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## GEORGIA WILDLIFE HEALTH PROGRAM AND THE GEORGIA SEA TURTLE CENTER

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### Abstract

Throughout my veterinary career I have been very interested in free-ranging wildlife. While working on St. Catherines Island (SCI) in coastal Georgia, I was able to develop the Georgia Wildlife Health Program. The primary goals of this program are to assist conservation organizations on various aspects of wildlife health and disease issues and to provide veterinary services to wildlife biologists and graduate students. The focus of the program has been on health related issues pertaining to wild reptiles and birds.<sup>1-3, 6-10, 12-16</sup>

In 2000, we partnered with the Wildlife Conservation Society's (WCS) Field Veterinary Program on a global sea turtle health assessment project.<sup>4</sup> Georgia was added as the North American site because of the relationship WCS had with SCI. The work in Georgia included establishing baseline health parameters for several of the life stages of the loggerhead sea turtle.<sup>5</sup> In addition to evaluating healthy turtles, we started to be called upon to do the initial evaluation of stranded sea turtles. Through this work, it became apparent that a sea turtle rehabilitation center was needed in coastal Georgia.<sup>11</sup>

The original site for the facility was to be on SCI, a remote barrier island which is only assessable by boat. I started to fund raise on my own by writing grants and developing some unique methods to raise awareness and funding. The "Turtle Crawl" was established in 2003 with the goal of creating awareness and raising funds for the Georgia Sea Turtle Center (GSTC). I eventually realized that I was not going to be able to generate enough money to build the center. Furthermore, it became apparent that public education was going to be a critical component of the GSTC's mission and this was going to be difficult due to the remoteness of SCI. We approached the Jekyll Island Authority (JIA) and the Jekyll Island Foundation (JIF) in 2003 about the idea of starting a sea turtle conservation center on Jekyll Island. With the full support of these organizations and the collaborative efforts of many others, the idea became a reality.<sup>11</sup> A fund raising campaign was initiated by the JIF which ultimately raised approximately three million dollars to cover the costs of the new facility.

The GSTC, a department of the JIA, officially opened its doors on June 16, 2007. One of the unique features of the GSTC is that it integrates rehabilitation, research, professional student training, and interactive education for the public. Approximately 100,000 visitors tour the GSTC annually. The center has a staff of thirteen full-time and several seasonal hourly staff. Additionally, AmeriCorps members, rotating veterinary externs, graduate students, and more than 150 volunteers frequent the Center on a regular basis.

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## Rehabilitation

Three species of sea turtles, the loggerhead, Kemp's ridley, and green, are commonly presented to the GSTC for rehabilitation. In addition to Georgia, turtles have come from Florida, North Carolina, and even Massachusetts. Some of the more common problems include boat strike and other traumatic injuries, fishing line and hook entanglements and ingestion, fibropapillomatosis, flotation abnormalities, and cold stunning. The GSTC also treats other wild turtles native to Georgia. The diamondback terrapin (*Malaclemys terrapin*) lives in the marshes surrounding Jekyll Island and has been another focus species for the GSTC.

## Education

Sea turtles are used as a flagship to represent the entire marine ecosystem. Creating interactive and engaging educational programs has been the key in spreading this message. For example, a window from the exhibit gallery looking into the turtle hospital allows visitors to observe treatments, diagnostic procedures and surgeries first hand. Seeing the turtles and their medical problems up close and personal has a lasting effect on both children and adults.

## Research

Research is the third pillar of the center's mission, which consists of a unique mix of veterinary medical and ecological research. Some examples include:

- A nesting sea turtle monitoring, management and research program includes night and dawn patrol for nesting sea turtles, primarily loggerheads. This program includes flipper and P.I.T tagging, morphometric measurements, sample collection for a variety of projects, and documenting nest location and providing protection from predators and inundation.
- Satellite telemetry of rehabilitated sea turtles to monitor their post-release behaviors and survivorship.
- Loggerhead nutritional health related research is being conducted to establishing normal values for vitamins, minerals, and lipids in free-ranging loggerhead sea turtles. Additionally, samples are taken from turtles coming to the GSTC for rehabilitation at set time periods as they recover. Common prey items have been analyzed nutritionally. We are now developing tube feeding formulas for critically ill sea turtles and a vitamin supplement based on the information gathered.
- Tramadol pharmacokinetics in loggerhead sea turtles.
- A diamondback terrapin conservation program focuses on efforts to reduce mortality from automobiles on the Jekyll Island Causeway. Rehabilitation, education, research, and mitigation efforts are all important for this program's success.

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## Professional Training

The GSTC veterinary externship program has grown to the point where we have one or more veterinary students working with us throughout the year. Students come from all over the US as well as a number of other countries.

In 2009, the GSTC was awarded a Corporation for National and Community Service/Georgia Commission for Service and Volunteerism AmeriCorps grant to expand our internship program. This grant has enabled us to have 11 members present for a full year and six to eight members present for 6 mo to work in the areas of husbandry, education, research, and volunteer coordination.

## International Programs

Our international programs are focused on training biologists and veterinarians, educating children and adults, and providing scientific expertise. The GSTC has developed extensive collaborative programs with our partners (Ross University School of Veterinary Medicine (RUSVM) and the St. Kitts' Sea Turtle Monitoring Network (SKSTMN)) in St. Kitts, West Indies. We are developing programs that focus on training sea turtle biologists and veterinarians on various aspects of sea turtle conservation. Staff from the GSTC have traveled to Costa Rica to provide expertise and training and biologists and veterinarians from Costa Rica have trained with us at the GSTC.

## ACKNOWLEDGMENTS

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## WILDLIFE CONSERVATION SOCIETY'S GREAT APE HEALTH PROGRAM

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### Abstract

In 1999, the Wildlife Conservation Society's Field Veterinary Program developed the Gorilla Preventive Health Program to establish a multi-disciplined health monitoring system for free-ranging western lowland gorillas (*Gorilla gorilla gorilla*). The overall objective was to establish preventive health care activities, and standardized and comparable health monitoring systems, for these critically endangered apes by partnering with existing projects in several central African countries. Through this grassroots approach, WCS veterinarians worked with local project managers and wildlife researchers to provide health care advice and services, and training in health monitoring techniques to protect the health of the gorillas in their regions. Primary focus was on health visual data collection, fecal parasite screening<sup>1</sup> and the creation of preventive medicine guidelines for protected area personnel and researchers. One aim of the program was to improve human health and livelihoods as a way to protect wildlife health by preventing anthroponotic disease transmission; a novel approach at the time, but one later recognized as the "One World - One Health<sup>TM</sup>" concept. In 2003, catastrophic Ebola Virus Disease outbreaks in apes and humans shifted much of the program's focus towards Ebola virus research and surveillance.<sup>2</sup> We describe the inception and evolution of the "Great Ape Health Program" reflecting on accomplishments, challenges and lessons learned.

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## REINTRODUCTION OF WOLVES (*Canis lupus*) IN IDAHO

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### Abstract

Wolves, *Canis lupus*, were extirpated from Idaho by 1930 and were listed under the Endangered Species Act (ESA) in 1976. The US Fish and Wildlife Service's (USFWS) established 3 recovery areas for wolves - northwestern Montana, central Idaho, and the greater Yellowstone area. Efforts to document wolves in central Idaho found no evidence of a viable population by 2002. A Record of Decision was issued on the Final Environmental Impact Statement November 1994 with reintroduced wolves defined as a nonessential experimental population (ESA Section 10j). The goal for delisting wolves was greater than 10 breeding pairs and at least 150 wolves in each of the 3 recovery areas for 3 successive years. Wolves were captured in Alberta, and 15 wolves were released in Idaho in January 1995. In 1996, 20 additional wolves captured in British Columbia were released in the same area. During spring 1996, 3 wolf packs produced pups. By 2000, Idaho met the delisting criteria and the population continued to grow with over 850 wolves by 2008 when wolves were initially delisted in Idaho and Montana. Numerous legal challenges occurred throughout the process and wolves were subsequently relisted and delisted multiple times between 2008 and 2011. Despite controversy and court challenges, successful reintroduction resulted in a robust population of wolves in Idaho. Following delisting, hunting and trapping seasons have been established and wolves are being managed as a big game species.

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## **PROGRAM SUPPORT FOR THE RESTORATION OF BIODIVERSITY IN GALAPAGOS NATIONAL PARK**

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### **Abstract**

The Galapagos archipelago is located approximately 1000 km west of mainland South America. Wildlife on these volcanic islands lived and evolved in isolation for millions of years prior to the arrival of humans in the 16<sup>th</sup> century. Since that time the wildlife has been exploited and many plant, invertebrate, and vertebrate species have been introduced. Some of these have become invasive and threaten the natural biodiversity of this living laboratory. The Galapagos National Park (GNP) was formed in 1959 in order to preserve the integrity of the natural ecosystems. Since that time the GNP has worked with the Charles Darwin Foundation, The Galapagos Conservancy, Island Conservation, and scores of scientists, universities, and NGOs to study, preserve, and restore the ecosystem. When requested, veterinary input is provided to support the GNP conservation programs. Support may include training of veterinary professionals, park rangers, or researchers; establishment of baseline health parameters, diagnosis and treatment of disease, development of novel control methods for introduced species, and development of protocols associated with translocations or reintroductions of wildlife. The GNP has successfully removed invasive species such as goats, donkeys, pigs, black and Norway rats, mice, and many other species from many of the islands in the archipelago, resulting in the recovery of vegetation and native wildlife populations.

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## NEPAL ELEPHANT (*Elephas maximus*) HEALTHCARE AND TUBERCULOSIS SURVEILLANCE PROGRAM UPDATE

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### Abstract

The Nepal Elephant Healthcare and Tuberculosis (TB) Surveillance Program was initiated by Elephant Care International in 2007 following the first comprehensive TB testing of Asian elephants in 2006. Previous reports have described the challenges that TB presents to wildlife, humans, and domestic livestock in Nepal <sup>1-3</sup> and a recent report has demonstrated the risk of transmission to the wild.<sup>4</sup>

The program is based near Chitwan National Park where a field office and lab are staffed by a full-time veterinarian. Program goals are to 1) mitigate transmission of TB to wild elephants, rhinos and other ungulates by controlling TB at the captive-wild interface, 2) ensure the health of government elephants used for anti-poaching patrols, rhino censuses, and other conservation purposes, 3) safeguard tourism that supports the national parks, 4) build wildlife veterinary capacity, 5) encourage the development of elephant TB control programs other Asian elephant range countries, and 6) advance our knowledge of TB in elephants.

Ninety-three percent of the captive population has been tested using the Elephant TB Stat-Pak<sup>®</sup> and / or DPP<sup>®</sup> Vet TB<sup>™</sup> assays.<sup>a</sup> Over 20 elephants have been treated prophylactically or therapeutically for TB based on serology results, culture, and /or exposure history.

The Program has facilitated multiple research projects, involving students and investigators from Tufts University, Michigan State University, Murdoch University, and the Institute of Agriculture and Animal Science (Nepal).

In 2010 the Ministry of Forestry approved the Elephant Tuberculosis Control and Management Action Plan (2011-2015), the first such plan in Asia. The plan is on-line at [www.elephantcare.org](http://www.elephantcare.org).

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**Products Mentioned in the Text:** <sup>a</sup>Chembio Diagnostic Systems, Inc, Medford, NY, USA 11763.

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## WHY ARE EAGLES FALLING FROM THE SKY? THE FIGHT AGAINST BIRD CRIME TO SAVE THE EAGLES OF HUNGARY

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### Abstract

The Imperial eagle (*Aquila heliaca*) and the White-tailed sea eagle (*Haliaeetus albicilla*) are among the most emblematic species of the rich Hungarian avifauna. The Hungarian breeding population of the Imperial eagle is approximately 150 pairs and is one of the most important cohorts of the species in Europe. The White-tailed sea eagle population size is close to 260 breeding pairs and is also very important in this part of Europe. The Golden eagle (*Aquila chrysaetos*) is a very rare (1-5 pairs) breeding bird in Hungary.

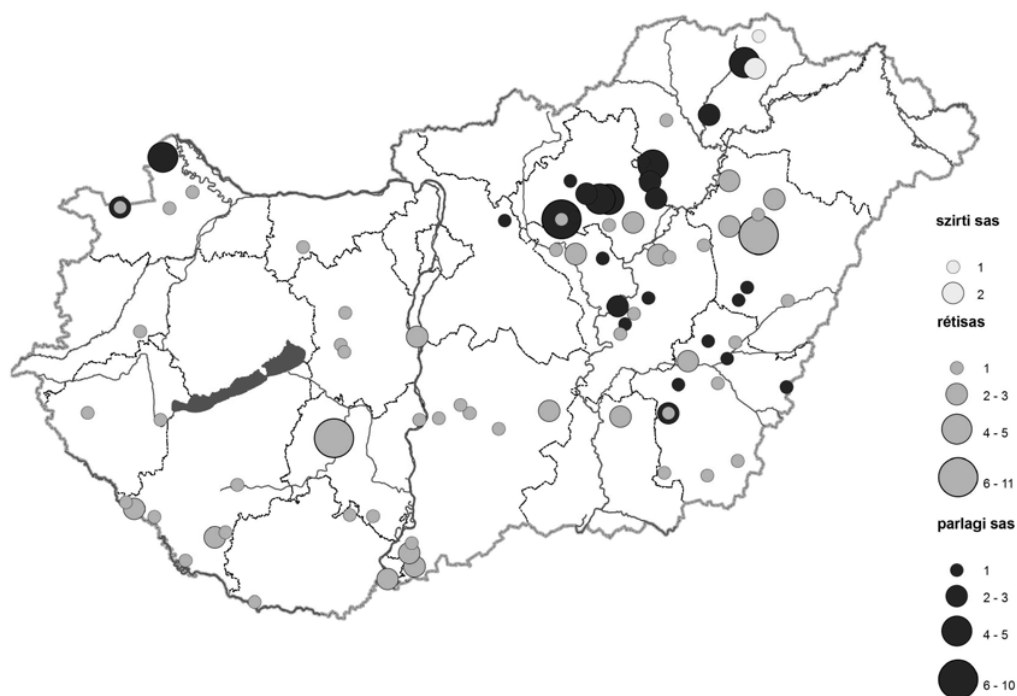
Despite tremendous conservation efforts, many factors threaten these vulnerable populations including habitat loss, disruption of breeding sites, poaching, electrocution and illegal poisoning, with poisoning occurring particularly frequently. A new LIFE Plus project involving the Budapest Zoo and Botanical Garden, is trying to manage poisoning in eagles. Unfortunately, most of the affected birds are found dead and thus, a detailed autopsy and toxicological examination are carried out in order to determine the possible intoxicating agent and the cause of death. A minority of the birds are brought alive to the Rescue Centre at the Budapest Zoo, where a physical examination and x-rays are done. Supportive therapy is initiated immediately and further toxicological examinations are performed depending on the health status of the bird. Our clinical experience demonstrates that some of the intoxicated birds present with typical clinical signs of organochlorines. The most commonly found agent is carbofuran, a pesticide banned in the EU since 2008. Nevertheless, this substance is still commonly used in other parts of the world and very often found as a toxin in poisoned birds.<sup>1,2</sup> Clinical signs of carbofuran intoxication in birds include leg spasms, vomiting and distended crops. The administration of atropine at dosages of at least 1 mg/kg is safe and effective even in emaciated birds. In some cases, the administration of atropine provided quick relief, but re-occurrence of the symptoms took place. Atropine can be repeated 2 times/day, but the prolonged effect of the toxins must be monitored thereafter through kidney and liver functions tests. X-rays are extremely helpful to exclude foreign bodies and possible lead poisoning in these cases. Eagles are quite often shot, but bullets or pellets are frequently not related to the acute onset of the clinical signs described above. Nevertheless, the x-rays have an important role in the forensic and legal procedures.

A summary of the intoxicated eagle cases between 1998 and 2012 is shown in Figure 1. The high numbers clearly indicate the huge impact on these bird species and the threatening trend.

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**Figure 1.** Intoxicated eagle cases in Hungary between 1998 and 2012. (szirti sas = Golden Eagle, retisas = White-tailed Sea Eagle, parlagi sas = Imperial Eagle).

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**ANESTHETIC PROTOCOLS, ASSOCIATED CHALLENGES, AND SUCCESSFUL OUTCOMES IN SPECTACLED BEARS (*Tremarctos ornatus*), SLOTH BEARS (*Melursus ursinus*), AND GIANT PANDAS (*Ailuropoda melanoleuca*) AT THE SMITHSONIAN'S NATIONAL ZOOLOGICAL PARK, 1995-2013**

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**Abstract**

Limited publications exist in the literature on spectacled bear (*Tremarctos ornatus*), sloth bear (*Melursus ursinus*), or giant panda (*Ailuropoda melanoleuca*) anesthesia. Between 1995-2013, the Smithsonian's National Zoological Park recorded a total of 117 anesthetic procedures on captive bear species (41 anesthetic procedures on 4.3 spectacled bears, 32 anesthetic procedures on 3.5 sloth bears, and 44 anesthetic procedures on 3.1 giant pandas). Induction protocols involved some combination of xylazine (n=39), medetomidine (n=46), ketamine (n=96), tiletamine-zolazepam (n=75), and/or midazolam (n=1). The most commonly used protocol for spectacled bears and sloth bears was tiletamine-zolazepam (1.0-3.0 mg/kg), ketamine (1.3-3.0 mg/kg), and medetomidine (0.012-0.016 mg/kg) (n=11 for each species), and for giant pandas it was ketamine (2.1-10.9 mg/kg) and xylazine (0.5-1.1 mg/kg) (n=17). Supplemental injections of ketamine, midazolam, telazol, and/or medetomidine were required to complete inductions in 39 cases. Anesthetic maintenance was most often performed with isoflurane, ketamine, or propofol and anesthesia was reversed with yohimbine, atipamezole and/or flumazenil when indicated. Atropine or glycopyrrolate were often administered to minimize oral secretions. Metoclopramide was given frequently in an attempt to minimize vomiting, with variable effect. Commonly reported complications included perianesthetic vomiting during various anesthetic protocols, and bradycardia and hypertension in response to alpha-2 agonists. Seizure activity was reported in some bears that received ketamine for induction. Hyperthermia occurred very rarely and was associated with prolonged induction times, and hypothermia occurred during prolonged anesthetics. No episodes of spontaneous arousal occurred.

