total volume of 6.2 m<sup>3</sup> and has a lift capacity of 30 kg (Dragonfly Innovations Inc., 2108 St. George Ave., Saskatoon, SK S7M0K7, Canada). The body mass of the craft is 30 kg. The ventral surface of the craft is covered with bubble wrap and the dorsal surface with nylon (Goodwind' kites, 222 Anderson Rd, Unit B, Mount Vernon, WA 98273 USA). The internal framework or skeleton consist of carbon fiber tubes with an outer diameter of  $6.25 \times 10^{-2}$  m diameter (Goodwind' kites). A 0.3 m  $\times$  1.858 m strip of ultra thin solar panel with a capacity of 1.2 amperes and 15.4 V is one source of energy. Other sources of energy are a wind hydrogen electric reactor, a solar hydrogen electric reactor and a rechargeable battery. The minimal kinetic energy out put of the aircraft is  $6.25 \times 10^{-3}$  J.

### Introduction

Autonomous Under water Vehicles (AUVs) powered by conventional batteries and solar energy have been developed for a variety of oceanographic uses.<sup>1,18</sup> Other drone vehicles (AAVs), drone aircrafts powered by batteries and solar energy, have also been developed for military and civilian applications.<sup>3,14</sup> Clark has conducted extensive fluid dynamic studies on models patterned after the manta ray (*Manta birostris*), with a major objective of applying the data derived from the studies to develop a plane with aerodynamically improved wings.<sup>4</sup> Brower used computer tomography scans of manta rays to develop and test actuators capable of simulating the motion of the pectoral fin of the manta.<sup>2</sup> In the area of application, bordered by AUVs and AAVs, is a sparsely explored locus, in which solar hydrogen electrical energy and wind hydrogen electrical energy can be used to power drone vehicles used in zoo and wildlife medicine, including aquatic veterinary medicine and ecological medicine. We have developed and continue to develop solar hydrogen electric (sHe<sup>-</sup>) drones. One sHe<sup>-</sup> drone is a 1 m water

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sampling boat and the other is an air ship with a wing span of 6m and head to tail length of 4 m (Figure 1).<sup>3</sup> Regular balloons and aircrafts are not effective in monitoring air samples in a city or some war zones, such as the current Gulf region states. The use of a scuba diver or technician in an inflatable for monitoring ponds, lakes and gigantic aquarium tanks is not economical. The drone water sampling boat and the drone airship were designed to fill the utility gap due to limitations of regular aircrafts and boats.

## Structure

The drone boat has a flat bottom hull made of aluminum and a roof covered by six 3V solar panels with a total current out put of 0.6 amperes. Bolted down in the center of the hull is a robotic winch, which is attached by nylon cable and electrical cable to a probe at the bottom of the boat.<sup>2</sup> A propeller with a shaft and rudder are attached to the stern of the boat. Three bottles, containing hydrogen, oxygen and water, are connected by a series of tubes to a proton exchange membrane (PEM). Electrical leads connect the PEM to the solar panels and the motor of the winch and motor of the propeller. The bottles of water and gas, along with the PEM and solar panels are components of the solar hydrogen reactor.<sup>9</sup> Two, 7.2 V, 1.9 ampere Nickle-Cadmium batteries are used a back-up energy supply (Tower Hobbies, Champaign, IL,61826-9076 USA).

The drone air ship is a much more complex machine (made at Brooklyn Technical High School, patent pending). Its shape mimics that of a manta ray (Figure 1).<sup>12,13</sup> It has two 15.4V, 1.2 ampere solar panels on the dorsal surface (www.powerfilm solar.com). At the center is a 38 cm, triple blade propeller. On the ventral and distal surfaces of each wing are 30 cm diameter propellers with motors. An air sampling unit consisting of three high efficiency tangential inlet axial outlet discharge (HETAD) micro-cyclone precipitators, extends from the center of the craft to the anterior (made at Brooklyn Technical High School, patent pending) (Figure 2). The anterior of the craft is funnel shaped and the inlet is closed during flight by a robotic window. Two one liter sample bags are attached to the gas outlets of the cyclone precipitators. Immediately posterior to the robotic window is a wind turbine, which is attached to an auxiliary hydrogen reactor (made at Brooklyn Technical High School, patent pending). A major solar hydrogen electrical reactor is located in the mid-ventral area of the craft. Data from image analysis studies and fluid dynamics investigation, including buoyancy and pressure studies were used in the construction of the craft.<sup>1,6-8,16</sup>

## Function

By means of a remote control panel, the boat can be auto-piloted. The submersible probe can be lowered to differing depths and water samples collected by gravity into four test tubes in the probe.<sup>9</sup> The helium balloons enable to aircraft to have a vertical ascent (Figure 1). The propellers at the wings are used for vertical and horizontal motion. They simulate the propulsive energy of the wings of a manta ray. Vertical descent is accomplished by ballast and the propeller in the center of the craft. Remotely controlled valves allow air flow into the sampling bags and the cyclone precipitators.<sup>16,17</sup>

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## Discussion

Extensive fluid dynamic tests will be conducted on both crafts to improve kinematics and durability. The aircraft is currently undergoing a second phase of re-engineering, in terms of design and development. In addition, submersible probes with larger sampling capacity will be developed for the drone boat. One question we plan to answer by experimentation is: How can developmental studies of sting rays yield insights into batoid wings for aircrafts?<sup>5,10,12,13,17,19</sup>

## Conclusion

The drone air craft utilizes hydrogen and oxygen, derived from the photo-electrolysis of water as fuel and has capabilities for collecting three ranges of particle sizes from the air and gaseous samples from the atmosphere. The craft uses a series of vortices for its locomotion, energy conversion and sample collection.<sup>6,11</sup> The drone water sampling boat is capable of collecting four water samples and four different depths or four different regions in a body of water.<sup>9</sup>

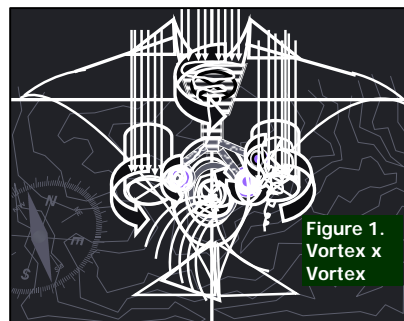
## ACKNOWLEDGMENTS

We are most grateful to the Brooklyn Technical High School Alumni Research Foundation, who funded our project. Technical support was provided by Dr. William Stopskof and Dr. Andrea Goodnight. Without the wizardry of the BHTS Robotic Team, the crafts may not have had full lives. Special thanks to Boris Yuditskiy, Daniel Simkin, David Chen and Galisa Allison. Last but not least we thank Mr. Gordon Williams, BTHS Robotics instructor.