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## HYPERKALEMIA IN EXOTIC FELIDS ANESTHETIZED WITH AN ALPHA - 2 ADRENOCEPTOR AGONIST, KETAMINE, AND ISOFLURANE

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### Abstract

Hyperkalemia is a poorly understood complication associated with general anesthesia in non-domestic felids.<sup>1</sup> In a multi-center retrospective study, eleven cases of hyperkalemia in six tigers (*Panthera tigris*), two cougars (*Puma concolor*), one cheetah (*Acinonyx jubatus*), one lion (*Panthera leo*), and one liger (*Panthera tigris-Panthera leo* hybrid) were evaluated. Anesthesia in all cats was induced with intramuscular ketamine and medetomidine (n=7) or dexmedetomidine (n=4), and was maintained with isoflurane. Mean  $\pm$  SD total anesthesia time was  $256.7 \pm 87.6$  min, and mean time to first evidence of hyperkalemia was  $186 \pm 40.1$  min. Mean dosage of dexmedetomidine was  $15.0 \pm 4.6$  mcg/kg and medetomidine was  $32.15 \pm 11.15$  mcg/kg. Mean potassium concentration at the time when hyperkalemia ( $\geq 5.5$  mmol/L) was recognized was  $6.5 \pm 1.5$  mmol/L, and mean blood glucose concentration at that time was  $256.5 \pm 115.4$  mg/dL (n=6). Treatment of hyperkalemia consisted of atipamezole (n=4), insulin (n=4), dextrose (n=4), calcium gluconate (n=4), and/or bicarbonate (n=5). Four of the eleven cases arrested and three of those died due to their hyperkalemia. Alpha-2 adrenergic agonists are known to inhibit insulin secretion, and a decreased amount of circulating insulin can have an effect on both plasma glucose and potassium concentrations. A study at the authors' institution indicated a significant increase in plasma potassium, glucose, and aldosterone concentrations over time in eleven anesthetized exotic felids (Reilly, manuscript submitted). In anesthetized exotic felids, the authors' recommend to measure blood glucose and potassium every 30 minutes, and the treatment of hyperkalemia if present.

### ACKNOWLEDGMENTS

The authors would like to thank the University of Florida College of Veterinary Medicine and the Houston Zoo Inc. for providing anesthetic data of exotic felids that developed hyperkalemia.

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## **SUCCESSFUL IMMOBILIZATION OF CAPTIVE AND FREE-RANGING PINNIPEDS: PRACTICAL TIPS TO IMPROVE SUCCESS**

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### **Abstract**

Immobilization of captive and free-ranging pinnipeds continues to be viewed as a “higher-risk” anesthesia by many zoo and wildlife veterinarians. Common concerns have included unpredictable quality and duration of anesthesia, apnea, bradycardia, prolonged recovery times, and death. However, numerous published and unpublished data sets illustrate that pinnipeds can be very safely anesthetized using newer anesthesia protocols and procedures. In a zoo setting, the degree of pre-anesthetic assessment that is possible varies greatly, but should not be overlooked. Such a luxury does not exist with wild specimens, but the success of various recent field anesthesia projects illustrates that lack of pre-anesthetic information should not be viewed as a valid reason to avoid an immobilization. Various combinations of medetomidine, butorphanol, and midazolam provide a safe, reliable protocol that can be used as a pre-anesthetic or complete induction agent based upon dosages. Other newer anesthetic drugs (e.g. alfaxalone) also show great promise. Gas anesthesia (isoflurane, sevoflurane) provides not only a reliable maintenance anesthetic, but can also be used as an induction agent with proper equipment (special nets, restraint devices, masks) and training. Efficient intubation and maintenance of proper ventilatory support are a necessity, often requiring modification of equipment for use in the field. Anesthetic monitoring is greatly enhanced through the use of Doppler monitors, capnography, arterial blood gas analysis, and reflectance pulse oximetry, all of which can require specialized knowledge or equipment to ensure success. Vascular access can be challenging, but with proper training, reliable arterial blood samples for gas analysis are obtainable, as well as efficient placement of central line catheters for intravenous access. With secure vascular access, continuous rate infusion of anesthetics and supportive drugs is possible in a zoo setting, which can further enhance patient safety. Overall, with proper planning and precautions, pinnipeds can be safely anesthetized for sustained periods of time. An increased comfort with such procedures will also help to further shift zoo philosophy away from the “anesthesia as a last resort” paradigm, leading to improved clinical care and healthier anesthetic candidates.

### **ACKNOWLEDGMENTS**

The author thanks Dr. Gwen Jankowski, Dr. Jennifer Langan, Dr. Jenny Meegan, and Susana Cardenas for their continued assistance and support of numerous pinniped projects and procedures in the zoo and in the field.

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## **A NOVEL NON-REBREATHING SYSTEM FOR REPTILIAN ANESTHESIA: A DESCRIPTION OF EQUIPMENT REQUIRED AND INDICATIONS FOR USE**

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### **Abstract**

Inhalant anesthesia in reptilian species presents many challenges including prolonged inductions and recoveries. Additional challenges can be encountered during the maintenance period, especially in patients intubated with small internal diameter endotracheal tubes. More specifically, endotracheal tubes with a small internal diameter can become occluded with mucous, which has the potential to create a one-way valve, allowing air into the lungs, but not back out.<sup>1</sup> This can result in hyperinflation of the lung and subsequent morbidity or mortality. Additionally, small internal diameter endotracheal tubes limit the volume of gas that can be adequately delivered to and exhaled by the patient. These concerns can be addressed by modification of a standard non-rebreathing system that provides constant fresh gas inflow and at the same time reduces airway dead space.

Non-rebreathing systems are indicated for use in reptiles weighing less than 10kg.<sup>2</sup> At the San Diego Zoo a Mapleson non-rebreathing system with a Norman mask elbow is used for most reptile procedures requiring anesthesia. Using this unmodified system, gas flow follows the path of least resistance and is delivered to the patient during inspiration and towards the reservoir during expiration.<sup>1</sup> We have modified this system by firmly inserting a red rubber catheter into the Norman elbow, advancing the red rubber catheter into the tracheal lumen, and placing a standard induction mask over the patients head to create an airtight seal. It must be noted that when using this system, the external diameter of the red rubber catheter must be smaller than the tracheal lumen to allow for gas to flow from the patient to the reservoir and an airtight seal between the patient and induction mask is essential. This modification creates unidirectional gas flow and offers many advantages over a traditional non-rebreathing system. Trauma to the glottis and tracheal mucosal damage is minimized due to the small size of the catheter relative to the lumen of the trachea and increased pliability of the red rubber catheter when compared to more commonly used endotracheal tubes. Additionally, the concern for mucous accumulation at the end of the endotracheal tube is minimized and because the gas flow is unidirectional, the necessity to overcome pressure limitations created by small internal diameter endotracheal tubes is greatly mitigated. When desired, positive pressure ventilation can be provided by increasing the fresh gas flow rate or temporarily occluding the exhaust gas airflow. When using this modified system, effective respiration, adequate oxygenation and elimination of CO<sub>2</sub>, appears to take place independent of spontaneous respiration or intermittent positive pressure ventilation.

We have successfully used this system in a variety chelonian and squamata species including venomous snakes and recognize this system has limitations including;

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incomplete airway protection, potential for pulmonary distension if exhaust gas outflow is occluded, and concerns for patient cooling and airway drying secondary to constant unidirectional gas flow.

The description of this technique should serve as a foundation for more detailed studies and future directions should include; blood gas analysis in anesthetized patients to better characterize ventilation, post-mortem examination of the pulmonary system to determine if this technique results in adverse changes to the respiratory epithelium, and investigation of the utility in avian and mammalian species.

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## CETACEAN SEDATION AND ANESTHESIA: REVIEW AND UPDATE

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### Abstract

Although still applied infrequently, sedation of managed populations, and even free-ranging cetaceans has become an acceptable risk. On the other hand, anesthesia of managed cetaceans is still considered high risk to most zoo and wildlife veterinarians. Historical failures and limited experience have lead to myths and misconceptions about anesthesia of cetaceans. As well, the high value and relative difficulty with invasive support and monitoring has lead to hesitation in performance of medically necessary invasive procedures, leading to negative outcomes, further perpetuating the these misconceptions.

Managed populations include many Killer whale (*Orcinus orca*), Beluga whale (*Delphinapterus leucas*), Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), False killer whale (*Pseudorca crassidens*) and others. Still, most experience has come from work with bottlenose dolphin (*Tursiops truncatus*). Despite clear differences in body mass, common concerns include endotracheal intubation and extubation, body positioning and padding, body temperature management, vascular access and anesthetic agent.

Combinations of benzodiazepines (diazepam, midazolam), opioids (butorphanol, meperidine) have been used for pre-anesthetic sedation. Injectable anesthetic agents such as thiobarbiturates and propofol have been used for rapid sequence induction allowing manual orotracheal intubation by rostral luxation of the modified larynx. Inhalation anesthetics (halothane, isoflurane, sevoflurane) have been used reliably for maintenance of anesthesia. Ventilation by “apneustic plateau” and conventional methods have been applied with variable success and implore review. Anesthetic monitoring and vascular access are complicated by anatomic differences from terrestrial species. Imaging techniques and advanced non-invasive monitoring methods will advance the care of these species under general anesthesia.

### ACKNOWLEDGMENTS

The author thanks Dr. Chris Dold, Dr. Eric Jensen, and Dr. Sam Ridgway for their assistance and support of numerous cetacean projects and procedures in the aquatic animal medicine.

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**VITILIGO ASSOCIATED WITH HYPOVITAMINOSIS D IN MALAYAN  
FLYING FOX (*Pteropus vampyrus*) AND ISLAND FLYING FOX (*Pteropus  
hypomelanus*)**

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**Abstract**

Eight Malayan flying fox (*Pteropus vampyrus*) and three island flying fox (*Pteropus hypomelanus*) presented for varying degrees of skin depigmentation of the wing membranes, face, and feet. Lesions were observed 6 mo after the bats were placed in a newly constructed indoor-only exhibit. Skin biopsies indicated segmental loss of melanin pigment within the epidermis; skin scrapes and culture results were negative for infectious agents. Routine blood work was unremarkable. Vitamin D values were low compared to values from captive flying fox with outdoor access. A diagnosis of cutaneous vitiligo was made. All bats were removed from the exhibit and housed in an off-exhibit holding area that was modified to contain several UV-B light sources. Bats were regularly seen basking, and slow re-pigmentation was noted in some individuals. Zookeepers recorded bat behavior to determine each bat's average proximity to the UV-B lights. Two months after the addition of UV-B lights, all bats were anesthetized to repeat blood work. Vitamin D values were 2-4 times higher than the original values. Based on the results, these two species of diurnal bat likely require UV-B light in order to synthesize Vitamin D.

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## FEED-RELATED HYPERVITAMINOSIS D IN A CAPTIVE FLOCK OF BUDGERIGARS (*Melopsittacus undulatus*): MORBIDITY, MORTALITIES AND PATHOLOGIC LESIONS

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### Abstract

In the spring of 2012, the Blank Park Zoo began suffering mortalities in a flock of 229 captive Budgerigars (*Melopsittacus undulatus*) housed in an interactive public-feeding aviary. Clinical signs included weakness, posterior paresis, inability to fly, central nervous signs or acute death. Gross and microscopic lesions were not apparent in acutely affected deceased birds. Many birds had evidence of trauma, but these traumatic events are now hypothesized to have been related to the birds' weakness. Investigation into the cause(s) of morbidity and mortality were complicated by the recent opening of the new interactive enclosure, so environmental and husbandry sources were heavily scrutinized. Later in the course of the investigation, microscopic examination of tissues revealed mineralization of soft tissues consistent with hypervitaminosis D.<sup>1-5</sup> Serum analysis of deceased birds also identified elevated vitamin D<sub>3</sub> levels. Analysis of the formulated diet detected elevated levels of vitamin D<sub>3</sub> 22.5-times the manufacturer's labeled content in the formulated feed (label = 1800 IU/kg, actual = 40,520 IU/kg). These findings eventually led to the manufacturer's recall of over 100 diets fed to a wide variety of domestic and captive wild animals in the United States and internationally. This case report highlights the complexities of determining the etiology of a toxic event in a zoological institution.

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## OBSERVATIONS ON WEIGHT LOSS AND FECAL CONSISTENCY IN GIANT ANTEATERS (*Myrmecophaga tridactyla*) DURING THREE TRANSITIONS FROM A MIXED NATURAL IN-HOUSE TO COMMERCIAL COMPLETE DIETS

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<sup>1</sup>*Clinic for Zoo Animals, Exotic Pets and Wildlife, University of Zurich, 8057 Zurich, Switzerland;* <sup>2</sup>*Zoo Zurich, 8044 Zurich, Switzerland*

### Abstract

Giant anteaters (*Myrmecophaga tridactyla*) are highly specialized insectivores with low energy requirements compared with similar-sized mammals and an exceptional feeding apparatus and behavior.<sup>6,7</sup> Common health problems which have been attributed to suboptimal nutrition are loose stool or diarrhea, anorexia, tongue tip constriction or vitamin K deficiency.<sup>4,8</sup> Traditional, natural in-house diets typically include dog kibble, cereals, honey, milk products, meat and insects.<sup>5</sup> Simpler (and more rational) diets consisting of a mixture of commercial dry feeds for cats and leaf eaters have been promoted particularly in the US.<sup>2</sup> In recent years commercial complete diets have become available. Potential advantages of such diets are nutritional adequacy, standardized quality, less preparation time and facilitated exchange of animals between institutions. However, it has been reported that anteaters may be very reluctant for a diet change and may react with prolonged refusal.<sup>1,3</sup> For veterinarians and curators it is difficult to decide what period of anorexia and what percent of weight loss is acceptable. Here we report observations from three consecutive dietary changes (Diet A, B, C; Table 1) made with three giant anteaters (Figure 1). The animals were clinically healthy and stool consistency was firm. The changes took place over a period of 3 yr. Prior to the diet change, giant anteaters at Zurich Zoo were fed with a mixed natural in-house diet of minced meat, dog pellets, low fat curd cheese, cereals, shrimp meal, honey, seasonal fruits, boiled eggs, chitin powder, mineral/vitamin supplement and sifted peat.<sup>8</sup> Diet A was completely refused by all three animals and the trial was stopped after three weeks of reduced and intermittent intake resulting in increased aggression against keepers and tapirs (which share the exhibit with anteaters). The loss of weight of 6% was considered not acceptable for two lactating females. After 14 mo on the previous traditional diet, a second attempt with diet B was made. This diet was accepted with increasing amounts over a period of 4 mo but a weight loss of 6-24% occurred after 18 mo on this diet, and all animals showed repeatedly loose stool. Due to high cost of diet B and irregular supply, a third change to diet C became necessary. Both female anteaters immediately started to eat diet C well and to increase weight. With diet C all animals showed loose stool, which was eliminated by adding peat to the diet (115gr to 260gr of diet C, per animal, twice a day), which represented an increase of the content of crude fiber from 13% to 17% in dry matter. Within 12 mo the two females increased their body weight by up to 33% (Figure 1). The male showed intermittent reluctance to eat the diet and eventually died of causes

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considered unrelated to the diet change.

A possible reason for the complete refusal of diet A may have been the fact that it was provided as granules first, which the animals did not seem to like, and only as ground powder later. Diets B and C were powdered from the beginning. It should be noted that diet A appears to be well accepted by anteaters at other institutions.<sup>8</sup> Therefore we think that the history presented here does not represent a test case of the three diets, but illustrates the problem in overcoming individual feeding preference inertia in this species. The transition from the traditional custom made to a commercial diet resulted in a timesaving effect of approximately 45 min per day.

In conclusion, we observed that the introduction of commercial complete diets may result in important weight loss of up to 20%. In lactating females a weight loss of more than 6% was considered not acceptable. Occurrence of loose stool appears to be a problem observed with introduction of commercial complete diets in giant anteaters in general and needs to be addressed with addition of fiber into the diet.

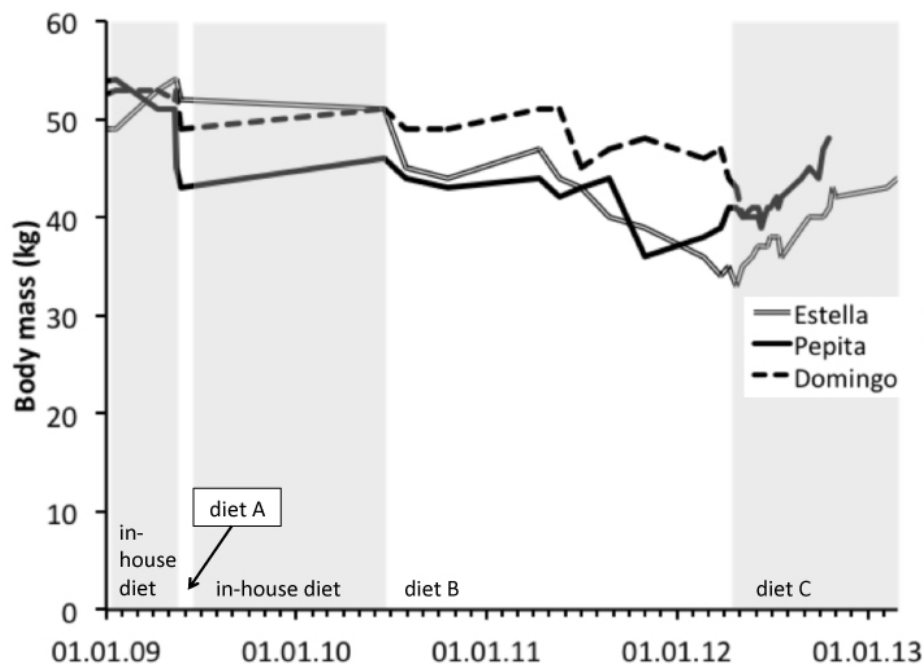
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**Table 1.** Composition of commercial diets for insectivorous mammals.

Company	Diet A “Mazuri Insectivore Diet 5MK8” Purina Mills, St. Louis, MO 63166 USA	Diet B “Mazuri Termant” PO BOX 705 Witham, Essex CM8 3TH, UK	Diet C “Insectivore” Kliba Nafag, Provimi Kliba AG, 4303 Kaiseraugst, Switzerland
Crude fat	12.0 %	10.2 %	10.0 %
Crude protein	28.5 %	28.3 %	29.7 %
Crude fiber	9.6 %	10.9 %	13.0 %
Ash	6.5 %	6.1 %	7.4 %

**Figure 1.** Changes in body mass of three adult giant anteaters (*Myrmecophaga tridactyla*) during three diet changes from a mixed natural in-house diet to a commercial complete diet.



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## METABOLIC CONSEQUENCES OF THE EARLY ONSET OF OBESITY IN COMMON MARMOSET MONKEYS

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### Abstract

We examined the patterns of fat mass gain from birth to 12 mo in common marmosets (*Callithrix jacchus*), and tested the hypotheses that juvenile marmosets with excess adipose tissue will display higher fasting glucose, decreased insulin sensitivity, and decreased ability to clear glucose from the blood stream. Lean and fat mass was measured by quantitative magnetic resonance at 1, 2, 6, and 12 mo for 39 marmoset infants. Circulating glucose was measured by glucometer; insulin and adiponectin by commercial assays. The QUICKI (a measure of insulin sensitivity) was calculated for subjects with fasting glucose and insulin measures. Oral glucose tolerance tests (OGTT) were conducted at 12 mo on 35 subjects.