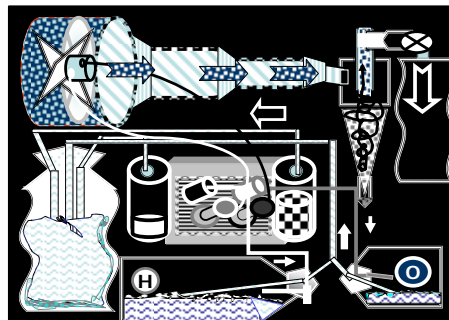
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**Figure 1.** The drone aircraft. The craft uses a series of vortices for its motion, air sampling and energy conversion. The air sampling unit mimics the architecture of an avian or mammalian respiratory system.



**Figure 2.** Air Sampling Unit and Auxiliary Solar Hydrogen Reactor. The wind energy rotates the turbine, which converts mechanical energy to electrical energy. The electrical energy splits the water in the reservoir into hydrogen and oxygen in the Proton Exchange Membrane. The gases are conducted by tubes to the respective gas bags, where they are collected under negative pressure. The air increases in velocity as it moves through the ducts of decreasing diameter. The air then enters the cyclone precipitator where particulates are separated out and gases collected in the gas sample bags. Once the bags are filled, a valve at the entrance of the bag closes it and the air leaves through the exit duct of the cyclone precipitator.

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## MINIMUM ALVEOLAR CONCENTRATION (MAC) OF SEVOFLURANE IN BALL PYTHONS (*Python regius*) and BURMESE PYTHONS (*Python molurus*)

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### Abstract

Despite the popularity of the inhalant anesthetics, only limited information on their performance in reptiles is available.<sup>2,3,7,-11</sup> The aim of this study was to determinate the minimum alveolar concentration (MAC) of sevoflurane in two common snake species: *Python regius* and *Python molurus*.

Sevoflurane is a potent inhalant anesthetic, with a mild odor (that causes minimal breath holding) that has a low solubility and ease of adjustment of anesthetic depth. Its low solubility results in rapid anesthetic induction and recovery. It has bronchodilator activity, doesn't irritate the airways, is a myorelaxant and cardiac output decreases with increased concentration of sevoflurane.<sup>6</sup> MAC is defined as the concentration of an anesthetic in the alveoli able to induce surgical anesthesia in 50% of the subjects. In this study, an electrical stimulus (4 mA, 12 V, 30 ms, 2 Hz) was delivered to subcutaneous electrodes by an alternating current (AC) electric stimulator (Vygon stimulator, Vygon Group, Padua, Italy).

Fourteen captive-bred snakes, seven *Python regius* (four males, three females) and seven *Python molurus* (four males, three females) with known clinical history were utilized for the study. A blood sample was collected from the ventral tail vein and/or from the heart<sup>4</sup> for every snake at the beginning, during, and at the end of the anesthetic period for blood gas analysis.

Anesthesia was induced with 8% sevoflurane in 100% O<sub>2</sub> delivered through a facemask. After induction, snakes were endotracheally intubated with uncuffed tubes. The endotracheal tube was then connected to a Bain nonrebreathing circuit and was mechanically ventilated with a ventilator at a rate of 6 breaths/min. The snake was kept in dorsal recumbency on an electric heating pad at 30°C. Temperature was monitored using a cloacal thermometer. In addition to blood gases, other measured parameters and methods utilized included: SpO<sub>2</sub> using an esophageal pulse oximeter probe (Multiparameter Patient Monitor UT4000f-pro, Goldway Industrial, Smithtown, NY, USA), heart rate using a Doppler apparatus (Ultrasonic Doppler Flow Detector, Model 811b, Parks Medical Electronics, Aloha, Oregon, USA), cardiac electrical conductivity, end-tidal CO<sub>2</sub>, and end-tidal anesthetic concentration. Heart rates ranged from 10-16 beats/min for *Python molurus* and 20-43 beats/min for *Python regius*.

Following intubation, inspired sevoflurane concentration was reduced to 2% and was allowed to equilibrate with end-tidal anesthetic concentration for 20 min. Then an electrical supermaximal

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stimulus was delivered to subcutaneous electrodes on the ventral aspect of the tail for 30 sec and the animal was observed for purposeful movement. The vaporizer was then decreased by 0.2% and equilibration and stimulation were repeated. This sequence was repeated until electrical stimulation caused observed purposeful movement. The MAC was calculated as the mean of the lowest end-tidal concentrations in which half of the subjects did not respond to the stimulus.

Data were analyzed using an ANOVA analysis and the Mann-Whitney U nonparametric test ( $p \leq 0.05$ : significance level;  $p \leq 0.01$ : high significance level). MAC values of sevoflurane were found to be between 0.9% and 1.3% with a mean and standard deviation of  $1.07 \% \pm 0.21\%$  for *Python molurus* and were found to be between 1.0% and 1.9% with a mean and standard deviation of  $1.31\% \pm 0.31\%$  for *Python regius*.

MAC of sevoflurane in our study is lower than values reported in lizards<sup>1</sup> and quite different to that reported in other snakes<sup>5</sup> where minimum infundibular concentration (MIC) was studied.

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**INVESTIGATING CRYOPRESERVATION TECHNIQUES FOR OKAPI (*Okapia johnstoni*) SPERM**

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**Abstract**

Development of assisted reproductive technologies in okapi (*Okapia johnstoni*) could contribute to increasing genetic diversity in the captive population. The aim of this study was to evaluate the use of two common cryoprotectant agents (CPA's), glycerol (Sigma Chemical Co., St Louis, MO 63103) and dimethylsulfoxide (Sigma Chemical Co., St Louis, MO 63103), at concentrations of 1%, 2% and 4%, to determine the optimal freezing conditions for okapi semen. Samples were assessed for motility and progressive status at the following stages: at initial collection, addition of extender, cooling to 4C, and post thaw. Prior to freezing, both cryoprotectants appeared to have similar motility and forward progressive status, ranging from 15-35%, average 26% with a forward status of 3.0/5. When comparing post freezing samples, the 4% glycerol dilution demonstrated the greatest post thaw motility of 18% and forward progressive status of 3.4/5, when compared to 3-14% motility and 1.6-2.5/5 progressive forward motility in other samples. To the author's knowledge this is the first report of cryopreservation of okapi semen.

**ACKNOWLEDGMENTS**

The authors would like to thank Dr. Scott Citino and the White Oak Conservation Center staff for their assistance in collecting these samples.

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## PHARMACOKINETICS OF ENROFLOXACIN IN AFRICAN ELEPHANTS (*Loxodonta africana*) AFTER A SINGLE RECTAL DOSE

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### Abstract

Captive African elephants (*Loxodonta Africana*) are susceptible to many types of gram negative bacterial infections such as *Escherichia coli*, *Mycoplasma* spp., *Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., and *Proteus* spp. Enrofloxacin (Baytril<sup>®</sup>, Bayer Health Care, Animal Health Division, P.O. Box 390, Shawnee Mission, KS 66201) is a potentially effective antibiotic for treatment of these bacterial infections in elephants. Very limited data exists on the pharmacokinetics of enrofloxacin in elephants<sup>2</sup> and most of the dosage regimes for gastrointestinal absorption are based on horse dosages since they share a similar gastrointestinal tract<sup>1</sup>.

Three African elephants from Wildlife Safari in Winston, Oregon, two females both 37-yr-old and one male 26-yr-old, were used to determine whether therapeutic levels of enrofloxacin could be achieved thru rectal administration of liquid injectable enrofloxacin (Baytril 100<sup>®</sup>, 100 mg/ml, Bayer Health Care, Animal Health Division, P.O. Box 390, Shawnee Mission, KS 66201) at a dosage of 2.5 mg/kg. A pretreatment baseline blood sample was collected. Following administration, blood samples were collected at 45 min, 1.5hr, 2.5hr, 5hr, 9hr, 23hr, 36hr to determine plasma enrofloxacin levels. Plasma enrofloxacin levels were measured at North Carolina State University, College of Veterinary Medicine using high performance liquid chromatography (HPLC) analysis. Plasma ciprofloxacin levels were measured concurrently. Results indicate plasma concentrations of enrofloxacin did not reach adequate bacteriocidal levels for any of the the following common bacterial isolates in captive elephants: *Mycoplasma* spp., *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., and *Proteus* spp. The study determined that a rectally administered dosage of 2.5 mg/kg of liquid injectable enrofloxacin was insufficient to obtain therapeutic levels in African elephants. The low plasma levels of enrofloxacin in all three elephants may be a result of poor absorption in the distal large intestine. A future study will determine if oral administration will provide a more efficient mode of drug delivery and absorption in African elephants. It is also possible that the current dosage of 2.5 mg/kg is too low to achieve adequate therapeutic levels.

### ACKNOWLEDGMENTS

I would like to thank the elephant and veterinary staff at Wildlife Safari for their participation in conducting this study. Thanks to Doctors: Modesto McClean, Jason Bennett, Andi Chariffe, Tessa Lohe, Benji Alacantar. Also thanks to Dinah Wilson, Carol Matthews, Anthony Karels, Mary Iida, Shawn Finnell, Patches Stroud, Katie Alayan.

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## **DISEASE SURVEILLANCE IN ZOOS: FINDINGS OF THE WEST NILE VIRUS SURVEILLANCE PROGRAM**

*Yvonne Nadler DVM MPH,\* Jan Mladonicky, BS, Dominic Travis, DVM, MS, and West Nile Virus Working Group*

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### **Abstract**

In June 2001, Lincoln Park Zoo in conjunction with the Centers for Disease Control and Prevention (CDC) hosted a meeting that brought together experts from USDA, United States Geological Survey (USGS), State and local public health, Association of Zoos and Aquariums (AZA) and American Association of Zoo Veterinarians to discuss surveillance for West Nile Virus in Zoological institutions. CDC recognized the potential role of zoos as public health sentinels, but until then lacked the ability to collect such data in a manner that ensured confidentiality for the Zoological community. With funding from the CDC, the West Nile Virus Surveillance System for Zoological Institutions was created. Under this pilot, 22,272 diagnostic samples were analyzed from 183 different institutions over 6 yr. Surveillance coverage included 47 states and one U.S. territory. The system identified 3,737 samples positive for West Nile virus.

Along with the valuable diagnostic information gained during the surveillance effort, other benefits of this system were realized. First, zoos can play an important role in surveillance for Public Health issues. This builds partnerships between animal health and Public health at the local level. Second, zoos can organize around an issue that affects not only their collections, but wildlife and the public as well. Third, valuable lessons were learned that should be considered with any future expansion of surveillance in zoos. Issues with taxonomic classification, case definition, animal identification and database design will be illustrated.

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## THE PREVALENCE OF *Leptospira interrogans* AND INTESTINAL PARASITES IN THE WILD RACCOONS (*Procyon lotor*) ON THE GROUNDS OF THE MINNESOTA ZOO

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### Abstract

The raccoon (*Procyon lotor*) is a known reservoir of zoonotic diseases throughout North America.<sup>2,5</sup> Since the raccoon is a potential host for many pathogens that can affect domestic and exotic species, its presence on the Minnesota Zoo grounds may present a disease risk to collection animals.<sup>1-4</sup> Enteric parasites and *Leptospira interrogans sensu lato* are the major pathogens of concern in this study. They have been diagnosed in collection animals and have the potential to be transmitted from raccoons to other animals or people. Information on the type and prevalence of these pathogens in indigenous raccoons is important in managing raccoon-human and raccoon-animal interactions. Raccoons were live trapped on the Minnesota Zoo grounds during the summer of 2007 and urine, blood and feces were collected for analysis. Blood was tested by microscopic agglutination testing for six serovars of *L. interrogans* and urine was tested by PCR for shedding of *L. interrogans*. Feces were evaluated by standard floatation and sedimentation methods, in addition to direct smears, to determine the prevalence and type of intestinal parasites.

Serum was analyzed from 20 raccoons and urine from 17. Only one raccoon had a positive serum *Leptospira* titer for the serovars Grippityphosa and Icterohemorrhagiae. This raccoon was PCR negative, as were all the others tested. The roundworm, *Baylisascaris procyonis*, was not as prevalent as expected, with only 2 out of 19 (11%) positive. The trematode, *Alaria* sp., was more prevalent, with 11 of 19 (58%) positive. This study will continue in the summer of 2008.