Results: Subjects classified as Fat (> 14% body fat) at 12 mo already had higher percent body fat by 1 mo and had both greater lean mass (198.4±6.2g vs 174.0±6.8g, p=.013) and fat mass (45.5±5.0g vs 24.9±3.4g, p=.002) by 6 mo. Percent body fat decreased between 1 and 12 mo in Normal subjects; in Fat subjects it increased.<sup>1</sup> At 6 mo Fat subjects already had significantly lower insulin sensitivity (mean QUICKI = .378±.029 versus .525±.019, N=11, p=.003). At 12 mo Fat subjects had lower QUICKI, higher fasting glucose (129.3±9.1 mg/dL versus 106.1±6.5 mg/dL, p=.042) and circulating adiponectin tended to be lower (p=.057). The OGTT results demonstrated that Fat animals had a decreased ability to clear glucose.<sup>2</sup>

Conclusions: Excess adiposity in captive marmosets develops by 1 mo. Early onset obesity in marmosets results in impaired glucose homeostasis by 1 yr.

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**BODY CONDITION SCORING INDEX FOR FEMALE AFRICAN ELEPHANTS  
(*Loxodonta africana*) VALIDATED WITH ULTRASOUND MEASUREMENTS OF  
SUBCUTANEOUS FAT**

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**Abstract**

A major problem of zoo African elephants is ovarian acyclicity, which preliminary studies suggest may be related to obesity. To test this relationship, methods to accurately quantify body condition and obesity in elephants are warranted. Body condition scoring (BCS), an indirect means of measuring body condition based on visual evaluation of subcutaneous fat and key skeletal elements, has become an integral part of assessing body fat in a variety of species as an effective and inexpensive method for quantifying condition. The objective of this study was to develop a BCS index for female African elephants and validate the index with ultrasound measures of subcutaneous fat. The new visual elephant BCS index consists of a list of body regions and the physical criteria for assigning a numerical score (1-5 point scale) indicative of body fat, and includes example photographs of elephants representing each BCS. Ultrasound measures of subcutaneous fat showed that as the BCS increased, the amount of fat also increased ( $P < 0.05$ ). The new BCS system was tested for reliability in a trial consisting of 3 assessors, and intra- and inter-assessor reliability ranged from 75-95%, indicating excellent levels of agreement. Furthermore, BCS was correlated ( $P < 0.05$ ) with reproductive cycling status, indicating body condition does play a role in ovarian acyclicity. Because of the increased role for BCS in elephant management and potential implications of management decisions based on BCS, this validated and reliable method of body condition scoring will be a valuable tool to those caring for elephants.



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## FAUNA: DIET MANAGEMENT SOFTWARE FOR HEALTHY ANIMALS

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### Abstract

We all want to feed our animals well; within zoos and aquariums different stakeholders concentrate on different aspects of feeding. Some may be mostly concerned with the nutrient content, others with the way food is presented. The physical and behavioral health of animals may be a principal focus and sustainability, efficiency and costs are becoming increasingly significant drivers. Recording and cataloguing feeding practices and the outcome of adjustments, along with the means for systematic retrieval of said records at some later point in time, would represent a significant advance in the evaluation and dissemination of effective feeding practices. Sharing knowledge about best feeding practices could improve global management of living collections, with anecdotal descriptions replaced by observation, documentation and analysis.

Format FAUNA™ is bespoke software designed by members of the EAZA Nutrition Group and AZA's Nutrition Advisory Group working with Format International. Our vision is a zoo diet management system for all those concerned with feeding our animals well that will ensure diet information is stored in a rigorous, standardized format. The application links to animal stock numbers, allowing comparison with food purchasing/accounts. FAUNA can be used for diet formulation, permitting the exchange of true 'diet' data – the nutrients that are being offered and consumed in specific quantities, not just a list of the food ingredients involved. It also allows for easy collation of diets used for many species at a single collection thereby fulfilling criteria for legal purposes or professional accreditation.

### ACKNOWLEDGMENTS

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## SEDATION AND ANESTHESIA IN THE GREAT APES- AN OVERVIEW

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### Abstract

Great ape veterinary care often requires the use of general anesthesia to insure patient and caregiver safety. Anesthetic protocols utilized in great apes have both similarities and differences from those used in domesticated species, as well as those used in human medicine. In addition, some unique anatomical and frequently encountered disease states may pose an increased challenge to the veterinarian when working with these animals.

### Introduction

General anesthesia in great apes is usually needed to accomplish thorough physical examinations, invasive procedures, complicated diagnostics and treatments, surgeries and dental care. Anesthesia carries inherent risks and the clinician must balance the risk of anesthesia with the benefit of improved diagnostics and treatment options for these large and powerful animals. There are a number of commercially available anesthetics that may be used alone, or in combination, to induce and maintain general anesthesia in great apes. Having a complete understanding of the animals' unique anatomy and physiology, paired with the proper anesthetics, can minimize the risks of anesthesia, especially when dealing with compromised individuals.

### Discussion

#### Induction of Anesthesia

Anesthetic inductions that minimize stress (e.g. hand-injection of induction agents rather than darting, when possible) are preferred, and all apes should be held off of food for 12-24 hr prior to anesthesia (realizing that this change of routine may make patients wary of veterinarians and staff). If hand-injections are not feasible, a remote drug delivery system can be used to deliver the anesthetic induction drugs intramuscularly. Other factors to discuss prior to anesthesia include animal and personnel considerations. Animal considerations include factors such as the safety of enclosures where anesthesia will be induced, method of induction, alternate approaches for induction, ability to separate animals. Personnel considerations include the safety of induction protocol and area, prediction of dart trajectory and clearance of personnel and what steps would need to occur if there were an accidental human drug exposure or injury. Also remember to assure that there are adequate amounts of induction drug and supplemental dosing available and review prior anesthetic records. Assuring clear exits and paths to work area,

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appropriate means for transport following induction, adequate preparation for anesthetic emergencies and emergency response procedures in the event of an animal awakening/escape during transport are all also vital to complete planning for anesthetic events in great apes.

### **Hypoxemia**

A major concern during the anesthesia of great apes is the incidence of hypoxemia. Maintenance of airways, especially in animals without endotracheal intubation and during the recovery phase of anesthesia, can be difficult- particularly in mature male gorillas and orangutans due to their large size, heavy neck musculature, and the sagittal crest. Hypoventilation may occur due to excessive ventroflexion of the head and subsequent airway occlusion, or secondary to large, gas filled intestines and abdominal pressure on the lungs. Positioning the apes on their sides or propping their upper bodies up at a slight angle if in dorsal recumbency with head and neck extended can help alleviate these issues. There is a tendency for these animals to collapse their chins towards their chests when recovering and this can result in a blocked airway. To avoid this, extubation should occur as far into the recovery period as possible and the animals should be placed in lateral recumbency with their down arm extended cranially and head extended.

There are a number of possible causes of hypoxemia in an anesthetized animal. In larger apes, atelectasis (focal areas of alveolar collapse) is a concern and may result from the immobilization itself, or from pressure from the large gastrointestinal tract on the lungs. It seems that pressure from the gastrointestinal tract is most notable when animals are in dorsal recumbency, and switching to a lateral position may help to alleviate some compression of the lungs. Obesity can also result in a decrease in functional reserve capacity or airway obstruction, and obese animals must have an airway secured as soon as possible following anesthetic induction. Hypoventilation as a result of anesthetic drugs may result in hypoxemia, and the provision of supplemental oxygen, whether via endotracheal tube or nasal cannulae, is recommended, even if inhalant anesthetics are not used. Dissociative anesthetics generally maintain respiratory rate, although tidal volumes may be decreased, and the respiratory pattern may be altered (apneustic breathing has been described following ketamine). Potent opioids are the drugs that are most commonly associated with respiratory depression, and this effect may be magnified in the presence of other anesthetic agents. The inhalant anesthetics (isoflurane, sevoflurane) may also contribute to hypoventilation, however if these agents are delivered in 100% oxygen, hypoxemia is unlikely. Monitoring using a pulse oximeter is an important way that blood oxygenation can be monitored, and it is recommended that a pulse oximeter is available for all immobilizations for this reason.

### **Unique Challenges**

In addition to aspects of anesthesia that may result in hypoxemia, the anesthetist must anticipate anatomical features that may predispose to hypoxemia in the great apes. Compared to other veterinary species, the trachea of the great apes is shorter, and long

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endotracheal tubes can easily pass the carina and result in one-lung ventilation. With only one lung intubated, both hypoxemia and hypoventilation may result, as atelectasis proceeds in the unventilated lung. Following intubation, the anesthetist should auscult both hemithoraces to verify that breath sounds are heard on each side (see below for more details).

The relatively long arms of the great apes require attention during anesthesia to make sure that they are not placed in a manner that will create strain or pressure points. In larger apes, the hands may be loosely tied together to prevent one arm from falling off of the procedure table, or they may be rested on the thighs. The forearm or upper arm may also be used for non-invasive blood pressure measurement.

Hypothermia, hyperthermia, pressure necrosis and potential nerve damage to peripheral limbs, and thermal burns from heating sources are all a risk in anesthetized great apes and should be avoided. Recovery on padded or heavily bedded surfaces is preferable. Care should be taken to lubricate the eyes well with sterile lubricating ointments before recovery on bedding to try to avoid corneal abrasions.

### **Intubation**

Intubation can occur once the ape is sedate enough to have slack jaw tone. Face masks and supplemental gas anesthetic delivery to attain this level of relaxation is sometimes required and it is important to monitor the animal closely for signs of regurgitation and aspiration during this phase of induction. When using injectable drugs alone, without intubation, supplemental oxygen supplied via a facemask or intra-nasally via an oxygen line will improve oxygenation.

Intubation can generally be performed with the ape in either in lateral or dorsal recumbency with the head extended to straighten the airway, and assistance in opening of the mouth and retraction of the tongue can make intubation easier.

A long curved laryngoscope and an airway exchange catheter can make intubation easier, especially in cases where the animal is regurgitating or when the animal has excessive laryngeal tissue, as is sometimes the case in large male orangutans and gorillas. Laryngeal spasms can occur in apes so the use of topical local anesthetic sprays may aid intubation.

In general, cuffed endotracheal tubes with an inner diameter ranging from 6 mm to 11 mm are appropriate for apes, depending on individual sizes. As mentioned previously, great apes have shorter tracheas than would be expected and it is easy to intubate a main-stem bronchus if not careful. Auscultation of all lung fields using positive pressure ventilation and/or thoracic radiographs should be used to confirm tube placement. If pulse oximetry readings are low during gas anesthesia in an intubated animal, withdrawal of the endotracheal tube by a few centimeters may be enough to return the blood oxygen saturation to normal. Cuffed endotracheal tubes should be used and reinforced endotracheal tubes with extended lengths work well in the larger animals. Either a

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straight long (14-18 cm) Wisconsin laryngoscope blade or a large (#5 or larger) mackintosh laryngoscope blade may be used to aid in intubation, the latter possibly more helpful if intubation will occur with the ape in dorsal recumbency. If anesthesia is not adequate for intubation (manifested by swallowing, arm movement, or retraction of the tongue), additional injectable anesthesia (generally ketamine) can be given, or inhalant anesthetics may be provided by mask until the patient has relaxed.

The great apes are also prone to laryngospasm which can complicate endotracheal intubation and delay oxygenation and ventilation. Laryngospasm may occur following extubation and all patients should be monitored closely during recovery, with adequate anesthetic drugs available to re-induce anesthesia if necessary. When performing direct laryngoscopy, it is important to avoid direct contact of the laryngoscope blade with the epiglottis or arytenoids. Placing the tip of the blade in the vallecula, at the base of the tongue, will allow the anesthetist to push down on the tongue, which will pull the epiglottis forward for a good view of the arytenoid cartilages. Lidocaine (maximum of 2 mg/kg) may be sprayed onto the arytenoids and may decrease laryngospasm during intubation. If bronchospasm occurs, the anesthetist may appreciate a distinct difficulty in ventilating for the patient, and hypoxemia and hypoventilation may occur.

Terbutaline (0.05 mg/kg IV or IM) may be used to treat bronchoconstriction, but may cause transient tachycardia. Laryngospasm can also result from aspiration of gastric contents as a result of emesis during induction.

The anesthetist should have many sizes of endotracheal tube available (usually at least endotracheal tubes that are one size smaller, and one larger, than the anticipated size). An extremely helpful tool is a small stylette or airway exchange catheter that may be placed between the arytenoids during difficult intubations and serve as a guide for endotracheal intubation by threading the end of the stylette through the Murphy eye of the endotracheal tube. Airway exchange catheters in particular (e.g. as manufactured by Cook Medical, Bloomington IN) may be helpful in large male apes that may have large amounts of redundant oropharyngeal mucosa. Large male orangutans in particular may have elongated soft palates, and with a mobile larynx and excessive perilaryngeal mucosal tissue, can present a difficult intubation. Non-cardiogenic pulmonary edema has been reported in a male orangutan, believed to have been secondary to airway obstruction during anesthetic induction.<sup>1</sup> The airway exchange catheters also can be attached to an anesthetic circuit and can be used to provide oxygen while intubation is proceeding. Prior to intubation, and following extubation, extension of the chin and neck may be necessary to allow maintenance of a patent airway. The chin should be extended until respirations are quiet and regular, and animals should be recovered in lateral recumbency to allow saliva to drain.

Mature orangutans have large air sacs, which connect directly to the trachea. Due to the propensity of orangutans to have extensive air sac infections, securing the airway with a cuffed endotracheal tube is essential in these animals when trying to prevent aspiration of infected materials. If an air sac infection is suspected or confirmed, the airway should be secured as soon as possible following anesthetic induction (even in the cage where the

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animal was immobilized) to prevent aspiration of air sac contents into the lungs. If the laryngeal air sac contains “fluid” secretions, these animals are at increased risk for aspiration of the fluid via the ostia which connect the air sacs to the trachea and the anesthesiologist should be prepared to suction, drain the airsac, and/or maintain upright positioning until intubation. Depending on the circumstances of the infection, the orangutan may be kept in a seated position until intubation can be achieved, with the hope that backflow of air sac secretions will be minimized. Other secretions such as saliva may pool in the oropharynx, especially with anesthesia using dissociative agents. Long sponge forceps with gauze or a suction device with Yankauer tip should be available to clear the airway if necessary.

### **Vascular Access**

In general, it is convenient to obtain intravenous (IV) access during long procedures. An IV catheter will allow administration of intravenous fluids, as well as provide an easy route for administration of additional anesthetics or other medications. The saphenous veins are usually visible on the posterior aspect of the calf, and the cephalic veins are usually easy to identify and support intravenous catheterization. For monitoring of direct blood pressure, or for arterial blood gas analysis, the metatarsal artery is easily accessed on the dorsal aspect of the hind limbs. The femoral artery is also easily located in the inguinal area, but hemostasis of this artery following venipuncture may be more challenging than the metatarsal artery, which may be bandaged following catheter or needle removal.

### **Sedation and Anesthesia**

Pharmacologic agents used for the sedation and anesthesia of the great apes can roughly be divided into the general classes: pre-anesthetic anxiolytics, induction agents and anesthetics. In several instances, especially during short procedures, many of these agents may also serve dual functions.

#### **Preanesthetic Medications Anxiolytics**

Anxiolytic agents are not widely used as preanesthetic agents in primates. Benzodiazepines are GABA<sub>A</sub> agonists and provide some degree of sedation. This sedation is not enough to allow safe handling of larger primates. Therefore, the benzodiazepines are used primarily in combination with the cyclohexamines to smooth the induction of anesthesia with these drugs. Telazol, a combination of the benzodiazepine zolazepam and the cyclohexamine tiletamine, is a frequently used induction agent. Miller et al. reported that oral administration of detomidine (0.5 mg/kg) and ketamine (10mg/kg) reduced the stress of sedation and reduced the reaction of six lowland gorillas to darting with no screaming or charging.<sup>2</sup>

#### **Opioids**

Opioids are used extensively in primate anesthesia to control pain. It typically is used

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intra- and post-operatively, but has been studied for use as a pre-operative sedative. Kearns et al reported the use of oral carfentanil alone and in combination with droperidol for induction of anesthesia.<sup>3</sup> Complete anesthetic inductions were achieved in 20 minutes, however, all apes showed respiratory depression and cyanosis. In human pediatric populations, using oral opioids causes a high incidence of nausea and vomiting and this may also be a risk in the great apes when these agents are used. This has been seen in one institution using oral fentanyl, and the increased risk of aspiration is a factor to be considered.

### **Alpha<sub>2</sub> Agonists**

Alpha<sub>2</sub> Agonists produce sedation and analgesia by presynaptically inhibiting the release of noradrenergic and serotonergic pathways of the brain and spinal cord. The most potent and selective of the Alpha<sub>2</sub> Agonists is medetomidine. In the humans the D-enantiomer, dexmedetomidine, provides excellent sedation. Dexmedetomidine may be used in conjunction with local anesthetics for human craniotomies where patients need to be able to respond to commands. Its use in the great apes has had varied levels of success and is predominately used as an adjunct to ketamine for induction of anesthesia.

### **Induction Agents**

#### **Ketamine**

Ketamine is often used as both a chemical restraint agent or as an induction agent. It has a wide safety margin and has been given in doses ranging from 0.5 to 20 mg/kg. In the great apes the dose range is usually in the 5 to 15mg/kg range. Ketamine can be administered IV, IM, PO or rectally. Ketamine is an NMDA receptor agonist in the thalamoneocortical and limbic systems. It produces a dissociated anesthetic state. Ketamine can increase heart rate, cardiac output and mean arterial pressure. In addition, it can cause elevated ICP and thus should be avoided in cases with elevated ICP. A disadvantage of Ketamine is that it produces profound salivation. This can be attenuated by the anticholinergic drug glycopyrrolate.

#### **Ketamine Combinations**

A common drug combination for induction of anesthesia is the sedative Telazol and Ketamine. The combination reduces the amount of Ketamine needed for induction (Telazol 2-4mg/kg and Ketamine 1-3 mg/kg). This combination produces a reliable smooth induction, stable cardiopulmonary function and good muscle relaxation.

The combination of Ketamine and Medetomidine is more controversial. Horne, et al. compared the effects of 30-40 mcg/kg medetomidine and either ketamine (2mg/kg) or telazol (1.25 mg/kg) in chimpanzees.<sup>4</sup> The authors found that both combinations produced sedation within 2-5 minutes and light anesthesia within 3-15 minutes. The medetomidine/ketamine combination produced a more rapid emergence and return of normal behavior than the medetomidine/telazol combination. Medetomidine caused a

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transient hypertension that usually resolved in about 15 minutes. In contrast to the report by Horne, et al., other reports have shown an unreliable depth of anesthesia and spontaneous arousal with the use of medetomidine.

### **Telazol**

As noted previously Telazol is a 1:1 combination to the cyclohexamine, tiletamine and the benzodiazepine, zolazepam and has been used in many great apes.<sup>5</sup> It can be used alone or in combination with ketamine and / or medetomidine. The usual dose ranges in apes varies from 1.5-6 mg/kg IM. Telazol typically produces a smooth and rapid induction and stable respiratory and cardiac conditions under anesthesia. Prolonged anesthetic recoveries have been reported.