### ACKNOWLEDGMENTS

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***Mycoplasma canis* DETECTED IN MEXICAN FREE-TAIL BATS (*Tadarida brasiliensis*)**

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**Abstract**

*Mycoplasma* are pleomorphic bacteria lacking a cell wall and enclosed by a single limiting membrane. A wide variety of animal species from reptiles to birds to mammals can play host to *Mycoplasma* species. Many of the 'pneumoniae' *Mycoplasma* are nonpathogenic, living on mucosal surfaces, particularly in the respiratory and genital tracts. Although a small number of species are significant natural pathogens, most produce disease in conjunction with other insults or disease agents.<sup>5</sup>

A captive colony of Mexican free-tail bats (*Tadarida brasiliensis*) experienced a sudden increased mortality. Appetite loss and weakness were noted in a small number of cases. Histologic examination of the bats identified a mild nodular lymphoplasmacytic peribronchitis and rarely, a mild tracheitis. Paraffin-embedded tissue samples were submitted for DNA sequencing, identifying *Mycoplasma canis*.

*Mycoplasma canis* is considered a commensal or urogenital tract pathogen of dogs<sup>4</sup> although it has been found in humans<sup>1</sup> and in the upper respiratory tract of cattle.<sup>2</sup> The lesions within the respiratory tract and identification of the associated *M. canis*, suggests that these bats suffered a mycoplasmal infection. It is unknown if the infection alone was severe enough to have resulted in death of the animals. Mycoplasmosis has not been reported in bats previously.<sup>3</sup>

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## REHABILITATION OF A BOBCAT (*Felis rufus*) INCAPACITATED BY A TASER GUN

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### Abstract

Electromuscular incapacitating devices (EMDs) are electronic immobilization weapons that have been available for over 30 yr. The purpose of these non-lethal weapons is to incapacitate humans or animals through the use of high-voltage electricity. Research on the effects of EMDs concluded that while EMDs may increase heart rate, they have no effect on cardiac rhythm or morphology in normal subjects.<sup>3,6,11</sup> Other studies investigated the changes in blood factors following single or repeated taser exposure and reported transient increases in acidosis, lactate, hematocrit, and other factors.<sup>2,4,10</sup> Potential injuries may include thoracic spine compression fractures.<sup>9,12</sup> However, no studies were found to suggest that EMDs cause paralysis or paresis of limbs in humans or animals. This is a case report of a 9-mo-old bobcat that was tasered at the T10 spinal level resulting in temporary hind limb paralysis and residual paresis. Routine rehabilitation of muscle weakness includes exercise and electrical stimulation. Research demonstrated mixed results on the effectiveness of these methods.<sup>1,5,7,8,13</sup> The bobcat received daily treatments consisting of range of motion exercises and sensory stimulation. One year post incident, electro-acupuncture and rehabilitation techniques (spinal and soft tissue mobilization) were implemented. Environmental modifications were designed and changed periodically to encourage pelvic limb muscle strengthening and to facilitate muscle re-education. After 4 mo of treatment, the bobcat could ascend/descend stairs and initiate walking. Although the movement patterns were not consistent, the bobcat continues to demonstrate progressive improvement.

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**SPECIES SUSCEPTIBILITY TO BLUETONGUE IN EUROPEAN ZOOS DURING THE BLUETONGUE VIRUS SUBTYPE 8 (BTV8) EPIZOOTIC AUGUST 2006 - DECEMBER 2007**

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**Abstract**

Bluetongue is a serious viral disease of domestic livestock – principally sheep. However little is known about the susceptibility of non-domestic ruminants to the disease. Though all ruminants are thought to be susceptible to infection, clinical expression is highly variable. It can range from subclinical infection, to fever, coronitis and laminitis, ulceration of the mucous membranes of the oronasal cavity, oedema of the head and neck, cyanosis and death. Clinical expression is dependant on species, breed/race, subtype of virus, previous exposure, stressors, environmental conditions and behaviour of the Culicoid midge vector<sup>2</sup>

European zoos are in a unique position to contribute to knowledge on disease caused by Bluetongue Virus subtype 8 (BTV8) in non-domestic ruminants, as they house and monitor a wide range of ruminant species that can be assumed to have been naïve to infection prior to the BTV8 epizootic in north-western Europe that began in August 2006. A web based survey was produced and received endorsement by the EAZA veterinary committee and the Infectious Disease Working Group of EAZWV. The survey was circulated in December 2007 to all EAZA and WAZA collections in the region and to other zoos via EAZWV and the regional zoo vet associations.

One hundred forty six responses were received – representing over 50% of EAZA collections. Of these, 49 zoos were within 20 km of confirmed BTV8 cases and could be classified as at risk of infection. These 49 zoos held over 1000 susceptible individuals of 53 different species and 7 ruminant families indigenous to Europe, North and South American, Africa and Asia. Clinical disease was seen in 62 individuals (6% of the at risk population), in 13 zoos (27% of at risk collections).

Preliminary analysis suggests that the Bovidae are the most susceptible family of ruminants to clinical disease, with four species showing morbidity of greater than 20% and mortality of greater than 10%. The average case fatality rate for the affected Bovidae species was 69%. All the affected ruminant species in this study were indigenous to Europe, Asia or the Americas. Clinical signs in these species are consistent with those recorded for BTV8 infection domestic livestock.<sup>1</sup> It is noteworthy that none of the over 200 African ruminants of 20 species being held by zoos in at risk areas were reported to be clinically affected. Follow up of the affected animals

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is planned to confirm the data presented here and to establish the proportion of animals listed in which a definitive diagnosis of BTV8 infection was established.

In conclusion, BTV8 infection can cause significant mortality or morbidity in some ruminant species particularly of those members of the family Bovidae indigenous to Europe and Asia. Zoo managers should focus protective measures on these susceptible species as a priority. Protective measures might include housing, use of insecticide, midge control and vaccination. Species not showing clinical disease may well act as reservoirs and multiplier hosts for BTV and managers should also consider instituting protective measures for these species. Investigation of levels of viremia in different species would help determine which of them posed the greatest risk. Further data collection over the coming midge season is planned and should help improve the accuracy and validity of this study.

#### **ACKNOWLEDGMENTS**

European Association of Zoos and Aquariums (EAZA) and European Association Zoo and Wildlife Veterinarians (EAZWV) Infectious Disease Working group for their support of this survey and all the respondents for their assistance.

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This material was first presented at the EAZWV meeting in Leipzig, May 2007. Its presentation at AAZV also is fully endorsed by EAZWV.

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## EUROPEAN EXPERIENCES OF CONTRACEPTION IN ZOO SPECIES: DEVELOPMENT OF A EUROPEAN CONTRACEPTION DATABASE

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### Abstract

Wildlife contraception is an important tool in our genetic and welfare management of zoo species. To maintain genetic diversity, appropriate population demographics must be retained in each sex and age class. This is achieved through highly cooperative and structured international breeding programs such as the European Endangered Species Program (EEP) and Association of Zoos and Aquariums (AZA) Species Survival Plan (SSP) Program. Contraception plays an important role in these programs by preventing over-representation of certain individuals, reducing inbreeding and modifying generation length. Contraception is also employed to modify inappropriate sexual behaviours, reduce the need for culling of unwanted young, decrease the repetitive physiologic demands due to pregnancy on selected individuals and ensure populations do not exceed available space within zoo collections. Although many methods of contraception are available, providing safe and effective contraception for species with highly variable reproductive physiologies is a major challenge.

In North America this challenge has been addressed by setting up a centralized source of contraceptive information and constructing a database of the efficacy of the different contraception methods. As a result, the AZA Wildlife Contraception Center (WCC) now has a database of more than 21,000 entries and has been able to produce guidelines for a variety of species. These guidelines are freely available to all on the internet ([www.stlzoo.org/contraception](http://www.stlzoo.org/contraception)); however, as not all North American contraception methods are available for use in the European Union, these guidelines have their limitations. European zoo veterinarians and collection managers also have considerable experience with a variety of contraceptive methods – some of which are not currently available in North America. By compiling and pooling European experiences with contraception in zoo species with that of the AZA WCC database we can both gain from each others' experiences.

To this end we propose to set up a European contraception group which will work in association with and compliment the AZA WCC. The European Group for Zoo Animal Contraception

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(EGZAC) would endeavor to gather data on use of contraceptive methods in European collections, increase awareness amongst European veterinarians, collection managers, and population managers of the most current recommendations on contraceptive use and fine tune these recommendations to best meet the specific needs of European collections.

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**A REVIEW OF FORMOSAN PANGOLIN (*Manis pentadactyla pentadactyla*)  
NECROPSIES AT THE TAIPEI ZOO**

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**Abstract**

There are eight species of pangolins identified in the world.<sup>1</sup> All pangolin species are toothless insectivores that prefer ants and termites as food sources. The Formosan pangolin (*Manis pentadactyla pentadactyla*) belongs to the subspecies of Chinese pangolin (*Manis pentadactyla*), which is distributed solely on the Taiwan Island. Raising and captive management of pangolins has been problematic and there is little information available on ecology, physiology and medical care of this species. Here we report on a 10-yr (1995-2004) retrospective review of 68 pangolin necropsy records in the Taipei zoo. The male-to-female ratio was about 2:1(44:24). However, the age of the necropsied animals could not be determined. Most animals had gross lesions involving more than 2 organs. Pneumonia (45/68) and gastric ulcers (42/68) were the most commonly found lesions. Histopathologic examination revealed bronchointerstitial pneumonia with marked syncytial cells and edema in some of the animals. These pulmonary lesions were determined to be positive for canine distemper virus using the non-biotin polymerized horseradish-peroxidase method.<sup>2</sup> This is the first detected contagious viral infection ever found in pangolins.

**ACKNOWLEDGMENTS**

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## PHYSIOTHERAPY TREATMENT AND OUTCOME OF A NEUROLOGIC DYSFUNCTION IN A KOMODO DRAGON (*Varanus komodoensis*)

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### Abstract

A 14-yr-old male Komodo dragon developed neurologic deficits after a brief incident of hyperthermia at Chester Zoo. Initial neurologic assessment revealed generalized poor muscle tone. The animal was unable to weightbear effectively on the right front limb and was also unable to hold its cervical spine in sufficient extension to facilitate normal walking. Poor abdominal tone was hindering locomotion and general movement, exacerbated by poor exercise tolerance and attention span. There was increased rigidity, and thus limited movement through the cervical and thoracic spine.

A neurologic assessment chart was developed to monitor progress objectively. This chart was clinical sign centric, in that we started with several behaviors we expect the animal to be able to accomplish, and highlight methods to test these.

The requirements for the Komodo dragon, as minimum outcomes from a series of physiotherapy sessions, were:

- To be able to walk unaided for more than five steps (and thus thermoregulate)
- To show unaided prehension of food
- To show interest in the females (and thus fulfill one of his main conservation purposes as a breeding animal)

The physiotherapy sessions included leg, neck and tail coordination and weightbearing exercises (developed by Equine and Canine Solutions (physiotherapists), Hartford, Northwich, Cheshire, CW8 1LP UK). A daily swimming routine was incorporated to help with coordination and to achieve extra focal stimuli. A large indoor pool in the enclosure was used for this hydrotherapy.

After 1 mo, improvements seen when compared to the initial presentation included:

- Increased exercise tolerance (from 10 to 30 min)
- Initiated walking, especially away from unpleasant stimuli
- Each limb would move independently during locomotion
- Range of movement of cervical and thoracic spine improved with external mobilization using a specifically designed neck brace.
- Abdominal muscle tone improved
- Attention span improved – possibly due to changing stimuli and/or surroundings

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- Strength improving, especially against resistance.

However, after 5 mo of physiotherapy treatment muscle wastage became obvious over several weeks. There was consensus between the reptile keepers, curators, vet team and physiotherapists that physiotherapy had reached a plateau of improvement with locomotion ability beginning to regress, associated with the obvious muscle wastage and weight loss.

The physiotherapists felt there was little chance of significant improvement and that they had exhausted therapy options. In view of this and that despite intensive therapy, he hasn't fulfilled the original criteria a decision was reached to euthanize him on welfare grounds (i.v. saturated potassium chloride (KCl) solution under general anesthetic). This was used rather than pentobarbitone to protect neurologic ultrastructure.

On gross post mortem there was marked muscle wastage over tail and limbs, despite having excellent coelomic fat stores. This could indicate a neuromuscular deficit or disuse atrophy. There were minimal changes elsewhere. Cranial MRI did not highlight any changes however histopathology indicated vacuolation and spongiosis of the tectum. The tectum refers to the dorsal portion of the midbrain. The midbrain processes information from the senses and controls how the animal orients itself towards them, such as turning towards a sound. Thus, the tectum is vital in allowing the animal to recognize patterns in its environment, via direct auditory and visual input. Damage to this area of the brain can at least partially explain the Komodo dragon's observed clinical signs. In mammals at least, the anterior part of the midbrain also incorporates the cerebral peduncle, which is a large bundle of axons travelling from the cerebral cortex through the brain stem and these fibres are important for voluntary motor function.<sup>2</sup> The peduncle communicates with the tectum to allow special orientation towards or away from a stimulus. The peduncle appears both part of the pyramidal and extrapyramidal tracts, although opinion is divided on this. Birds and reptiles predominantly utilise the extrapyramidal system for motor control.