### **Propofol**

Propofol is an alkylphenol dissolved in a lipid solution. Propofol is a sedative hypnotic that produces sedation as a GABA agonist in the CNS. Propofol is ultra short acting due to its metabolism by the P450 enzymes throughout the body and is metabolized nearly ten times more rapidly than thiopental. Propofol is a potent vasodilator and can directly decrease cardiac contractility, which can result in hypotension. In addition, it is a potent respiratory depressant and can produce apnea. Propofol can be used for both induction of anesthesia and for maintenance of anesthesia. Interestingly, the author's experience has been that the dose of propofol needed to maintain anesthesia in the great apes is 5-10 times less than in humans.

### **Maintenance of Anesthesia**

Anesthesia can be maintained either through inhalational agents or through intravenous medications. Considerations such as duration of anesthetic event, what needs to occur during anesthetic event (painful procedures, high risk, etc), transport of animals, may all influence the choices when deciding how to maintain anesthesia. In cases where the ape needs to be transported, total intravenous anesthesia (TIVA) may be the method of choice.

### **Inhalational Anesthetics**

All the common inhalational anesthetics have been used in primates. Isoflurane has traditionally been the preferred agent. Halothane is no longer produced in the U.S. for human use. Sevoflurane has almost completely replaced halothane and isoflurane for use in humans. The potency of inhalational anesthetics is measured in minimal alveolar concentration (MAC), which is the minimal concentration required to prevent movement to noxious stimulus in 50% of subjects. Sevoflurane is less potent than isoflurane. Its MAC in great apes is around 2.0% compared to 1.2% for isoflurane. The solubility of sevoflurane is less than that of isoflurane. Therefore, the onset and recovery from sevoflurane should theoretically be faster with sevoflurane. Sevoflurane and isoflurane have minimal effects on cardiac function, but can cause dose dependent reduction in



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systemic vascular resistance (SVR).

### **Propofol**

As noted earlier, propofol can be used for maintenance of anesthesia in primates. In humans, the usual dose for maintenance of anesthesia is 150-250 mcg/kg/min, whereas in the authors experience anesthesia can be maintained in the great apes with doses as low as 25 mcg/kg/min. It has been shown that propofol alone has no significant analgesic properties and therefore should be used in conjunction other drugs to reduce the physiologic response to pain.

### **Opioids**

As stated earlier opioids are often used with either inhalational or intravenous anesthetics to reduce pain associated with surgical stimulation. Opioids have minimal effects on cardiac contractility even at very high concentrations. Use of opioids lowers the MAC requirement for inhalational anesthetics.

### **Morphine**

Morphine is one of the most commonly used analgesics. It produces analgesia and sedation. Studies have revealed a myriad of opiate receptors in the central nervous system. Specific receptors are implicated in the various effects and side effects of morphine.

### **Fentanyl**

Fentanyl is approximately 100 times more potent than morphine. It is much more lipid soluble and crosses the blood brain barrier. It can be given in low doses (1-2 mcg/kg) for analgesia as an adjunct to general anesthesia or at high doses (50-150 mcg/kg) as a sole anesthetic. Even at large doses fentanyl has minimal effects on cardiac output.

### **Novel Drug Use in the Great Apes**

The author's experiences at the Fort Worth Zoo with anesthetizing the great apes have shown the apes to be extremely sensitive to anesthetic agents. They seem to be particularly prone to hypotension both with inhalational and intravenous drugs. As a response to this it has become this author's practice to routinely start an infusion Dopamine. Dopamine stimulates dopaminergic, alpha and beta adrenergic receptors. Doses of 2-10 mcg/kg/min have an inotropic effect, increasing cardiac contractility. This can significant improve blood pressures during surgery.

In addition to using inotropic agents, the author also routinely uses the non-depolarizing muscle relaxant cis-atracurium. Cis-atracurium competitively inhibits acetylcholine at the motor end-plate in muscle. It is the relaxant of choice for subjects with hepatic or renal dysfunction because it is eliminated by hydrolysis and Hoffman degradation. Using

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cis-atracurium allows the apes to be maintained at a lower depth of anesthesia without them moving. This is particularly helpful in apes who are hypovolemic as it reduces the cardiac effects produced by high concentrations of anesthetics.

Finally, at the Fort Worth Zoo the author has recently employed the use of the ultra-short acting opioid remifentanyl. Remifentanyl has a rapid onset and a half-life of 3-6 minutes. Because of the ultra-short half-life remifentanyl is administered as a continuous infusion. It has the same potency as fentanyl and is easily titrated for effect.

### **Cardiac Disease**

There is a significant amount of active research into the incidence and causes of cardiac disease in the great apes. What is known is that affected animals develop systolic cardiac dysfunction that can progress to congestive heart failure, and may cause clinical signs such as exercise intolerance, lethargy, and tachypnea. In apes with suspected cardiac disease that require anesthesia, medetomidine decreases cardiac output by increasing cardiac afterload through intense peripheral vasoconstriction. Even though dissociative agents can cause tachycardia and mild decreases in cardiac output, they are probably the safest drugs to use as a basis for an anesthetic protocol for apes with cardiac disease, from the perspective of both the handlers and the ape. If a cardiac exam will be performed during the anesthetic, an anesthetic protocol should be used that will allow comparison to previous exams. In general, telazol (3-4 mg/kg IM) or ketamine (6-10 mg/kg) should allow safe initial immobilization, and apes can be subsequently maintained on an inhalant anesthetic protocol for the remainder of the exam, although this may not always be necessary (and will also affect the echocardiographic exam). Inhalant anesthetics can cause hypotension due to vasodilation, but intravenous fluids should be used sparingly if at all in apes with cardiac disease to prevent volume overload.

### **Dental Procedures**

Much as in other animals, anesthesia for dental prophylaxis and surgery can be maintained using inhalant anesthesia, but may be supplemented by dental blocks which will result in a smoother, more reliable anesthesia and provide some analgesia following the procedure. In general, lidocaine (a total of 2-4 mg/kg for all blocks) is preferred to bupivacaine both to limit possible toxicity, and because it is relative short acting, preventing self-trauma from prolonged loss of sensation.

### **Ophthalmologic Surgery**

Ophthalmologic surgery may require the use of neuromuscular blocking agents to maintain a central eye position to allow intraocular surgery. The use of all neuromuscular blocking agents must be monitored using a nerve stimulator, and this therapy may be reversed at the end of the procedure if the effects of the drug are still present. The author prefers intermittent boluses of cis-atracurium to maintain eye position due to a short duration of action and rapid breakdown by Hoffman degradation. Frequently, smaller doses can be used than those that cause full neuromuscular blockade,

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and the ability of the patient to ventilate adequately must be evaluated prior to recovery from anesthesia.

## **Conclusion**

Techniques and drugs used to sedate and anesthetize the great apes are many. This allows the anesthetist to tailor an anesthetic to fit the needs of the patient being anesthetized. With the advent of newer, safer drugs, even elderly apes can now be safely anesthetized.

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## REGULATION OF IRON BALANCE IN RHINOCEROSSES

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### Abstract

In captivity but not in the wild, black and Sumatran rhinos are susceptible to iron overload disorder while white and Indian rhinos are resistant.<sup>3,6</sup> The difference in susceptibility aligns with wild forage diets, either browser or grazer, and may reflect evolutionary adaption to the low iron browser diet in the wild.<sup>4</sup> Iron overload likely contributes to increased morbidity and premature death of affected rhinos in captivity. To date, one black rhino single nucleotide polymorphism (SNP), HFE S88T has been reported, the functional consequences of which remains undetermined.<sup>1</sup> Our goal is to identify the iron regulatory differences underlying the observed species difference in susceptibility to iron overload.

The sequences of African white and black liver and spleen mRNAs were assembled using Trinity RNA-Seq software.<sup>2</sup> The SIFT computer algorithm was used to compare rhino with human sequences and identify possible disease causing mutations.<sup>5</sup> Candidate SNPs were independently validated by genomic sequencing in four rhino species. Candidate mutations that may be associated with primary iron disorders or hemolytic anemias known to occur in black rhinos were identified in the following genes:

SLC28a2: adenosine transporter may contribute to low erythrocyte ATP levels

STEAP4: metalloreductase – may contribute to metal homeostasis by reducing vacuolar iron and copper, thereby allowing their transfer across vacuolar membranes

EPB41: mutations are associated with hereditary hemolytic anemia

The functional consequences of these candidate mutations are being determined.

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## CHARACTERIZATION OF IMMUNE RESPONSES IN TUBERCULOSIS POSITIVE ASIAN ELEPHANTS (*Elephas maximus*)

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### Abstract

Tuberculosis is an important health concern for Asian elephant (*Elephas maximus*) populations worldwide. Most infections are due to *Mycobacterium tuberculosis*, though mechanisms underlying tuberculosis susceptibility are unknown.<sup>3,4</sup> In humans and other species, tuberculosis susceptibility is dependent on the host immune response following infection. Disturbances in the balance between host cell-mediated and humoral immune responses are central to disease pathogenesis.<sup>5,7</sup> Recent studies have begun to describe contributions of elephant immune responses to tuberculosis. Real time RT-PCR measurement of cell-mediated (T<sub>H</sub>1) and humoral (T<sub>H</sub>2) cytokine levels in baseline peripheral blood samples from tuberculosis positive and negative elephants showed trends towards higher expression of some T<sub>H</sub>1 cytokines in positive elephants, though findings were not significant.<sup>1,2</sup> Subsequent related studies were conducted to examine functional responses of peripheral blood mononuclear cells from 8 tuberculosis positive and 8 negative elephants following mycobacterial antigen stimulation. Results showed that samples from positive elephants exhibited enhanced proliferation and greater production of T<sub>H</sub>1 cytokines, including tumor necrosis factor- $\alpha$ , interleukin-12, and interferon- $\gamma$ , than tuberculosis negative elephants. Additionally, examination of elephant pulmonary tuberculosis lesions (n=14; 9 affected 5 controls) using light microscopy, immunohistochemistry, and *in situ* hybridization illustrated distinct patterns of inflammation with morphology and local cytokine expression reminiscent of both human latent and active tuberculosis lesions.<sup>6</sup> Altogether, results of these investigations have indicated the elephant immune system does play a role in tuberculosis pathogenesis. Ideally, the foundation of knowledge established by these findings will serve to promote continued investigation of elephant tuberculosis immunopathogenesis for the long-term conservation of this endangered species.

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**PHARMACOKINETICS OF A SINGLE ORAL OR RECTAL DOSE OF CONCURRENTLY ADMINISTERED ISONIAZID, RIFAMPIN, PYRAZINAMIDE AND ETHAMBUTOL IN ASIAN ELEPHANTS (*Elephas maximus*)**

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**Abstract**

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a concern in captive Asian elephants (*Elephas maximus*). Treatment of TB in elephants utilizes multidrug protocols including isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and/or ethambutol (EMB). In this study, a single combined dose of INH<sup>a</sup> 5 mg/kg, RIF<sup>b</sup> 10 mg/kg, PZA<sup>c</sup> 30 mg/kg and EMB<sup>d</sup> 30 mg/kg was administered orally to six Asian elephants, and a single combined dose of INH, RIF, and PZA was administered rectally to five elephants. Blood samples were collected at 14 time points. PZA and EMB concentrations were determined using validated gas chromatography assays. INH and RIF concentrations were determined using validated high performance liquid chromatography assays. Rectal INH produced an early T<sub>max</sub>. Oral INH produced comparatively lower C<sub>max</sub> but higher AUC values. Oral RIF and oral EMB were well-absorbed, with RIF C<sub>max</sub> values approaching the human normal range and EMB C<sub>max</sub> values at the upper end of the range. Rectal RIF was not absorbed. Rectal PZA produced faster but lower median C<sub>max</sub> values, at the low end of the human normal range. Oral PZA produced comparatively higher C<sub>max</sub> and higher AUC values. Results of this study indicate that currently recommended monitoring times for rectal INH and oral RIF do not provide an accurate assessment of PK parameters for these drugs.<sup>1</sup> This study demonstrates notable individual variability indicating that dosing of these medications requires individual elephant monitoring and adjustment. This study provides an update to previous pharmacokinetic studies of these medications in Asian elephants.

**Products Mentioned in the Text:** <sup>a</sup>Isonicotinic Acid Hydrazide, Spectrum Chemical Mfg. Corp., 769 Jersey Ave., New Brunswick, NJ 08901-3605 USA. <sup>b</sup>Rifamycin AMP, Spectrum Chemical Mfg. Corp., 769 Jersey Ave., New Brunswick, NJ 08901-3605 USA. <sup>c</sup>Pyrazinamide, Grove Pharmacy, 3050 S. National Ave, Ste 109, Springfield, MO 65804 USA. <sup>d</sup>Ethambutol hydrochloride tablets, USP, West-Ward Pharmaceutical Corp., 401 Industrial Way West, Eatontown, NJ 07724 USA.

**ACKNOWLEDGMENTS**

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## **RADIOGRAPHIC EVALUATION OF CARPAL AND TARSAL JOINTS ASSOCIATED WITH OSTEOARTHRITIS IN A GROUP OF SEVEN AFRICAN ELEPHANTS (*Loxodonta africana*): SUGGESTIONS FOR A CHANGE IN STRATEGY WHEN EVALUATING ELEPHANT HEALTH**

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### **Abstract**

The AZA standards for elephant management require institutions holding elephants to obtain baseline sets of radiographs of the feet for evaluating potential infections, fractures or metabolic conditions.<sup>1,4</sup>

In January 2012 a 51-yr-old female African elephant (*Loxodonta africana*) was euthanized due to severe progressive degenerative osteoarthritis. The elephant was maintained on chondroprotectants and analgesics. The carpal and tarsal joints from all four limbs were radiographed then imaged with Computed tomography (CT). OsiriX<sup>®</sup> software was utilized to develop three-dimensional images of the carpal and tarsal joints. Gross evaluation was then compared to radiographic and CT images. In all images, severe degenerative joint disease (DJD), including ankylosis, joint space collapse, enthesiophytes, and osteophytes were observed. Extensive subchondral bone exposure was observed in most articulating surfaces.

Radiographic techniques were developed to image the carpal and tarsal joints in a group of six female African elephants ranging in age from 28-45 yr. All elephants demonstrated varying degrees of DJD without significant changes to the digits. Some lesions were comparable to findings observed in the aged female. Only three of the six elephants demonstrated clinical signs comparable with osteoarthritis and all elephants were placed on chondroprotectants; three individuals were placed on analgesics at previously documented pharmacokinetically studied doses.<sup>2</sup> Banked sera was evaluated for *Mycoplasma sp.* titers which have been associated with DJD in elephants;<sup>3</sup> all were negative.

It is strongly suggested to consider inclusion of carpal and tarsal radiographs when evaluating the appendicular health of elephants. Further evaluation of appendicular health in these elephants is ongoing.

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## SHOE APPLICATION AND ITS EFFECTS ON RESOLUTION OF HOOF PROBLEMS IN A GIRAFFE (*Giraffa camelopardalis*)

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### Abstract

The need for hoof care and maintenance in captive giraffe is well-known.<sup>1</sup> A variety of farriery techniques are used in domestic animals<sup>3,4</sup> and have the potential for application in exotics. While mention is made of using blocks in giraffe,<sup>2</sup> little mention is made of the use of shoes or more aggressive interventions.

A 2-yr-old Rothschild/reticulated giraffe hybrid (*Giraffa camelopardalis rothschildi/reticulata*) was immobilized for hoof trim of overgrown hind feet. Over the course of the next 2 mo, her hind feet continued to overgrow and the distal extent of the lateral toe of the right hind foot became elevated off the ground. This animal was immobilized for repeat hoof trim. Radiographs showed rotation of the third phalanx (P3) in the lateral toe of the right hind foot with bony lysis medially and a lucency at the distal extent of P3. Ultrasound showed fiber disruption in the deep digital flexor tendon with a probable tear on the lateral aspect. The hooves were trimmed but it was not possible to fully correct the lateral claw on the right hind foot.

Just over 11 weeks later this animal was immobilized for additional trimming. Radiographs showed rotation of P3 in both hind feet and the right front foot, with the right hind being the most affected. After trimming all four feet, an aluminum shoe was glued in place on the right hind foot and a steel band was placed across both claws, attached with screws, and sealed with a glue layer to aid in support of the foot and encourage equal hoof growth. Despite concern for possible complications, this animal showed only one mild, isolated period of lameness that resolved without intervention.

Immobilization for hoof trim almost 8 weeks later showed radiographic improvement of all feet. The aluminum shoe and steel band were removed and wooden shoes were placed on both hind feet. The shoe on the right hind foot was wedged to relieve DDF tension, while the shoe for the left hind foot was level. Both shoes were attached to the feet by screws in the wood that gripped but did not penetrate the edges of the hoof wall. Cast material (3M<sup>TM</sup> Scotchcast<sup>TM</sup> Plus casting tape, 3M, St. Paul, MN 55144, USA) coated with an acrylic glue (Equi-Thane Superfast, Vettec Hoof Care, Oxnard, CA 93033, USA) was wrapped around the hoof wall and shoes to help secure the wood in place. The left shoe fell off 11 days later, the right stayed on for a total of 3 weeks. At the end of this time period, all digits were in contact with the ground and the hooves on the hind feet were more normally shaped.

Nine months later when this animal was immobilized for hoof trimming, radiographs

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showed slight rotation in P3 but it was possible to trim the hooves into a normal shape. The holes, originally 2cm from the coronary band for metal band application, were completely trimmed away in this immobilization.

In conclusion, more advanced farriery techniques can be used successfully in the case of captive wildlife despite lack of behavioral training and in the absence of a controlled environment.

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## **METASTATIC MINERALIZATION IN 16 CAPTIVE TWO-TOED SLOTHS (*Choloepus* spp.)**

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### **Abstract**

Metastatic mineralization was diagnosed in 16 captive two-toed sloths ranging in age from 2 mo to 26 yr old (mean 11.9 yr) and housed at facilities throughout the United States. Gross and histological lesions were characterized as well as concurrent disease processes. Gross mineralization was detectable at necropsy in 5 of 16 sloths, and was most prominent in the great vessels and particularly the aorta. Affected vessels were diffusely dilated, firm and brittle with tan plaques that partially occluded the lumen. Histologically, vascular mineralization was detected in 9 of 16 sloths and varied from moderate to severe including osseous metaplasia, smooth muscle hyperplasia and degeneration consistent with arteriosclerosis. Mineralization was detected throughout viscera, most commonly in the stomach mucosa (13 of 16 sloths), kidneys (12 of 16 sloths) and lungs (6 of 15 sloths), and was associated with mononuclear inflammation and local tissue destruction. Fourteen of 16 sloths had significant renal disease including 2 which were treated for clinical renal failure prior to death. Nine of 12 had mild to severe cardiomyopathy, possibly secondary to severe vascular compromise. Metastatic mineralization is caused by prolonged hypercalcemia. Common causes include high calcium diets, dietary or metabolic calcium: phosphorus imbalance, hypervitaminosis D and primary or secondary hyperparathyroidism. Severe vascular mineralization occurred in sloths in the absence of renal disease.