This case highlights an environmental issue that, while rectified speedily, still produced severe neurologic damage. A paucity of information is available on clinical neurology in reptiles.<sup>1,3,4</sup> This type of neurologic condition had not been recorded in Komodo dragons before.<sup>2</sup>

As far as the authors are aware, the methods of physiotherapy employed in this case have never been employed in a large lizard before. Although we saw good initial improvement, especially when increasing the positive stimulation from the environment, the damage to the tectum discovered on histopathology precluded a favorable outcome. Unlike mammalian brains, lizard brains appear to retain significant ability for neurogenesis, including regeneration of injured neurons, particularly in the forebrain,<sup>5</sup> but the lesions were too severe in this case.

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## MANAGEMENT OF ATOXOPLASMOSIS IN A MIXED SPECIES AVIARY

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### Abstract

*Atoxoplasma* spp. are protozoa of the Eimeriidae with a direct life cycle,<sup>2,6</sup> affecting a number of passerine species.<sup>1,3</sup> They appear to be host specific.<sup>6</sup> *Atoxoplasma* constitutes an extremely difficult and controversial parasitic pathogen to manage in captive passerine birds.<sup>3</sup> Unlike other Eimeriidae species, the asexual life cycle of *Atoxoplasma* takes place in internal organs (often the liver) and not in the intestinal mucosa,<sup>6</sup> making ante mortem diagnosis challenging. Polymerase chain reaction (PCR) studies have shown *Atoxoplasma* to be common subclinically.<sup>1</sup> This disease is known to be circulating in UK passerines, but is not thought to be associated with clinical disease.<sup>4</sup> A PCR test for *Atoxoplasma* developed in the USA<sup>3</sup> is unavailable in the UK, but we are investigating the possibility of developing one in the UK.

This case is the first recorded and confirmed report of *Atoxoplasma* in the pied starling (*Spreo bicolor*).

Archived pathology records at Chester Zoo (1999 to 2007) were reviewed. Atoxoplasmosis was first confirmed in a Bali mynah (*Leucopsar rothschildi*) in quarantine in 2000. Atoxoplasmosis was not positively identified within the collection until 2006 in juvenile pied starlings in a naturalistic outdoor aviary, (Arcade), and in 2007 in a recently fledged emerald starling (*Lamprolornis iris*) in an indoor tropical free flight aviary (Table 1). Hepatomegaly was the most consistent feature on gross post mortem evaluation. Histopathology revealed most sinusoids were expanded diffusely by moderate numbers of mixed leucocytes with many hepatocytes and macrophages containing abundant hemosiderin. Merozoite-like protozoans were found within macrophage cytoplasm and less overtly free in the liver, spleen and small intestinal mucosa.

All these animals were parent raised in the respective aviaries. There was no movement of birds between these aviaries during this time, as part of our management plan. On review, there had been 13 pied starling deaths with possibly associated with atoxoplasmosis (Table 1). There have been no 'sick birds' in the collection with atoxoplasmosis or coccidiosis in general, making confirmed ante mortem diagnosis challenging.

Fecal examinations for parasites in the tropical free flight aviary have been negative for coccidian oocysts from 2001 until November 2007 when oocysts were found on a general sample. This remains the only positive sample from this aviary despite repeated sampling from

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June 2007 to March 2008, as we enter a new breeding season. There have been no further confirmed deaths due to atoxoplasmosis in any species since mid 2007. However, if there is a low circulating infection in the tropical free flight aviary, there is evidence that exposure to low levels of infection during chick development may help immune systems cope when challenged with a higher burden.<sup>3</sup>

## Management

### Husbandry

Hand rearing chicks to prevent exposure to *Atoxoplasma*. There is no evidence of vertical spread of atoxoplasmosis, but thorough cleaning of egg, (done at Chester Zoo using F10 disinfectant (Health and Hygiene (Pty) Ltd, Sunninghill, South Africa) and UV sterilization) and removal of fecal material prior to incubation is essential. Note however that birds not previously exposed to the organism may be more susceptible to high mortality when they are exposed.<sup>4</sup>

### Targeted Therapy

Treatment to reduce the coccidial burden to increase the chance of juvenile birds surviving past fledging includes:

- Feces are monitored for coccidial burdens monthly.
- 1.25 mg Chlozuril (Appertex™ Janssen Pharmaceutica, Beerse, Belgium) per bird based on Samour 2000.<sup>5</sup> The first year of using this treatment in pied starlings (2006) was the first in 5 yr that several chicks fledged successfully and 2007 was the first year all fledglings survived.

### Quarantine

Newly acquired passerines are quarantined and screened with multiple fecal flotations (minimal of three every 7 days) for coccidian oocysts. If any passerines show clinical signs in quarantine, or fecals show *Isospora* like oocysts, blood collection and examination of buffy coat smears for *Atoxoplasma* will be undertaken. This test is specific but not very sensitive, unless the bird is showing clinical signs, thus the investigations to produce a PCR.

## Overall Recommendation

Birds in large multi-species aviaries such as Chester's tropical free flight are difficult to treat effectively. Thus, this aviary should be primarily for display purposes and not house *Atoxoplasma* susceptible species or individuals that are critical to breeding programs.

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**Table 1.** *Atoxoplasmosis* findings (confirmed and suspected) at Chester Zoo 1999-2007.

Species	Suspect vs Confirmed <i>Atoxoplasmosis</i>	n	Aviary Location	Age	Time of Year
Pied starling	Suspect (undetermined)	10	Arcade	8 day – 2 mo 12 days (juveniles)	June to August 1999 – 2001
Pied sStarling	Suspect (Coccidiosis)	3	Arcade	1 mo 10 days – 1 mo 18 days (recently fledged)	Mid June to Mid July 2001 – 2003
Bali mynah	Confirmed	1	Quarantine	9 mo 17 days (young adult)	End August 2000
Pied starling	Confirmed	1	Arcade	1 mo 20 days (recently fledged)	Mid July 2006
Pied starling	Confirmed	1	Arcade	16 days (nest bound)	End July 2006
Emerald starling	Confirmed	1	Tropics Freeflight	28 days (fledgling)	June 2007

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## VETERINARY REQUIREMENTS OF PRIMATE RELEASE: A NEW APPROACH FOR THE INTERNATIONAL UNION FOR CONSERVATION OF NATURE SPECIES SURVIVAL COMMISSION GUIDELINES

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### Abstract

Reintroduction processes carry a risk of introducing disease to release areas. This risk is significant for great apes who share many common diseases with human beings. Potential exposure of great apes to pathogens during captivity makes understanding and managing this risk critical. In addition, released animals can be affected by an endemic disease for which they are not prepared.

This paper highlights a flexible risk analysis approach to the veterinary requirements adopted by the International Union for Conservation of Nature (IUCN) Best Practice Guidelines for the Re-introduction of Great Apes. It combines science and policy to address two major issues:

- The likelihood of individuals or groups surviving in the new habitat.
- Minimizing pathogen spread in new environments.

Specifically, this approach uses a logical framework to determine the following:

- Adverse health events and means of introduction and spread (hazard identification).
- Likelihood of such events occurring. Methodologies, assumptions and uncertainties need to be commonly understood by stakeholders. (risk assessment)
- Strategies to reduce the likelihood of an adverse outcome.
- Strategies to manage the consequences of such an outcome occurring (risk management).

Four underlying principles of this approach are: no situation is without risk; socio-ecological variables make health assessment an adaptive process; introduced and recipient populations need to be assessed; and, all stakeholders should be involved in identifying mitigation strategies.

Wildlife rehabilitation and release back to the wild is a complex process. Successful great ape reintroduction is possible<sup>5,11</sup> but disease investigation associated with this process must be thorough.

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The potential for transmission of diseases is increased during the re-introduction process as animals and humans have frequent close contact with conspecifics and with one another under increasingly stressful conditions. Animals held in captivity or transported, even for a short period of time, may be exposed to a variety of pathogens for which they have no immunologic experience. This may result in disease, with resultant effects on fecundity, death, and/or an increase in the number of carriers of infectious organisms. Thus, releasing apes to the wild may result in the introduction of disease to conspecifics or unrelated taxa with potentially severe effects.<sup>2,3,7-10,12</sup>

There are many established protocols for moving non human primates between captive facilities around the world. In these cases, veterinary protocols are designed with a great deal of historical knowledge about the relevant facilities and animals and are fairly straight forward. In contrast, there are no standard protocols for movement of non human primates for the purpose of reintroduction, supplementation or translocation. In these cases there are many more variables to be considered and much more uncertainty. Emerging infectious diseases have been shown to significantly adversely affect biodiversity in general<sup>4</sup> and primates in particular.<sup>13</sup> Wild and recently wild primate populations remain a largely unexplored source of information regarding disease and may hold clues to the origins and evolution of some important pathogens. Primates can act as reservoirs for human pathogens and vice versa. Since infectious diseases pose serious risk to threatened primate species,<sup>3</sup> studies of these diseases in primate populations can benefit conservation efforts and may provide the missing link between laboratory studies and the well recognized needs of early disease detection, identification and surveillance.

Removal into captivity, such as into sanctuaries, may result in nonhuman primates becoming infected with agents to which they were not exposed in their natural habitats. For example, many helminth parasites have poorly developed host specificity, some affecting both primates and species widely separated from primates.<sup>2</sup>

As projects move towards reintroduction programs, or in some instances, continue to monitor the health of animals already released, screening for “normal (endemic) diseases” would ideally be done in both introduced animals and the recipient population (if there is one). Those diseases present in both populations or those that are not pathogenic may be of lower concern. This is difficult to interpret because relatively common diseases in one population may be emerging diseases to another. As a minimum, apes to be introduced are being screened for infectious agents not found naturally in wild populations of the taxon of concern (such as pathogens acquired from people or other apes) and agents that may result in the introduction or spread of potentially dangerous diseases.<sup>3,10</sup> The basic process is as follows.

### **Hazard Identification and Risk Assessment**

A list of potential diseases of concern is drawn up after a comprehensive literature search, based on susceptibility and historical findings in the species, as well as field data collected from the relevant field site. A qualitative ranking of these diseases, based on epidemiologic, pathologic and social parameters results in a “traffic light protocol” where those diseases highlighted in red and yellow are thought to be of highest risk and therefore must be screened for and/or prevented.

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This rough assessment is adapted from work developed by the Conservation Breeding Specialist Group (CBSG) of the IUCN.<sup>1</sup> The reliability and certainty of the information gathered is also highlighted, so future research into the diseases can be directed to any knowledge gaps. Diseases of concern will rank higher if there is certainty that a disease is detrimental to the species, or if there is a high level of uncertainty about the known effects, both to the individual, and to the population. The diseases of concern list is not meant to be comprehensive, but rather reflect those that pose the greatest risk to potential release. Thus the testing regime in this case is viewed as an absolute minimum requirement for pre-release disease monitoring, and is specific to each release program.

### **Risk Mangement**

Once the hazard identification and risk assessment phases establish the level of risk (qualitatively or quantitatively) associated with each disease, risk mitigation strategies, and associated costs, are described. Specific recommendations can then be included in the overall veterinary recommendations for the importation of animals into the release site.

### **Risk Communication**

Vitally, all stakeholders are to be made aware of the findings of the assessment including updated information. This is part of a communication network that must be in place for any release program.

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## OKAPI (*Okapia johnstoni*) AND GLUCOSURIA

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### Abstract

Urinalysis of 26 okapis in four European zoos revealed glucosuria in 58%. Mainly animals from the age of four onwards are glucosuric without any difference in urinary specific gravity between non-glucosuric and glucosuric animals. Diabetes mellitus is not the cause<sup>4</sup> and neither is Fanconi syndrome,<sup>1</sup> as animals have normal values for urinary specific gravity, phosphatemia and kalemia and do not show any clinical symptoms. Benign renal disease, which is a genetic disease, seems very unlikely, as there is practically no inbreeding in the zoo population. Retrospective analysis of longevity of animals, born and raised semi-wild in the Station of Epulu (Democratic Republic of Congo) with a natural diet restricted to leaves only, and then imported into SSP (Species Survival Plan) and EEP (European Endangered Species Programme) institutions, revealed a significant difference between animals that had been living in Epulu for only their first 2 yr and those that stayed longer in Epulu. Considering the importance of diet in the anatomic and physiologic development of the young ruminant stomach,<sup>7</sup> we changed our previous diet (i.e., fruit, vegetables, concentrates, lucerne hay) to a diet with less easily digestible carbohydrates (i.e., no fruit, addition of unmolassed beet pulp and leaves). Urinary glucose/creatinine ratio decreased over time in two adult females. More analyses of the natural diet (leaves) are necessary to adjust the diet of our okapis even more.