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## HEALTH SCREENING OF RED-NECKED WALLABIES (*Macropus rufogriseus*) USING COMPUTED TOMOGRAPHY (CT) IN ADDITION TO CONVENTIONAL DIAGNOSTIC METHODS

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### Abstract

Four adult red-necked wallabies (*Macropus rufogriseus*) within a collection of 24 adults in the Blank Park Zoo were identified with granulomas or abscesses containing acid-fast organisms in 2012. The animals were diagnosed late in the course of disease,<sup>1</sup> with lesions greater than 6 cm detected by digital radiography or palpation. *Mycobacterium avium*, subsp. *hominissuis* and *Mycobacterium intracellulare* were identified by mycobacterial culture and genotyping by the National Veterinary Services Laboratory. *M. avium* subsp. *hominissuis* and *M. intracellulare* are ubiquitous in the environment. *M. avium* subsp. *hominissuis* is commonly isolated from non-tuberculous infections in humans and swine.<sup>2</sup> Antemortem diagnostic tools for the detection of mycobacterial infections in macropods are largely unavailable. Intradermal tuberculin testing produces inconsistent results and is therefore of limited diagnostic value.<sup>3</sup> Radiography is useful for localizing the site of infection in cases where osteomyelitis or calcified lymph nodes are present.<sup>4</sup>

In 2013 a health screening protocol for the red-necked wallaby population within the zoo was created which includes computed tomography (CT), in addition to physical exam, serology, hematology, and digital radiography to identify animals that may have subclinical illness. The results of this study have identified the normal and abnormal CT anatomy of wallabies, assisted in the identification and treatment of health conditions, and assessed the prevalence of wallaby retrovirus<sup>6,7</sup> in the evaluated animals. Skull and body CT scans identified abnormalities (dental, cardiac) which modified management of these individual animals, but no lesions suggesting subclinical mycobacteriosis have been observed.

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## HEPATOCELLULAR NEOPLASIA IN SLENDER-TAILED MEERKAT (*Suricata suricatta hahni*): A RETROSPECTIVE CASE SERIES

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### Abstract

From 2000 to 2012, tissues from 178 meerkats were submitted from zoological collections throughout the continental U.S.A. for histopathologic evaluation. Of these cases, 8 (4.5%) were diagnosed with hepatocellular carcinomas, 2 (1.1%) with hepatocellular adenomas, and 2 (1.1%) with nodular hepatocellular hyperplasia. One case had been previously diagnosed as a hepatocellular adenoma, and was subsequently diagnosed as hepatocellular carcinoma on excisional biopsy 1 yr later. In another case, both a hepatocellular carcinoma and adenoma were identified on distinct liver lobes of the same patient. All individuals were adults ranging in age from 8 – 13 yr, with a median age of 11 yr. The predominant clinical signs were lethargy, weight loss and palpable abdominal masses. Histologically, hyperplastic lesions exhibited a well demarcated, nodular appearance, with mild anisokaryosis and anisocytosis, but no identified mitotic figures. Hepatocellular carcinomas were well differentiated, the majority of which were characterized either by a trabecular pattern of neoplastic hepatoid cells or cords and sheets of hepatoid cells, with mild to moderate anisokaryosis, large nucleoli, and no to occasional mitotic figures. Metastasis to the local mesentery was observed in one case, and to the lungs and adjacent thoracic lymph node in another. Based on this report, hepatocellular tumors, in particular, hepatocellular carcinomas appear to occur commonly in the captive meerkat population. These findings may be consistent with low genetic diversity within the captive population or underlying infectious etiology. Further investigation into a possible viral etiology of hepatocellular carcinomas in captive meerkats is being pursued at this time.

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## NECROTIC ENTEROCOLITIS AND TYPHLITIS IN LEMURS

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### Abstract

A syndrome of necrotic enterocolitis and typhlitis has been documented in 50 free-ranging ring-tailed lemurs (*Lemur catta*) on St. Catherine's Island (SCI) over the last 15 yr, and in 14 semi free-ranging lemurs (*Lemur catta*, *Varecia rubra*, and *Eulemur mongoz*) at the Lemur Conservation Foundation over a 7 yr period. A workshop on this syndrome was hosted by SCI Foundation in November 2012 involving all of the authors. The following conclusions were reached: Younger lemurs 4-15 mo of age are more commonly affected although not exclusively. The case fatality rate is approximately 80-90%. The following case definition for this syndrome was developed: 1) Lemurs present with acute onset abdominal pain, lethargy, anorexia, +/- diarrhea or no defecation with hypoalbumemia. 2) Necropsy findings include mucosal to transmural necrosis of the cecum, small intestine and/or large intestine.

The histopathology seen with this syndrome resembles human appendicitis, and suggests that ischemia may be the underlying pathophysiologic mechanism. Although numerous bacteria and occasional fungal organisms are observed on histopathology and recovered by culture, they appear to be the result of opportunistic invasion rather than a primary cause. No specific pathogens have been consistently recovered, although findings have included an Adenovirus-like organism, a Parvo-like virus, a  $\beta$ -Coronavirus, *Lawsonia* spp, or *Salmonella* spp. Workshop participants concluded that 1) localized ischemia and/or hypomotility may be predisposing lemurs to develop this syndrome, 2) an infectious cause is unlikely, and 3) ingestion of a plant or toxin is a possible primary cause but further exploration is needed.

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## THE VIRTUAL APE PROJECT: MORE THAN A SOURCE OF ANATOMY FOR VETERINARIANS, ANTHROPOLOGISTS AND PRIMATOLOGISTS

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### Abstract

The concept of the “frozen zoo” or the preservation of zoo animals post mortem as museum specimens is widely recognized and its importance for science, conservation and teaching unquestioned. Here we present a new concept of collecting and preserving data of valuable species, the virtual ape project at the University of Zurich, Switzerland. The aim is to save three-dimensional digital whole body images of dead large primates, ideally before being subjected to post mortem examination. The project is coordinated by the Anthropological Institute and involves local zoo veterinarians, forensic medicine and diagnostic imaging specialists. Data is collected by combined computed tomographic and magnetic resonance imaging techniques, with the animal in an artifact-free body bag, to keep the biohazard risks to a minimum. For data acquisition standard protocols for humans such as for virtual dissection (e.g. Virtopsy) are applied. To date five Sumatran orangutans (*Pongo pygmaeus abelii*), five Western Lowland gorillas (*Gorilla g. gorilla*), and 11 Chimpanzees (*Pan troglodytes*) have been evaluated. Of these 10 were males, 11 females, 15 juveniles, six adults; four were fresh specimens and 17 were formalin-fixed or frozen specimens. The data is used in a multitude of areas, including comparative anatomy and evolution of primates.<sup>1</sup> The collected information increases our knowledge of large primate anatomy, an asset for zoological medicine clinicians. It can also improve the diagnostic quality of the post mortem examination. In the future the establishment of a network of digital data would be desirable, which the University of Zurich would volunteer to host.

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## THE USAID EMERGING PANDEMIC THREATS PREDICT PROJECT: DETECTING AND DISCOVERING ZOO NOTIC VIRAL PATHOGENS IN WILDLIFE TO MITIGATE EMERGING INFECTIOUS DISEASE PANDEMICS IN PEOPLE

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### Abstract

Most emerging infectious diseases (EIDs) are zoonotic, and a high proportion originates in wildlife in the developing world. Population growth and environmental change bring people into contact with wildlife in unprecedented ways and more frequently, yet impoverished countries lack the resources and infrastructure necessary to detect and respond to EIDs in a timely, effective manner. The USAID Emerging Pandemic Threats PREDICT project, led by the UC Davis One Health Institute with EcoHealth Alliance, Metabiota, Inc., Wildlife Conservation Society, and Smithsonian Institution, is advancing capacity for EID detection and control by conducting targeted pathogen surveillance in high-risk wildlife at human-wildlife interfaces in EID hotspots. This global approach to surveying wildlife for zoonotic pathogens has never before been attempted on this scale. PREDICT is establishing infrastructure (laboratories, protocols, equipment), training hundreds of health professionals, and promoting the integration of human, domestic animal and wildlife health across governmental sectors in 20 countries. This is enabling the detection of known and new viral pathogens that could impact human health, but also increasing capacity for wildlife health diagnostics and protection in these biodiverse regions. Since 2010, more than 35,000 wild animals have been humanely sampled resulting in the discovery of more than 200 novel viruses. PREDICT has played a key role in investigating the cause of human and wildlife disease outbreaks, and is creating mathematical models as tools for predicting zoonotic pathogen emergence. Looking ahead, all partners are aiming for full operationalization and sustainability of One Health, ultimately benefiting people, their domestic animals, and wildlife.

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## BUILDING AN EBOLA ANTIBODY SURVEILLANCE STUDY OF GREAT APES IN THE REPUBLIC OF CONGO

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### Abstract

Mathematical modeling suggests that gorilla numbers in Lossi Sanctuary dropped over 90 percent between 2002 and 2003 due to the Ebola virus (EBOV) and hemorrhagic fever (EHF).<sup>1</sup> Despite its high mortality rate during this outbreak, surprisingly little is known about exposure and immune responses to EBOV in western gorilla (*Gorilla gorilla*) and chimpanzee (*Pan troglodytes*) populations.<sup>2</sup> Using noninvasive fecal sampling and analysis our team is nearing completion of a study to measure prevalence of EBOV exposure and understand the relationship between prior EBOV exposure and population density in these great ape populations. We describe the path from research inception and diagnostic development to field deployment. In particular we show how mathematical modeling, ecology, and epidemiology were used to inform research design and support surveillance.<sup>3</sup> Our experience and sampling success highlight the importance of model-guided research design and surveillance.

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**DIAGNOSIS, TREATMENT, MONITORING AND RELEASE IMPLICATIONS  
OF CHLAMYDOPHILA ASSOCIATED ENCEPHALITIS IN HOUSTON TOADS  
(*Anaxyrus [Bufo] houstonensis*)**

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**Abstract**

The Houston Zoo participates in a local conservation program for the endangered Houston toad (*Anaxyrus [Bufo] houstonensis*) through hormone assisted captive propagation, maintenance of a captive assurance colony, and collection of eggs from the wild for head starting/release purposes. In spring 2012, 42% (22/52) of young toads from an indoor, captive bred group presented either neurologic signs such as head pressing and abnormal gait or acute death. Histologically, these toads had marked encephalomyelitis associated with an intracellular organism morphologically consistent with *Chlamydophila* sp., which was confirmed via electron microscopy. Preliminary molecular evaluation suggested that Chlamydial isolates from nervous tissue of affected toads were most consistent with *C. pneumoniae*. A polymerase chain reaction (PCR)-based assay was used to determine the most clinically relevant samples for screening toads for chlamydial nucleic acid. The clinical and histologic changes, and treatment and pre-release screening protocols we developed for screening toads for this chlamydial organism will be presented. Further inquiry will include identification of the strain of *C. pneumoniae* and an epidemiologic investigation to identify the possible source of infection.

**ACKNOWLEDGMENTS**

The authors would like to thank the staff at the University of Georgia Infectious Diseases Laboratory for their continued diligence in processing toad samples. Thanks also to Houston Zoo, Inc. staff: registered veterinary technicians Karina Vercic, Rynne Henigar and Eva Smoot, and administrative assistants Lindsey Parker and Kathryn Lippman for assistance with sample collection, organization, and shipping.

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## **PRESENCE OF *Paragonimus westermani* IN FREE RANGING LEOPARDS (*Panthera pardus kotiya*) IN SRI LANKA**

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### **Abstract**

Lung flukes, of the genus *Paragonimus*, are a pathogenic agent of human paragonimiasis, one of the medically important food-borne trematoda diseases in tropical, subtropical, and some temperate countries. This infection is often confused diagnostically with tuberculosis. The prevalence of human paragonimiasis is currently estimated at 20 million. To date, four species of *Paragonimus* have been reported in Sri Lanka and are identified, based on the morphological features of adult flukes, such as the branching pattern of ovaries and the arrangement of cuticular spines. Humans and other mammals become infected by eating raw or undercooked crayfish or freshwater crabs that harbor the parasites. Paragonimiasis most frequently involves the lungs, but can affect other organs, including the brain and skin. In this investigation, we examined the prevalence of *Paragonimus* worms in the lungs of free ranging leopards (*Panthera pardus kotiya*) in Sri Lanka. The eight animals examined originated from different areas of the country. The causes of death of these animals included health complications associated with noose trapping (n = 2), gunshot (n = 1), vehicle collision (n = 1), and intraspecies trauma (n = 4). Worms were identified in the lungs and determined to be *Paragonimus westermani*. Infected animals originated from the Nawalapitiya and Yala National Park indicating the worm has widely distributed. This is the first report of *Paragonimus* among free ranging leopards of Sri Lanka. Further studies are currently underway to determine the prevalence and mode of transmission in leopards, and its zoonotic importance in Sri Lanka.

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## DETECTION OF EEHV IN TRUNK WASH SECRETIONS FROM CAPTIVE AFRICAN ELEPHANTS (*Loxodonta africana*)

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### Abstract

Elephant endotheliotropic herpesvirus (EEHV) can cause fatal disease in endangered Asian elephants (*Elephas maximus*) though latent infection and shedding appears common in normal animals in captivity and in range countries. EEHV's have been noted in captive African elephants (*Loxodonta africana*) in necropsy samples and in wild African elephants recently surveyed for endogenous viruses via trunk nodule biopsy. The prevalence and type of latent EEHV infection in healthy captive African elephants is relatively unknown. To address this issue, trunk wash samples were collected from two African elephant herds. DNA extraction and qPCR was performed for EEHVs 1, 2, 3/4, 5, and 6. Preliminary results indicate that in one herd, 4 of 6 elephants were positive for EEHV 3/4. One elephant in this herd was positive for EEHV 1 and had previously been housed with Asian elephants. Each animal in the second herd was only positive for EEHV 6, although the sample timeline was very limited. Further collection for DNA sequencing will differentiate between EEHV 3 and 4, and characterize it along with the EEHV 1 and EEHV 6 shed by the other elephants.



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## CURRENT UNDERSTANDING AND HEALTH IMPLICATIONS OF KOALA RETROVIRUS IN ZOOLOGIC COLLECTIONS

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### Abstract

Koala retrovirus (KoRV) is an RNA virus that incorporates into the host genome and has the potential to cause malignancies and immunosuppression.<sup>4</sup> Viral particles morphologically consistent with a retrovirus were first documented in the bone marrow of a leukemic adult female koala in 1988 and subsequent nucleotide sequencing confirmed the presence of KoRV in koalas (*Phascolarctos cinereus*).<sup>1</sup> KoRV is unique in that it is capable of both endogenous transmission, by incorporating into the germ line, and exogenous transmission, through horizontal spread.<sup>2,3</sup> A newly-emergent variant of KoRV (KoRVB) was recently detected in a US zoo and it may be more pathogenic than the originally isolated variant (KoRVA).<sup>5</sup>

The discovery of this variant prompted the organization of an international workshop at the San Diego Zoo (April 17-18, 2013) to facilitate discussion between basic scientists and clinicians regarding KoRV and implications for koala health and management. Through discussions on evolution, genomics, prevalence (zoo-based and free-ranging), epidemiology, disease expression, anti-retroviral drugs, vaccine development, and the zoonotic potential of KoRV, we were able to identify potential health, management, and future research strategies to reduce the impact of KoRV on koalas. The outcomes of this workshop greatly improve our understanding of KoRV, an infectious disease of emerging importance in zoo-based and free-ranging koalas.

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## **PREDISPOSITION TO PROLIFERATIVE HEPATOCELLULAR LESIONS IN THE FENNEC FOX (*Vulpes zerda*)**

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### **Abstract**

Hepatocellular tumors are overrepresented in humans, woodchucks (*Marmot monax*), Richardson's ground squirrels (*Uroditellus richardsonii*), black tailed prairie dogs (*Cynomys ludovicianus*), and meerkats (*Suricata suricatta*). The tumors are associated with hepadnavirus infection in humans, woodchucks and ground squirrels, and the woodchuck is an animal model for human hepatitis B. A review of the archives at Northwest ZooPath identified 54 Fennec fox (FF) submissions, of which 15 (28%) (9.4.2) had 1 or more types of proliferative hepatocellular lesions, including hyperplasia (9), adenoma (8) and carcinoma (8). Of the remaining 147 archived foxes representing 12 other species, only a single hepatocellular adenoma was identified. Average age at diagnosis was 10.3 yrs (range = 8-13 yrs). One adenoma and one carcinoma had trabecular and pelioid patterns, and all others were trabecular. Metastasis (omentum and lymph node) was seen in one case. Zoo listserve solicitation identified several more cases. Prospective and retrospective serologic and metagenomic studies are being conducted on serum from affected animals and unaffected cohorts, and on frozen tissues or paraffin blocks, for exposure to potentially oncogenic infectious agents.

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## AN UPDATE ON RANAVIRAL DISEASE IN AMPHIBIANS AND REPTILES

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### Abstract

Iridoviruses in the genus *Ranavirus* are large double-stranded DNA viruses that infect amphibians, fish and reptiles. Although the type species Frog Virus 3 was described in the late 1960s, only in the last 15 yr have ranaviruses been recognized as a significant cause of mass mortality events of wild amphibians and more recently, chelonians. Although *Ranavirus* infections are not as clearly linked to population declines as another emerging amphibian pathogen, the chytrid fungus *Batrachochytrium dendrobatidis*, there are ongoing studies and concern that small threatened populations may be especially vulnerable to repeated outbreaks of ranaviral disease. There are six officially recognized species of *Ranavirus* and each species likely has several distinct strains that can vary in virulence and host range. Most amphibian ranaviruses fall into 1 of 3 species groups: the Frog Virus 3-like (FV3) viruses, the *Ambystoma tigrinum*-like (ATV) viruses and the Bohle iridovirus-like (BIV) viruses. There are a few reports of *Ranavirus* infection in captive amphibians; however, the prevalence and significance to zoo collections are unknown because of an overlap of clinical and pathologic findings with other infectious diseases and a lack of well-validated antemortem tests. Although *Ranavirus* infections are relatively easily diagnosed by molecular diagnostic testing (e.g. PCR) in sick animals with high viral loads, the ability to reliably detect subclinical infections using tissue samples, oropharyngeal swabs or cloacal swabs is debatable. Complicating disease risk assessments for amphibian translocation and reintroduction programs is the detection of subclinical infections during disease surveillance efforts without an efficient method to distinguish between different ranaviral strains. Treatment of sick animals is largely supportive, but early studies suggest that environmental temperature elevation may be helpful in some cases.