### Material and Methods

From May 1999 until March 2008 urinalyses of 26 okapis in four European zoos (Antwerp, Cologne, London, Marwell) were performed on midstream urine samples. A mechanical fluid densitometer (urinometer: 1.000 – 1.060) was used to determine the specific gravity (SG) in 116 samples of eight okapis in Antwerp. Glucose and creatinine were quantitatively determined in 87 samples of nine okapis in Antwerp using dry biochemistry (Kodak - Johnson & Johnson) and the ratios calculated to reduce the influence of urine volume.<sup>3</sup> In August 2006 a drastic diet change was carried out, which was gradually implemented during the following 6 mo. Unmolassed beet pulp and dried leaves of six different species were introduced; fruit was omitted and only small amounts of vegetables low in sugar and starch (i.e., celery, chicory, savoy cabbage, endives) remained. Two adult glucosuric females could be followed regularly whilst receiving this new diet and the glucose/creatinine ratio was calculated in a total of 11 and 28 urine samples, respectively, during a 19 mo observation period. One calf was confronted with the changed diet during its early pre-weaning period and 13 urine samples were analyzed during 16 mo follow-up. One subadult animal changed to the new diet at an age of 22 mo and follow-up lasted for 7.5 mo, during which 11 urine samples could be analyzed.

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The Okapi International Studbook provided information on dates and places of birth and death of all okapis. Longevity (disregarding young animals that died within 6 mo after birth) was calculated with the confirmed ages of all animals in Epulu (Democratic Republic of Congo) from 1940 till 2001 (n=34) and from 1975 till 2005 in SSP (n=25) and EEP (n=52).

## Discussion and Conclusion

One of the causes of glucosuria is diabetes mellitus, which does not appear to be the cause in okapis, since Fleming et al<sup>4</sup> observed that glucosuric animals have normal serum levels of insulin, glucose and fructosamine.

Pathologic renal disease with dysfunction of the proximal tubules (Fanconi syndrome) also leads to glucosuria with low specific gravity, hypophosphatemia and hypokalemia and clinical illness.<sup>1</sup> The okapis in our collection never showed hypophosphatemia nor hypokalemia and urinalyses of four glucosuric and four non-glucosuric animals demonstrated a normal specific gravity comparable to that in cows, sheep and goats (SG = 1.015 – 1.045)<sup>3</sup> with no statistical difference between glucosuric and non-glucosuric animals. A total of 116 urine samples of 8 okapis revealed a mean SG of 1.020 (SD = 0.007), of which 74 glucosuric samples had a mean SG of 1.020 (SD = 0.005) and 42 non-glucosuric samples had a mean SG of 1.019 (SD = 0.008).

Benign renal disease is known in humans as a genetic disease with glucosuria in the absence of hyperglycemia, the incidence in the United States is estimated at 0.16 – 6.3 %.<sup>2</sup> With 47% glucosuric animals in the SSP okapi population and practically no inbreeding,<sup>4</sup> benign renal disease seems highly unlikely. In the EEP okapis there is no inbreeding of any significance either<sup>9</sup> and still 58 % of examined animals were glucosuric with no difference between gender, but very significant difference considering the ages. It seems that most (92 %) of the adult animals from 4 yr of age onwards are glucosuric, with an odds ratio of 40.0 and a relative risk of 8.80.

In Epulu station (Democratic Republic of Congo), all okapis are non-glucosuric and receive only a natural diet of 23 kg leaves of diverse plant species.<sup>4</sup> On the contrary, in zoos a substitute diet has been developed with only small amounts of leaves. This significant difference may therefore bear an influence on their age. The mean longevity of 11 yr is identical for animals born in the two captive populations (SSP SD = 5 yr; EEP SD = 6 yr), but significantly different from the mean longevity of 16 yr (SD = 8 yr) for those born in Epulu. Further analysis revealed a significant positive correlation ( $r = 0.41$ ;  $P < 0.05$ )<sup>8</sup> between the number of years spent in Epulu and longevity of animals that were imported from Epulu and died in SSP and EEP (n=34). All SSP animals (n=11) spent their first 3 yr in Epulu and had the same longevity as those EEP animals (n=14) that also spent 3 yr in Epulu. Yet, those EEP okapis that spent only their first 2 yr of life in Epulu (n=9) died at a significantly ( $P < 0.01$ ) lower age than animals that came later in the EEP (n=14).

Langer<sup>7</sup> describes the ontogenic development in the true four-chambered ruminants (cervids, giraffids, antilocaprids and bovids). The postnatal anatomic development is directly affected by the nature of the food. The epithelial development of the ruminal wall is influenced by

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constituents of the gastric content. The cooperation between internal ontogenetic and external trophic influences is necessary to form a functioning adult organ.

In true ruminants, the nature of the food items ingested early in life is very important for normal morphologic and functional development.<sup>7</sup> In the wild only fresh young shoots are eaten (like in Epulu station) and okapis prefer leaves which are low in fiber content.<sup>5</sup> In zoos this is totally different, as a diet purely consisting of leaves is hardly feasible. On dry matter base, the former Antwerp diet consisted of: vegetables 2%, fruit 5.5%, pellets 13.5%, lucerne hay 79%. The fibre analysis of this diet corresponds with the natural leaves diet, but contains also high amounts of easily digestible carbohydrates. Implementing research results,<sup>6</sup> major improvements have been obtained, in order to adapt the diet better to a natural fermentation with rapid passage through the rumen. The new diet in Antwerp consists of: vegetables 0.3%, fruit 0%, pellets 11%, leaves 14%, unmolassed beet pulp 17%, lucerne hay 58%, which has the same comparable amount of fibres, but much lower amounts of sugars and starch, i.e a decrease of approximately 260 g per day. Two adult okapis showed a decrease of the glucose/creatinine ratio over time with a negative correlation  $r = -0.62$  ( $P < 0.001$ ) and  $r = -0.58$  ( $P > 0.05$ ), respectively. One young calf had no detectable amounts of urinary glucose, except on two occasions (ratios of 1.19 and 1.21 at the ages of 13.5 and 14 mo, respectively) during its 16 mo follow-up. One subadult remained non-glucosuric during the whole observation period.

In order to provide still more proof for adapting our diet there is a need for more profound analyses of the natural plants in Epulu, including easily digestible carbohydrates and secondary compounds.

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