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## **PREPUTIAL DIVERTICULITIS AND RUPTURE IN A BABIRUSA (*Babyrusa celebensis*) AT THE WILDLIFE CONSERVATION SOCIETY'S BRONX ZOO**

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### **Abstract**

Urogenital tract disorders of domestic suids may occur in exotic species, although no case reports exist in the literature. The preputial diverticulum, unique to swine, may present medical and reproductive complications. Two species at the Bronx Zoo (BZ) have experienced preputial swelling. Intermittent self-resolving preputial swelling in one geriatric and multiple juvenile red river hogs (*Potamochoerus porcus*) was associated with urine retention, likely a normal species variation. Distal preputial ulceration in a 5-yr-old male babirusa (*Babyrusa celebensis*) was noted on preshipment examination prior to arrival at BZ. After 8 mo at BZ, an inability to extrude the penis for breeding and intermittent preputial swelling were observed. The animal was immobilized for diagnostics, including electroejaculation to ascertain reproductive function. Debridement of the preputial diverticulum removed caseous debris; inner preputial mucosal biopsy identified posthitis with chronic irritation secondary to preputial diverticulitis. Local irritation responded to topical antimicrobial spray (Genta-Spray, gentamicin sulfate with betamethasone valerate; Butler Schein, Norcross, GA 30093 USA, topically s.i.d. to b.i.d. for 14 dy) and reproductive rest. Preputial swelling recurred in 5 mo, and immobilization 48 hr after onset revealed abscessation and necrosis of the prepuce with preputial diverticulum rupture. Aggressive surgical debridement and subsequent preputial reconstruction using oral mucosal autograft were undertaken, but graft failure occurred after 2 wk. Multiple immobilizations for wound care and stent placement successfully preserved the remaining prepuce and its patency, although reduced length resulted in permanent paraphimosis. Penile integrity has been maintained by spray-delivery lubricant (Priority Care Non-Spermicidal Sterile Lubricating Jelly, First Priority, Inc., Elgin, IL 60123 USA; applied topically, initially up to 6 times daily, tapering to s.i.d. to b.i.d). This case demonstrates successful management to preserve reproductive viability and highlights an under-recognized condition of exotic boars, emphasizing early intervention to avoid complications.

### **ACKNOWLEDGMENTS**

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## **SURGICAL REPAIR AND POST-OPERATIVE MANAGEMENT OF A LE FORT I FRACTURE AND FACIAL LACERATIONS IN A PEDIATRIC WESTERN LOWLAND GORILLA (*Gorilla gorilla gorilla*)**

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### **Abstract**

A 3-mo-old Western lowland gorilla (*Gorilla gorilla gorilla*) (3.5 kg) was presented for severe traumatic maxillary-facial wounds. Following emergency immobilization, it was determined that a downward force had degloved the nasal planum from the underlying bones and fractured the rostral maxilla and nasal bones, consistent with a Le Fort I presentation.<sup>1</sup> Following radiographic assessment of the skull, reconstructive surgery was undertaken. Surgical wire was used to stabilize the primary maxillary fracture, while soft tissue wounds along the nasal bridge and intra-orally were apposed in multiple layers. Bilateral nasal stents were placed and secured to the lateral nasal alar folds to maintain patency post-operatively.

Protracted hospitalization for wound care and hand rearing followed surgical stabilization. Flunixin meglumine (Flunixinject; Butler Schein Animal Health, Dublin, Ohio 43017, USA; 0.5 mg/kg, i.m., s.i.d.), famotidine (Baxter Healthcare Corporation, Deerfield, Illinois 60015, USA; 1 mg/kg i.m., s.i.d.), cefazolin (West-Ward Pharmaceuticals; Eatontown, New Jersey 07724, USA; 8.3 mg/kg, s.c., t.i.d.), amoxicillin trihydrate/clavulanate potassium (Clavamox Drops; Pfizer Animal Health, New York, NY 10017, USA; 15 mg/kg, p.o., b.i.d.), and topical 50:50 silver sulfadiazine cream (SSD; Dr. Reddy's Laboratories Louisiana, Shreveport, Louisiana 71106, USA) and Preparation-H ointment (Pfizer; Madison, New Jersey 07940, USA) were utilized during the recovery period. The infant was fed liquid formula (Similac PM 60/40; Abbott Nutrition; Abbott Laboratories, Columbus, Ohio 43219, USA) via oro-gastric intubation every 3 hr for the first week post-operatively. Self-feeding with assistance by a spooned-bottle began as continued improvement of superior labial mobility was noted. By 96 hr post-operatively, healthy granulation tissue was noted along the laceration repair with subsequent contraction of skin edges. Nasal stents were removed seven days post-operatively, with resolution of surgical swelling and unobstructed nasal breathing both noted.

Controlled interactions with members of the infant's natal troop were utilized to encourage social development over the following months, with gradual troop reintroduction.<sup>3,4</sup> Ongoing monitoring managed concerns associated with dental eruptions

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over its pediatric years and assessed long-term resolution of the maxillary fracture.<sup>2</sup>

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# DENTAL ONLAY BRIDGE-LIKE PROSTHESIS IN THREE KOALAS (*Phascolarctos cinereus*): THE USE OF PREMISE™ TRIMODAL COMPOSITE TO PREVENT INTERPROXIMAL *Eucalyptus* spp. RETENTION AND INFECTION

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## Abstract

Food accumulation in interproximal dental spaces facilitates bacterial colonization that incites periodontal disease, including marginal gingivitis, plaque accumulation, periodontitis, and localized alveolar bone loss that may eventually require dental extraction. We report three cases of severe interproximal *Eucalyptus* spp. retention in koalas (*Phascolarctos cinereus*) at the San Diego Zoo. Premise™ trimodal composite (Kerr Corporation, 1717 West Collins, Orange, California 92867, USA) was used in these cases to eliminate open and enlarged interproximal contact spaces where debris accumulation was observed.<sup>1</sup> Following removal of the partially masticated leaf material, the interproximal surfaces of the teeth were conventionally prepared with a course diamond burr, cleaned, and acid etched prior to the placement of a composite restoration that eliminated the interproximal defect. One case required minor crown contour alteration utilizing traditional dental cavity preparation techniques to increase the functional stability and retention of the composite prosthesis within the interproximal space. Aesthetically, the prosthesis replicates normal tooth coloration and appearance. Our observations indicate the prosthesis wears at a similar rate to captive koala teeth while preventing interproximal accumulation of masticated leaf material. A dental onlay bridge-like prosthesis using the Premise™ trimodal composite is a successful method to prevent interproximal *Eucalyptus* spp. accumulation in koalas. Repair or replacement of this prosthetic device may be required, so it should be examined visually and radiographically on a routine basis.

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## AORTIC ANEURYSMS IN ASIATIC BLACK BEARS (*Ursus thibetanus*) RESCUED FROM BILE FARMS IN CHINA

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### Abstract

Animals Asia has rescued 277 Asiatic black bears (*Ursus thibetanus*) from bile farms in China, where bears experience serious welfare issues.<sup>6</sup> Aortic aneurysms in animals, while not commonly reported, can be associated with heredity, parasites, nutrition, and hypertension.<sup>2,4,5,7</sup> Aortic aneurysm was diagnosed in nine bears at the China Bear Rescue Centre (CBRC) (Longqiao, Chengdu, China) through radiographs, computed tomography, and necropsies. Eight of the nine bears died, with dissecting aortic aneurysm as the cause of death in four. One of the deceased bears had hemopericardium without evidence of aortic dissection. Necropsies confirmed aortic aneurysm in three bears euthanized due to other conditions: spinal paresis, a nasal tumor, and unresponsive head-pressing. Clinically, one of the deceased bears coughed, likely related to severe aortic dilation. The surviving bear remains asymptomatic. Preliminary histopathology of necropsied bears revealed no primary aortic abnormalities. High prevalence of retinal and renal pathology in farmed bears may be attributed to hypertension and suggests an association with aortic aneurysm in this population. Seven (4.6%) of 152 bears at CBRC receive oral enalapril (0.5 mg/kg b.i.d., Enalapril, Yabao Pharmaceutical Group, Shanxi, 044602, China) and amlodipine (0.05-0.1 mg/kg s.i.d./b.i.d., Norvasc, Beijing Yimin Pharmaceutical, Beijing 101300, China) as treatment for suspect hypertensive retinopathy that was identified by fundic examination. In humans, aortic aneurysms and associated hypertension is well documented.<sup>1,3</sup> To the authors' knowledge, this is the first report of aortic aneurysms in ursids. Understanding the etiology of aortic aneurysms in this population is of importance considering the severe health implications when rehabilitating farmed bears.

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## HOW TO READ AN ELEPHANT BLOOD FILM: A VALUABLE DIAGNOSTIC TOOL IN SICK ELEPHANTS (*Elephas maximus*, *Loxodonta africana*)

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### Abstract

Although normal elephant hematology has been described,<sup>1</sup> hemogram changes in disease are uncharacterized in the literature. Band heterophils in blood films of sick elephants, even in low percentages of 1 to 2%, seem to be significant indicators of systemic inflammation. Because band heterophils are observed with such diseases as Elephant Endotheliotropic Herpesvirus (EEHV), salmonellosis, and surgical colics, their presence and morphology warrant further clinical investigation as diagnostic and prognostic indicators of disease. Observation of trends, such as relative increases and decreases in total numbers of lymphocytes and monocytes, as well as changes in the appearance of these cells, including reactivity and vacuolization, also have clinical relevance in the detection of systemic inflammation.

Elephant erythrocytes can show alterations in morphology, size and color in association with various inflammatory, infectious, and neoplastic diseases. Polychromasia and reticulocytes, not normally seen in blood films of healthy elephants, indicate a regenerative anemia. Platelets, normally abundant in elephant peripheral blood can decrease dramatically with certain diseases, particularly EEHV. Since elephant platelets tend to clump in vitro, blood film evaluation is more likely to provide evidence of developing thrombocytopenia.

Appropriate sample handling and processing of elephant blood samples are essential for obtaining accurate results. A larger gauge needle, at least 18 gauge, is recommended to avoid hemolysis. This recommendation precludes the use of commercial 'butterfly needles' which are currently available at a maximum size of 19 gauge. Additionally, preparing a blood film immediately after phlebotomy can significantly improve blood sample quality and aid in its interpretation.

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**EFFECT OF MANUALLY PRE-HEPARINIZED SYRINGES ON PACKED CELL VOLUME AND TOTAL SOLIDS IN BLOOD SAMPLES COLLECTED FROM AMERICAN ALLIGATORS (*Alligator mississippiensis*)**

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**Abstract**

The hemodilution effect of manually pre-heparinized syringes was determined by evaluation of packed cell volume (PCV) and total solids (TS) from blood samples collected from 50 American alligators (*Alligator mississippiensis*). A volume of 0.2 ml of blood was drawn into each of three 1 ml syringes: control with no heparin, heparin drawn to 0.1 ml then expelled, and heparin drawn to 0.2 ml then expelled. The order of blood collection into each syringe was randomized and the investigators were blinded to the heparin content of each syringe. PCV and TS values were determined from each syringe immediately after collection. Mean PCV and TS were compared between the control and heparin groups using linear mixed modeling. The syringes coated with heparin resulted in a significantly lower mean PCV and TS compared to controls with no significant difference between the heparin groups ( $P < 0.001$ ). This dilution effect was also found to be inconsistent and not accurate from one syringe to another. An adjunct method of collecting 0.5 ml of blood into 1 ml syringes coated with heparin drawn to 0.2 ml then expelled also showed a significant decrease in PCV and TS when compared to the control samples, although to a lesser extent ( $P < 0.001$ ). As a result, it cannot be recommended to manually heparinize syringes prior to collection of small blood samples from reptiles because significant and unpredictable hemodilution is likely to occur.

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## THE USE OF CLASS IV LASER THERAPY IN ZOO AND WILDLIFE MEDICINE

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### Abstract

Low level laser therapy (LLLT) has been used clinically for more than 30 yr,<sup>3</sup> with a steady increase in utilization within veterinary medicine and, specifically, with exotic species.<sup>4</sup> LLLT causes photobiomodulation of tissue, leading to an increase in metabolism, accelerated healing time, pain relief, improvement in circulation and reduction of inflammation.<sup>1,2</sup> Four classes of therapeutic laser exist, designated I - IV, with an increase in power output throughout the groups. Class IV laser systems classify any laser system that is greater than 0.5 watts, and include military anti-missile lasers. Laser therapy systems can provide up to 15,000 mW of power output, with wavelengths typically ranging from 808 nm - 980 nm. These higher powers afford the delivery of an effective dose to deeper tissues, in an efficient and clinically-relevant time period.

A Class IV therapy laser (Companion Therapy Laser CTL-10, LiteCure, Newark, Delaware 19702, USA) was used as an adjunct to traditional management of extensive thermal burns in a Savannah monitor (*Varanus exanthematicus*), a keel wound in a Bald eagle (*Haliaeetus leucocephalus*) and cheek patch dermatitis in an Asian elephant (*Elephas maximus*). A standard schedule was used (three applications in week one, two in the second week, and once weekly thereafter). A pre-set therapeutic dose relevant to each lesion was used in each situation. Although no control cases exist for the situations described, the laser therapy appeared to reduce both healing time and inflammation compared with other clinical experiences. In particular, the affected tissue of a Savannah monitor that suffered a thermal burn demonstrated minimal wound contracture within the first 11 mo of routine wound care, before showing considerable improvement to full healing over the subsequent 4 mo with LLLT. No adverse effects attributable to the laser therapy were seen in any of these cases. Wound healing and pain management can be difficult problems to manage clinically, and laser therapy offers a non-invasive, drug free option to aid in these cases.

### ACKNOWLEDGMENTS

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## CLINICAL DIAGNOSIS AND MANAGEMENT OF IDIOPATHIC EPILEPSY IN A BLACK RHINOCEROS (*Diceros bicornis*)

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### Abstract

Clinical epilepsy is an infrequent diagnosis in zoological collections with the predominance of cases being documented among primates.<sup>1,2</sup> An 8-yr-old male black rhinoceros (*Diceros bicornis*) with no prior history of illness presented acutely with a series of eight, 2 minute seizures over a 10-hr period. Seizures presented in a predictable pattern of pre-ictal disorientation and hypersalivation, followed by ataxia, erratic head movement, lip curling, and stargazing. Seizure episodes culminated in pelvic weakness and the rhinoceros falling to a “dog-sitting” posture before recovery. Treatment was initiated with anticonvulsants and intravenous phenobarbital (Westward Pharmaceuticals, Eatontown, New Jersey 07724, USA) to control the initial seizures prior to the beginning of oral maintenance therapy. Serology was negative for *Sarcocystis neurona*, *Toxoplasma gondii*, and viral encephalitides, as well as negative for environmental toxicants by gas chromatography. Analysis of feed revealed no contaminants or food-borne pathogens to explain the clinical signs. Based on the signs and laboratory results, a diagnosis of epilepsy was made and treated with oral phenobarbital (Qualitest pharmaceuticals, Huntsville, AL 35811, USA) with dosing gradually decreased from 2 mg/kg to 1 mg/kg over 4 mo. Eight months following the initial presentation, breakthrough seizing occurred. Following rescue therapy with parenteral diazepam (Hospira Inc., Lake Forest, Illinois, 60045, USA), levetiracetam (9.9 mg/kg PO BID, Lupin Pharmaceuticals Inc., Baltimore, Maryland 21202, USA) was added to the maintenance therapy. This case suggests the first known case of seizure disorder in a black rhinoceros and highlights the unique challenges of epilepsy in a megavertebrate.

### ACKNOWLEDGMENTS

The authors would like to thank Deborah Paperd, LVT, the rhinoceros zookeeper staff of Potter Park Zoo, The Docents of Potter Park Zoo, the Indianapolis Zoo, the Department of Large Animal Clinical Sciences of the Michigan State University Veterinary Teaching Hospital for their expertise and assistance with this case.

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## APPLICATION AND LIMITATION OF ADVANCED DIAGNOSTIC AND THERAPEUTIC MODALITIES IN ZOOLOGIC SPECIES WITH MALIGNANT MELANOMA

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### Abstract

Melanomas are tumors of melanocytes (pigment-producing cells) and have been reported in both domestic and non-domestic species.<sup>1,2,3</sup> A diagnosis of melanoma is made by histopathologic evaluation of affected tissue. Information on diagnostic and therapeutic modalities of malignant melanomas in zoological species is scant. In most cases, treatment of choice is surgical excision of the mass. Further staging usually includes routine hematology, plasma biochemistry, aspiration of local lymph nodes, thoracic radiography, and abdominal ultrasound to determine evidence of metastatic disease.<sup>4</sup> Adjunctive treatment consists of radiation therapy, chemotherapy and/or immunotherapy. In this study, a lion (*Panthera leo*) with dermal melanoma, a tiger (*Panthera tigris*) with conjunctival fornix melanoma, and a black footed penguin (*Spheniscus demersus*) with melanoma of the uropygial gland were treated with immunotherapy (n=3), strontium-90 brachytherapy (tiger) or external beam hypofractionated radiation therapy (lion). In all cases a complete blood count, plasma biochemistry panel, thoracic or whole body radiographs, and a computed tomography (CT) scan (lion and tiger) or PET/CT scan (penguin) were performed to evaluate for distant or regional metastasis. Masses in the penguin and the tiger were surgically excised prior to the start of immunotherapy. In addition, strontium-90 brachytherapy was also administered for one session to treat microscopic disease at the surgical site of the tiger. The lion received weekly external beam hypofractionated radiation therapy for four treatments in conjunction with immunotherapy to reduce the size of the melanoma prior to surgical excision. Following treatment, the melanoma was reduced in size by approximately 50%. No adverse effects associated with radiation therapy were observed.

Immunotherapy is a novel treatment for the management of canine melanomas that utilizes the human tyrosinase enzyme to stimulate the host active immune system against tyrosinase enzyme producing cells. There are no reports of its use in non-domestic species at this time. The lion and the tiger received 0.5 ml of the melanoma vaccine (Oncept, Merial, Duluth, Georgia 30096, USA) intramuscular once a week for four initial treatments followed by boosters every 6 mo for the remainder of the their lives. The penguin only received two doses of the vaccine (0.5 ml i.m.) as it was euthanized due to poor quality of life concerns. On post-mortem examination of the penguin, metastatic disease was present in the heart, lung, liver, kidneys, adrenal glands, gastrointestinal tract, skin, and brain. Both immunotherapy and radiation therapy were effective in treating the

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tumors found in the lion and the tiger as there is no physical evidence of metastatic disease present at 356 days (lion) and 138 days (tiger) since surgical excision of their respective masses. No adverse reactions to the melanoma vaccine such as pain, redness or swelling were observed in any of the patients of this report. The effectiveness of immunotherapy in developing an immune response in non-domestic species is currently under investigation. In the lion and tiger, computed tomography identified primary tumor margins while a PET/CT scan of the penguin did not identify the presence of metastatic disease of the melanoma. Further studies are indicated to evaluate the accuracy of advanced imaging modalities to identify metastatic disease and the application and limitation of radiation therapy and/or immunotherapy in zoological species with melanoma.

#### **ACKNOWLEDGMENTS**

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## METRONOMIC CHEMOTHERAPY FOR SARCOMA TREATMENT IN A KORI BUSTARD (*Ardeotis kori*)

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### Abstract

A 25-yr-old, wild-caught male Kori bustard (*Ardeotis kori*) developed a large, slow-growing subcutaneous mass over the keel. The mass was surgically debulked and described as a spindle cell sarcoma most consistent with a myxosarcoma. Local tumor regrowth with no evidence of metastasis occurred within 3 mo of the procedure. A second surgical procedure was performed to debulk the tumor and place twelve carboplatin-impregnated calcium-based matrix beads (Matrix III beads, Royer Biomedical Inc., Frederick, Maryland 21703 USA; containing carboplatin 4.6 mg/bead; Wedgewood Pharmacy, Swedesboro, New Jersey 08085, USA). The beads were placed in a grid pattern approximately 1.5 cm apart, radiating out from the incision and across the tumor site. Despite the therapies employed, moderate local tumor regrowth was clinically appreciable four weeks post-operatively from the second surgery. The bird was then started on metronomic chemotherapy with once daily oral cyclophosphamide (0.42 mg/kg; Cyclophosphamide, Taylors Pharmacy, Winter Park, Florida 32789 USA) and meloxicam (0.1 mg/kg; Metacam oral suspension, Boehringer Ingelheim Vetmedica Inc., St. Joseph, Missouri 64507 USA). Metronomic chemotherapy utilizes low, chronic dosing of chemotherapy agents to minimize toxicity while preventing tumor angiogenesis.<sup>1,2</sup> Five months post-operatively (4 mo after initiation of oral chemotherapy), the tumor was no longer appreciable on palpation, hematology and serum chemistry analysis did not reveal any derangements, and the bird remained clinically normal. To the authors' knowledge, this is the first report of a myxosarcoma and of successful metronomic chemotherapy in the Otididae family.

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## **SLENDER-TAILED MEERKAT (*Suricata suricatta*) FATALITIES ASSOCIATED WITH INTESTINAL TREMATODIASIS (*Prosthodendrium naviculum*)**

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### **Abstract**

*Prosthodendrium naviculum* (Trematoda) is a common parasite of bats in North America, which has occasionally also been reported in raccoons and mink.<sup>1</sup> Lecithodendriid trematodes are multiple life stage parasites which use snails as the first intermediate host and aquatic arthropods (insects and crayfish) as the infective intermediate host. Meerkats at the Toledo Zoo have traditionally been offered crayfish (*Cambarus diogenes*) as seasonally available enrichment. In June 2012, three of four slender-tailed meerkats (*Suricata suricatta*) developed acute lethargy, cardiac and respiratory distress, and then died either shortly after empirical treatment was initiated or before any treatment could be initiated. Histology revealed that all three animals had lymphoplasmacytic inflammation in the lamina propria of the small intestine and colon and two animals had large numbers of trematodes in the small intestinal lumen. Death was attributed to systemic lymphoplasmacytic inflammation, most notably in the heart, kidneys, liver, lungs, and skeletal muscle. Two animals had reactive lymphoid hyperplasia and medullary histiocytosis in the lymph nodes, and one had splenic microgranulomas. Histologic examination of the feeder crayfish identified large numbers of metacercariae in the muscles. PCR testing of frozen tissue from all three meerkats identified the organism *Neorickettsia risticii*, the causative agent of Potomac Horse Fever. Sequencing for the rickettsia in the flukes and crayfish is ongoing, as is sequence identification of the flukes in the crayfish and meerkats. This disease, which has proven fatal in equids, has not been previously reported in meerkats and identifies an unexpected risk of live enrichment feeding.

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## HYPERBILIRUBINEMIA IN CLINICALLY HEALTHY CAPTIVE WATERBUCK (*Kobus ellipsiprymnus*) AT THE SAN DIEGO ZOO SAFARI PARK

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### Abstract

Captive waterbuck (*Kobus ellipsiprymnus*) have been found with unexplained high total (tBili) and direct bilirubin (dBili) concentrations compared with wild and domestic ruminant species. We hypothesized that increased bilirubin concentrations in waterbuck are age related and not associated with laboratory or clinical evidence of liver disease.

Retrospective data from clinically healthy (n = 46) and diseased (n = 26) individuals at the San Diego Zoo Safari Park were compared and subdivided into neonate (< 8 dy), juvenile (8 dy to 2 yr), and adult (≥ 2 yr) groups. Paired t-tests and Chi square analysis showed that diseased waterbuck had significantly lower tBili, dBili, GGT, HCT than healthy waterbuck (P < 0.05). No significant association was found between icteric serum and tBili for either population, but the mean tBili for healthy waterbuck (7.60 mg/dL) was significantly higher than reference limits for cattle and other Reduncinae species (P < 0.05).<sup>1</sup> Healthy juvenile and adult waterbuck had significant positive correlation between clear serum and hyperbilirubinemia (≥ 3.8 mg/dL), and diseased juveniles had significantly higher mean tBili than diseased neonates (P < 0.05).<sup>1</sup> Diseased waterbuck overall had a significantly lower mean dBili to tBili ratio than healthy (P < 0.05).

Although tBili tended to be higher in adults, significant associations between hyperbilirubinemia, age, and disease status were not seen. Further studies are needed to elucidate the cause of altered tBili concentrations in captive waterbuck, and to verify the presence and type of bilirubin and its association with icterus.

### ACKNOWLEDGMENTS

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## DERMATOPHILUS CONGOLENSIS INFECTION IN A HERD OF SEMI FREE-RANGING CAPTIVE ADDAX (*Addax nasomaculatus*)

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### Abstract

*Dermatophilus congolensis* infection is documented in many species of animal and humans.<sup>2-5</sup> It is associated with warm, moist weather conditions, skin abrasions or other disruptions, and tick infections.<sup>1</sup> Clinical disease from *D. congolensis* was seen in a collection of semi free-ranging captive addax (*Addax nasomaculatus*) located on a 400 acre mixed species pasture. From 2008 to 2012, *Dermatophilus sp.*, was definitively diagnosed via culture in only one animal. However, one other animal had lesions diagnostic for *D. congolensis* infection and two additional animals had lesions either consistent with or compatible with *D. congolensis* infection on histopathology. Nine additional animals had a presumptive diagnosis of *D. congolensis* infection. The most severely affected animals were treated with a single dose of oxytetracycline (Liquamycin LA-200, Zoetis, New York, New York 10017 USA; 20 mg/kg i.m. or s.c.), after which clinical signs resolved in all but one animal. Infection with this organism should be self-limiting but spores contained in scabs that remain in the environment may remain viable for several months.<sup>6</sup> Of interest, none of the other exotic species housed in the pasture with these animals developed detectable infection, which may reflect preferences for certain areas by this species that are not frequented by other species or could possibly reflect differences in species susceptibility.

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## UNIQUE OCULAR ANATOMY OF THE BLACK PACU (*Colossoma macropomum*): GROSS, HISTOLOGIC, AND DIAGNOSTIC IMAGING

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### Abstract

The eyes of teleost fish follow the basic vertebrate blueprint consisting of scleral, uveal, and neural layers, a crystalline lens, and clear media (aqueous and vitreous humors).<sup>6</sup> In the least derived species, nutrition to the posterior segment is provided solely via diffusion from the choroid. Most teleosts possess an outgrowth of the choroid into the vitreous humor, termed the falciform process, as well as a vascular rete mirabile (originally and mistakenly termed the “choroidal gland”), which receives oxygenated blood from the pseudobranch and is thought to enhance oxygen tension in the posterior segment.<sup>1,3,7,8</sup> The choroid of the black pacu or tambaqui (*Colossoma macropomum*), a relative of the piranha and native to the Amazon River basin, also contains a thick pad of adipose tissue (C. Murphy, unpublished data). This presence of fat in the choroid appears to be nearly unique among vertebrates, as yet undocumented in the published literature, and of unknown function. This study correlates findings from imaging of intact pacu with gross and histologic anatomic features.

Seven black pacu averaging approximately 13 kg were examined in this study. Three fish were anesthetized using tricaine methane sulfonate (MS-222) for ultrasonographic<sup>a</sup> imaging of the eyes. Four additional fish were euthanized using an overdose of MS-222. Computed tomographic<sup>b</sup> images were made immediately post-mortem on three fish, and high-resolution magnetic resonance<sup>c</sup> images were made of exenterated eyes of one fish. In all modalities, the cornea, anterior chamber, lens, vitreous humor, and retina were identified and had an appearance typical of the vertebrate eye, with the exception that the retina was located at approximately half of the anterior-posterior (A-P) depth of the globe and had a flatter contour than the posterior sclera (Figure 1). The optic nerve was visualized well in all modalities except ultrasound. In all modalities, the majority of the globe posterior to the retina had an appearance consistent with fat. The choroid rete was identified within this fatty region in all specimens; as is typical of many teleosts,<sup>6</sup> it took the shape of an inverted U dorsal to the optic nerve head.

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The eyes of five of the above animals were fixed in formalin for gross and histologic examination. Eyes were decalcified and sectioned parasagittally, photographed, and embedded in paraffin. Sections were stained with hematoxylin and eosin. Slides from two archived specimens (courtesy D. Dubielzig) were also examined. The cornea, iris, lens, retractor lentis muscle, falciform process, retina, optic nerve, choroidal rete, and sclera (including scleral cartilage and ossicles) were identified and all had a gross appearance and histological architecture typical of the teleost eye (Figure 1).<sup>5,6</sup> The fatty choroid was grossly apparent, and histologically was composed mostly of adipocytes; choroidal fat was roughly 25% of the A-P volume of the globe in a single small specimen, and roughly 50% in larger adult fish. The choroidal rete was located entirely within the fatty region and dorsal to the optic nerve, as seen via imaging. Meridionally oriented blood vessels within the anterior 25% of the fatty region fed the choriocapillaris at the inner limit of the choroid, just posterior to the retina. The interface between the fatty choroid and the inner sclera was lined by a thin band of connective tissue and pigmented cells. The optic nerve coursed ventrally toward the retina, and in some sections a band of larger-caliber, thin-walled vessels and associated connective tissue paralleled this course. Throughout the optic nerve, inner sclera, and outer choroid were numerous eosinophilic granular cells. While their exact histogenesis and function are unclear, these are considered a normal component in some tissues in fish (e.g. gills) and can be increased in inflammation.<sup>4</sup> The presence of eosinophilic granular cells in normal ocular tissues has not previously been described, to our knowledge.

Varying amounts of fat have been noted within the choroid of ocular specimens from other fish species (D. Dubielzig, unpublished data), but this report in the black pacu is the first to carefully document this unique attribute. The function of choroidal adipose tissue is unknown. A role in insulation or heat production seems unlikely in a species with subtropical to tropical distribution. Additional hypotheses to consider include local energy storage, as has been hypothesized for lipid and glycogen inclusions within the choroidal cells of lamprey;<sup>2</sup> local storage of fat-soluble nutrients; a role in buoyancy; modification of the optics of the eye; and damping functions against extrinsic stimuli possibly deleterious to the visual process. For the moment, however, the fatty choroid of the black pacu remains an anatomic enigma.

**Products Mentioned in the Text:** <sup>a</sup>Philips iE33 linear 11-3 MHz and curvilinear 9-4 MHz probes, Philips Medical Systems, Andover MA, USA. <sup>b</sup>GE Lightspeed 16, 2.5 and 0.625 mm slices, GE Healthcare, Princeton NJ, USA. <sup>c</sup>BioSpec 7T, Bruker BioSpin MRI GmbH, Wein, Austria.

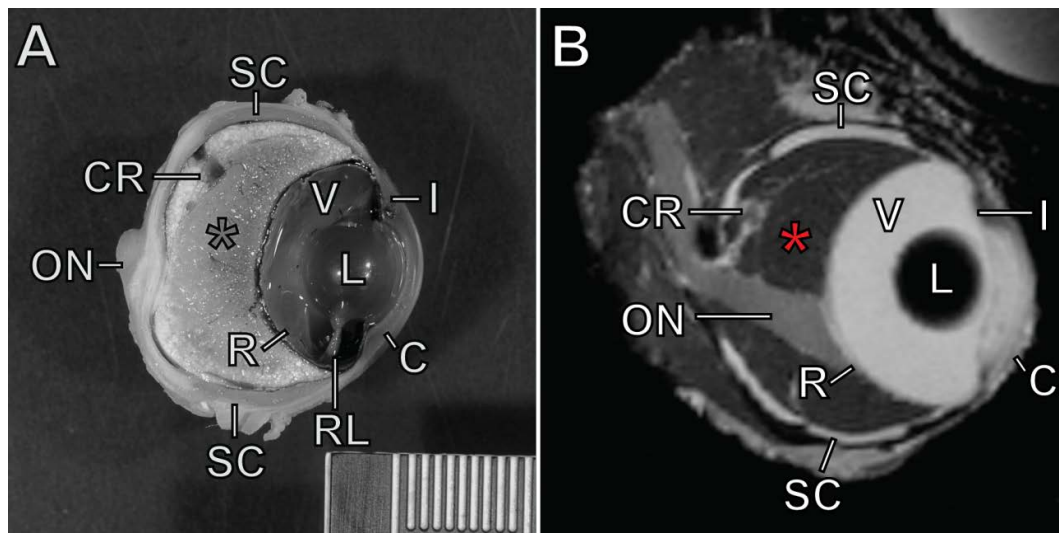
## ACKNOWLEDGMENTS

The authors thank the Steinhart Aquarium, California Academy of Sciences, for donating the black pacu used in this study.

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**Figure 1.** Ocular anatomy of the black pacu (*Colossoma macropomum*). A) Formalin-fixed eye in parasagittal section. B) MRI image of eye. See text for methods. C = cornea; I = iris; L = lens; V = vitreous humor; RL = retractor lentis muscle; SC = scleral cartilages; R = retina; CR = choroid rete mirabile (dorsal portion seen cross section); ON = optic nerve. Red asterisk = choroid, occupying the space posterior to the retina and anterior to the sclera. Scale bar = 1 mm hatches.

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## DIAGNOSIS AND ATTEMPTED TREATMENT OF *Metarhizium Anisopliae* FUNGAL INFECTION IN A ZEBRA SHARK (*Stegostoma fasciatum*)

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### Abstract

An adult male zebra shark, *Stegostoma fasciatum*, presented with a markedly swollen right clasper. The shark had been by himself in a reserve tank for the prior 13 mo due to chronic tankmate aggression. The clasper was swollen, with injected skin capillaries and multifocal, coalescing, purple, raised lesions. Repeated physical examination, fine needle aspirates, biopsies, bacterial and fungal cultures did not reveal an etiology, only locally extensive granulomatous inflammation. Treatment with antimicrobials was ineffective. On the third round of biopsies, special stains on deep edges of a soft tissue swelling that developed adjacent to the affected clasper confirmed branching fungal elements. Panfungus PCR was positive and a 200bp DNA fragment sequence was compatible with an Ascomycota. A pure culture of the fungus yielded a 50bp amplicon from 18s rRNA sequencing that demonstrated 99% homology to *Metarhizium anisopliae* (Genbank JN13140.1). Voriconazole<sup>a</sup> therapy was started at 5.32 mg/kg PO q48h based on prior successful treatment of fusariosis in a bonnethead but was eventually titrated to an effective dosing regimen of 10.64 mg/kg PO SID based on serum testing.<sup>3</sup> Transition to generic formulation<sup>b</sup> after 2 mo of therapy did not impact serum levels or efficacy. Therapy was continued for 9 mo with full resolution of signs. *Metarhizium anisopliae* is an entomopathogenic fungus that is commonly used commercially as a biopesticide, though not at the Shedd Aquarium.<sup>15,16</sup> This agent is not considered a risk to humans or laboratory animals, however there are emerging reports of infections in both humans and animals.<sup>1,2,4-14</sup>

**Products Mentioned in the Text:** <sup>a</sup>Vfend, 200mg tablets, Pfizer Ireland Pharmaceuticals, Ringaskiddy, County Cork, Ireland. <sup>b</sup>Voriconazole, 200mg capsules, Diamondback Drugs, Scottsdale, Arizona 85251 USA.

### ACKNOWLEDGMENTS

The authors wish to thank the incredible efforts of the staff of the Shedd Aquarium's Animal Health Department as well as the extraordinary aquarists in the Fishes Department, most notably Lise Watson and Heather Thomas for their tireless work on this case. We would also like to thank the experts in the University of Illinois Veterinary Diagnostic Laboratory and Zoological Pathology Program as well as the Fungus Testing Laboratory at the University of Texas Health Science Center for their consultation and work on the identification of the pathogen.

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***Fusarium solani* SPECIES COMPLEX IN A COLLECTION OF CAPTIVE AMERICAN HORSESHOE CRABS (*Limulus polyphemus*)**

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**Abstract**

Captive American horseshoe crabs (*Limulus polyphemus*) at the National Aquarium, Baltimore presented with severe shell and gill lesions over a 3-yr period (2006 to 2009). Carapace lesions were located on both the dorsal and ventral prosoma and opisthosoma. Carapace lesions included multifocal circular areas of tan discoloration, ulcerations, and/or pitting lesions extending from superficial to full thickness. Gill lesions involved both the book gill cover and individual book gill leaflets and included multifocal circular areas of tan discoloration, tan to off-white opaque proliferative lesions, and/or areas of black discoloration. Histopathology revealed septate, irregularly branched fungal hyphae throughout the thickened and irregular cuticle of the carapace with associated amebocytic inflammation, and occasional penetration into subcuticular tissues. Book gill leaflets were infiltrated by fungal hyphae and contained necrotic debris and amebocytes. Thirty-nine animals had histopathologic analyses performed with 38 (97.4%) having evidence of fungal hyphae. Fungal cultures of carapace and gill lesions were attempted in 26 tissue samples from 15 individuals and grew in 13 samples (50%), with ten cultures (76.9%) having successful genus identification. *Fusarium* sp. was identified in eight of the ten cultures (80%). *Fusarium solani* species complex was identified in six of these cultures via polymerase chain reaction amplification of two different ribosomal specific sequences of isolated fungal DNA. Histopathology revealed fungal disease was more prevalent than fungal cultures indicated and was a major cause of morbidity and mortality in this collection. Based on anecdotal reports from other facilities, the condition may be common in captive American horseshoe crabs.

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## EVALUATING THE EFFICACY AND SAFETY OF GUAIFENESIN AS AN INJECTABLE ANESTHETIC AGENT IN ELASMOBRANCHS

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### Abstract

Performing anesthetic procedures in elasmobranchs can offer a variety of challenges based on the mode of administration, environmental factors, individual animal metabolism, and lack of approved drug protocols.<sup>2,5</sup> Many accepted techniques are extrapolated from mammalian species and often differ in expected efficacy and duration.<sup>6</sup>

Guaifenesin, a muscle relaxant with an unknown mechanism of action, has widely been used in anesthetic protocols of terrestrial species<sup>1,3,4</sup> but its use has not yet been documented for aquatic animals. It is the goal of this case series to determine the practicality of a guaifenesin bolus as an anesthetic agent in elasmobranchs.

Five southern stingrays (*Dasyatis americana*) and one bowmouth guitarfish (*Rhina ancylostoma*) were anesthetized with 50-75ppm MS-222, moved into fresh saltwater, and then received a bolus of guaifenesin intravenously at varying doses between 10-40.5 mg/kg. Heart rate in all animals was monitored continuously via ultrasound during and immediately following guaifenesin administration; all five stingrays experienced brief episodes of asystole during injection which spontaneously resolved with no lasting ill-effects. Other cardiopulmonary effects observed in association with guaifenesin administration were bradycardia and tachypnea.

Although a surgical plane of anesthesia was not obtained with intravenous guaifenesin, heavy sedation with muscle relaxation was achieved for short term procedures. Further studies are required to determine safe dosing ranges and to explore protocols for guaifenesin use as a continuous rate infusion.

### ACKNOWLEDGMENTS

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**ADENOVIRAL DIVERSITY AND POSSIBLE HOST JUMPS IN A COLONY OF SOUTH AMERICAN FUR SEALS (*Arctophoca australis*) AND HUMBOLDT PENGUINS (*Spheniscus humboldti*)**

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**Abstract**

Adenoviruses are found in diverse vertebrates, with high level of host fidelity. In general, the most significant pathology due to intranuclear DNA viruses is associated with host jumps. To date, members of the genus *Mastadenovirus* have only been found in mammal hosts, and the genus *Aviadenovirus* has only been found in birds.<sup>1</sup> There has been a large investigative bias towards human adenoviruses, leading to a poor understanding of greater adenoviral diversity and ecology. Only 2 adenoviral species have been described in Carnivora, Canine adenovirus and California sea lion adenovirus.<sup>2,3</sup> Canine adenovirus may be a host jump from a bat adenovirus.<sup>3</sup> Little is understood about cospeciation of adenoviruses in the Carnivora lineage. We investigated the diversity of adenoviruses in breeding colonies of South American fur seals (SAFS, *Arctophoca australis*) from two populations, one in Peru (Punta San Juan Marine Protected Area, 15°22'S, 75°12'W) and the other in Chile (Guafo Island, 43°36'S, 74°43'W). Screens using nested pan-adenoviral primers have identified 4 Mastadenoviruses and 4 Aviadenoviruses. Concurrent investigation of adenoviruses in Humboldt penguins (HP, *Spheniscus humboldti*) at the same Peruvian site identified 3 Mastadenoviruses, 2 Aviadenoviruses, and 3 Siadenoviruses. One Aviadenovirus has been detected in both HP and SAFS. This is the first report of Aviadenoviruses in mammals or Mastadenoviruses in birds, and suggests that further viral diversity studies in sites with high density mixed species populations is warranted.

**ACKNOWLEDGMENTS**

The authors wish to thank Dr. Gwen Jankowski, Dr. Matt Allender, Marco Cardeña, and Franco Garcia for their assistance with this project. Partial funding for sample collection in Peru was provided by the Chicago Zoological Society, the Chicago Board of Trade Endangered Species Fund, and the Saint Louis Zoo WildCare Center. Funding for sample collection in Chile was provided Society for Marine Mammalogy.

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## THE ANIMAL WELFARE ACT: OVERVIEW OF THE MARINE MAMMAL REGULATIONS

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### **Abstract**

Facilities that are licensed with the United States Department of Agriculture must be in compliance with the Animal Welfare Act. USDA APHIS Animal care lists specifications for the humane handling, care, treatment and transportation of marine mammals in Subpart E in the Animal Welfare Act and Animal Welfare Regulations, Part 3 - Standards.

Common questions about the regulations governing marine mammals include questions about facilities, feeding, water quality, employees, veterinary care, separation, and space requirements. Indoor facilities must be properly ventilated and have ample lighting. Both indoor and outdoor facilities must be able to have the means to regulate the water temperature in a range that meets the specific needs of the species contained. Space requirements are specific to the species maintained and must be calculated utilizing the charts included in the Animal Welfare Regulations. Animal health and husbandry standards are clearly stated. The section on feeding includes how fish should be stored and that it must be stored in freezers that are maintained at maximum temperatures of 18°C (0°F) and that fish thawed under refrigeration must be fed to the marine mammals within 24 hr of thawing. Water quality standards include bacterial standards, the addition of chemicals to the water, the frequency necessary for testing pH and any chemical additives, the necessity of water quality records, salinity requirements and filtration. Employees must be adequately trained and work in concert with the attending veterinarian to maintain the prescribed level of husbandry set forth in Subpart E. Marine mammals known to be primarily social in the wild must be housed in their primary enclosure with at least one compatible animal of the same or biologically related species. Animals housed separately must have a written plan approved by the attending veterinarian that includes the justification for the length of time the animal will be kept separated or isolated, information on the type and frequency of enrichment and interaction and provisions for periodic review of the plan by the attending veterinarian. Veterinary care regulations include requirements for isolation or quarantine of newly acquired marine mammals, holding facilities, detailed medical records, and necropsies. Detailed transportation standards are also included in the Animal Welfare Regulations.

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## BOX TURTLE ECOLOGY AND HEALTH IN MISSOURI, USA: COMPARISON BETWEEN AN URBAN AND RURAL SITE

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### Abstract

The Saint Louis Box Turtle Project was initiated in the spring of 2012 in response to the growing threats, and lack of awareness, facing box turtles in the Midwest, the increasing disconnect between young people and the outdoors, and the need for scientific information to help inform box turtle conservation in the region. The key goals to the project are to promote conservation of urban and rural box turtles in the Saint Louis area by improving our understanding of the ranging patterns, ecology, and health status of these ecologically important animals, and by developing an education and outreach program. The study sites include Forest Park (one of the nation's iconic urban parks that lies in the heart of Saint Louis), and the Tyson Research Center, a protected 809ha natural area, 25km from Forest Park. In the first year of this long-term project, we deployed 20 radio-tracking tags and recorded home-range sizes at both study sites.<sup>1</sup> Additionally, we collected health data for 85 individuals including hematology, chemistry profile, corticosterone levels,<sup>1</sup> *Mycoplasma* spp. serology, and PCR results for herpesvirus, ranavirus, adenovirus, and *Mycoplasma* spp. from a subset of turtles. Seven veterinary and undergraduate students participated in the project and we introduced numerous school age kids to box turtles and nature. Additionally, we garnered media support by television, newspaper and web based outlets. Results and conservation value from the first two seasons, along with comparative data from our sister project with Galapagos tortoises in the Galapagos, will be presented.

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**PLASMA ACUTE PHASE PROTEIN CONCENTRATIONS IN THE EASTERN BOX TURTLE (*Terrapene carolina carolina*) AND INFLUENCES OF AGE, SEX, SEASON, AND GEOGRAPHY**

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**Abstract**

Acute phase proteins (APP) are the foundation to the innate immune response and have been shown to be valuable biomarkers, as increases can occur with inflammation, infection, neoplasia, stress and trauma. In reptilian medicine, little is known about acute phase protein responses and if these protein levels can be used to distinguish health compromise within a wildlife population. The purpose of this study was to characterize the plasma concentrations of the acute phase proteins (haptoglobin and serum amyloid A (SAA)) and protein electrophoretic profiles in free ranging Eastern box turtles (*Terrapene carolina carolina*) and assess any possible correlations between varying age class (adult vs. juvenile), sex (male, female, or unknown), season (spring, summer, or fall), or geographical location. Blood samples were obtained from 324 Eastern box turtles (*Terrapene carolina carolina*) from 2010 to 2012 at three sites in Illinois and one site in Tennessee, USA. Reference ranges were created for each age class, sex, and location. Significant differences were noted with SAA (age class, sex, season, state), haptoglobin (age class, sex, state, IL location), TP (sex, season, state, IL location), albumin (age class, season, state, IL location), alpha-1 (sex, season, IL location), alpha-2 (sex, season, state, IL location), beta globulins (age class, sex, season, state, IL location), gamma globulins (sex, season, state, IL location). As a non-specific marker of inflammation, acute phase protein testing is a valuable tool for health assessment of wildlife populations and may prove useful as a method of health status surveillance in free-ranging reptiles.

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## COINFECTION WITH MULTIPLE INFECTIOUS AGENTS IN A GROUP OF CONFISCATED EASTERN BOX TURTLES

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### Abstract

Chelonians have low fecundity, low juvenile survival rate, and a long adult lifespan; a life history strategy where loss of adult animals (such as loss by disease) has a significant impact on populations.<sup>2</sup> Frog virus 3, a *Ranavirus*, is strongly associated with mass mortality events in eastern box turtles (*Terrapene carolina*).<sup>3</sup> A currently unnamed *Mycoplasma* sp., distinct from other known species, has been associated with upper respiratory tract disease in *T. carolina*.<sup>1</sup> More recently, a novel adenovirus has been identified in association with enterohepatic disease in eastern box turtles, and a herpesvirus has been identified in animals with concurrent ranaviral disease. The diversity and significance of infectious diseases beyond this are just beginning to be understood. Frog virus 3, Box turtle adenovirus 1, *Terrapene* herpesvirus 1, and an unnamed *Mycoplasma* sp. were identified in a group of confiscated eastern box turtles. Coinfection was common in this group, and may have played a significant role in the expression of disease. An overview of these agents, along with *Terrapene* herpesvirus 2 and the intranuclear coccidiosis agent of tortoises will be presented.

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## EVALUATION OF SALMONELLA IN REPTILES AT THE WILDLIFE CONSERVATION SOCIETY'S BRONX ZOO, 2000-2012

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### Abstract

Few reptile population *Salmonella* prevalence studies have examined the association between positive culture and clinical disease.<sup>1,2</sup> *Salmonella* culture results from captive reptiles at the Bronx Zoo were reviewed for a 13 yr interval. In total, 175 samples from 182 individuals (n = 4 samples from 11 crocodilians, n = 91 samples from 78 snakes, n = 57 samples from 59 lizards, n = 23 samples from 34 chelonians) yielded 182 isolates. Serotype distribution differences were noted for sample type, family, and animal origin and health. *Salmonella enterica* subspecies *enterica* was most common (44.6%, 78/175) and found across all taxa, especially serotypes in the O:7 and O:8 antigen groups. *Salmonella enterica* subspecies *diarizonae* was common (24.0%, 42/175), and found almost exclusively in snakes (n = 33), many with clinical illness (n = 17). Where submission totals were recorded (2008-2012), snakes had highest antemortem prevalence (32.8%), with all positives from fecal samples (n = 21). Clinically ill animals were the source of 35.2 % *Salmonella* isolates (64/182). Bony changes (n = 15), dermatitis (n = 15), and anorexia (n = 14) were the 3 most common clinical signs in these cases. Snakes (n = 39/91) and turtles (n = 10/23) with positive cultures had a higher likelihood of illness than lizards (n = 13/57; *P* = 0.01). *Salmonella* positive ill animals were more likely to be confiscated in origin than from captive or commercial sources (*P* < 0.01). Conclusions from this study can guide management of *Salmonella* positive captive reptiles.

### ACKNOWLEDGMENTS

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## EVALUATION OF LOW-LEVEL LASER THERAPY IN A MODEL OF CUTANEOUS WOUND HEALING IN BEARDED DRAGONS (*Pogona vitticeps*)

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### Abstract

Wounds are a common presenting complaint for reptiles, often requiring a prolonged course of treatment. Low-level laser therapy (LLLT) is gaining support as an adjunct treatment for wound management in human and veterinary medicine,<sup>1,3</sup> but there have been no published studies in reptiles. A cutaneous wound healing model was developed in the bearded dragon (*Pogona vitticeps*) based on an existing murine model; in the reptile, quantification of healing by image analysis was challenging.<sup>2</sup> In this study, 2 full-thickness 4 mm diameter punch biopsies were created on the dorsum of each animal, splinted with silicone rings to minimize dermal contraction, and bandaged with semi-occlusive dressing. In the LLLT<sup>a</sup> group (n=5), 1 wound on each animal received 4 Joules of 670 nm (red) light. In the control group (n=4), 1 wound received sham treatment. The remaining wound on all animals was untreated. Each wound was digitally imaged daily for 4 days. The inner wound margin was traced, and the wound area calculated.<sup>b</sup> Median change in wound area was 32% for sham-treated wounds and 21% for LLLT, but this difference was not significant in a 2-sample rank sum test.<sup>c</sup> Wounds were resected *en bloc* on day 4 and examined histologically. Unlike the murine model, the wound margins visible in gross images were not correlated with histologically identified epithelialization. Wound resection was feasible as a survival procedure, allowing histologic evaluation of epithelialization in this model of cutaneous wound healing in reptiles.

**Products Mentioned in the Text:** <sup>a</sup>Luminex Ultra, Respond Systems Inc., Branford CT, USA. <sup>b</sup>ImageJ, US National Institutes of Health, Bethesda MD, USA. <sup>c</sup>R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

### ACKNOWLEDGMENTS

The authors thank Respond Systems Inc. for donating the laser unit used in this study.

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## COMPARISON OF FOUR EUTHANASIA METHODS IN AMERICAN ALLIGATORS (*Alligator mississippiensis*)

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### Abstract

Recent events in the European exotic skin trade created a need to re-evaluate euthanasia techniques employed for reptiles in the commercial trade. A lack of studies on euthanasia of reptiles led us to evaluate different euthanasia methods in American alligators, a significant commercial reptile species.

Twenty-four captive hatched and reared American alligators with an average snout to tip of the tail length of 146.29 cm were used in this study. Six animals were randomly assigned to each of four groups: 1. spinal cord severance (CORD), 2. spinal cord severance and pithing of the brain (PITH), 3. penetrating captive bolt (PCB), 4. non-penetrating captive bolt (NPCB). An electroencephalogram (EEG) reading was obtained from each alligator at three different time points, while awake (AWAKE), in light plane of anesthesia (ANES), and upon euthanasia (POST) in order to record brain activity. The awake and euthanasia recordings were obtained 24 hr after anesthesia to ensure there was no anesthetic affect on the readings.

The results of the study revealed that NPCB and PCB techniques are capable of significant depression of brain activity in American alligators beyond that of a light plane of anesthesia. The PITH procedure is also capable of depressing brain activity although the effect was not as significant. Cord severance alone did not suppress brain activity below that of a light plane of anesthesia.

In conclusion, NPCB, PCB, and PITH can be considered appropriate euthanasia techniques for American alligators. Spinal cord severance alone should be considered inappropriate for euthanasia of alligators.

### ACKNOWLEDGMENTS

We would like to thank the Louisiana Department of Wildlife and Fisheries for their assistance during this project.

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## TISSUE CONCENTRATIONS OF ENROFLOXACIN AND CIPROFLOXACIN AFTER ORAL AND TOPICAL TREATMENT IN HOUSTON TOADS (*Anaxyrus [Bufo] houstonensis*)

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### Abstract

Enrofloxacin is a fluoroquinolone antibiotic with activity against many of the gram negative bacteria that cause illness in amphibians, however there are few studies evaluating its oral or transcutaneous absorption in this group of animals. This study evaluates the liver tissue concentrations of enrofloxacin, and its active metabolite ciprofloxacin, after oral and topical dosing in endangered Houston toads (*Anaxyrus [Bufo] houstonensis*). Liver concentrations were used as a surrogate marker for plasma concentrations to assess absorption. Administration of enrofloxacin by dusting crickets with powdered enrofloxacin 68 mg liver-flavored tablets<sup>a</sup> at target dosages of 10 mg/kg resulted in combined enrofloxacin/ciprofloxacin tissue concentrations above 1.0 ug/gm, up to 24 hours post treatment. Administration of a 5 mg/L enrofloxacin liquid<sup>b</sup> to the backs of toads by use of a spray bottle also produced enrofloxacin/ciprofloxacin tissue concentrations above this value for up to 10 hours post treatment. In addition, these methods of administration once daily for up to 12 weeks produced no signs of adverse effects in these toads. Increased concentration of the topical spray to 10 mg/L enrofloxacin did not proportionately increase tissue concentrations. These methods of administration can be of benefit to veterinarians for amphibian conservation programs which house large numbers of small amphibians where individual treatment of animals is time-prohibitive, provided additional pharmacokinetics of topical and oral enrofloxacin in amphibians can be performed to refine the dose.

**Products Mentioned in the Text:** <sup>a</sup>Baytril 68 mg liver flavored tablets (Bayer Health Care LLC, Shawnee Mission, KS 66201 USA. <sup>b</sup>Baytril 100 mg/ml injectable solution (Bayer Health Care LLC)

### ACKNOWLEDGMENTS

This study was funded by a grant from the Houston Zoo Staff Conservation Fund. The authors would like to thank Dr. Joseph Flanagan for assistance with sample collection, Aleyda Galan and Chris Bednarski for assistance with enrofloxacin dosing and toad husbandry, and Houston Zoo Veterinary Staff Stephanie Fannin, Kara LaVictoire, and Ryanne Henigar for assistance processing and packaging samples.

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## AN UPDATE ON AMPHIBIAN CHYTRIDIOMYCOSIS: IN THE FIELD, DIAGNOSTIC LABORATORY, AND CLINIC

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### Abstract

The skin disease chytridiomycosis, caused by infection with the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), is now a well-recognized contributor to the global amphibian extinction crisis. In addition to the consequences of *Bd* infection for susceptible wild amphibian populations, there are also challenges for veterinarians that treat captive amphibians in settings that vary from rescue populations on front line of disease outbreaks in the tropics to well-equipped clinics supporting modern cosmopolitan zoo collections.

In the 15 yr since the first description of chytridiomycosis there have been significant advances in the understanding of *Bd* biology, genetics, host defense and pathogenesis. Although *Bd* is widely distributed and reported from every continent with amphibians, research suggests that some strains or genotypes are more damaging than others and provides support for efforts that aim to minimize movement of *Bd* infected animals to new locations (e.g. listing of *Bd* by the OIE under the Aquatic Animal Health Code).<sup>3,7</sup> *Bd* has a very wide host range with reports of infection in over 300 species of frogs, salamanders and caecilians to date; however, there are significant differences in host susceptibility to the disease chytridiomycosis. The range of species that can become subclinically infected with *Bd* provides ample reservoir sources of infection for highly susceptible species. Elements of host defense against infection can include cutaneous antimicrobial peptides, composition of cutaneous microbial flora, acquired immunity, differences in MHC IIB alleles and host thermoregulatory behavior.<sup>6,8</sup>

The methods used to diagnose and control *Bd* infections will vary depending on factors such as the species affected, the number of animals in the group, and resource availability. Rapid diagnosis of clinically-significant infections can be accomplished in minutes by wet mount or cytologic examination whereas subclinical infections will only be reliably diagnosed by molecular methods such as PCR.<sup>5</sup> Although PCR is very sensitive, low-level subclinical infections can sometimes require multiple tests before detection. Treatment methods for infected animals have received a lot of recent attention including some controlled experimental trials of safety and efficacy. Despite this treatment outcomes are variable depending on the species, lifestage (e.g. tadpole vs. metamorph vs. adult), and the laboratory or institution administering treatment. The most widely used treatment methods are itraconazole baths and elevation of environmental temperature. Recent reports suggest that lower concentrations of itraconazole (25 to 50 mg/L) than has been previously used (e.g. 100 mg/L) can be effective and in some



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situations minimize treatment-associated side effects.<sup>1,4</sup> Heat treatment (30°C or more) is useful, but only if tolerated by the species.<sup>2</sup> Use of chloramphenicol baths has been successful, but requires more experience in a wider range of species.<sup>9</sup> Regardless of the method chosen successful treatment requires good husbandry and hygiene. Very sick animals can benefit from supplemental electrolytes as lethal infection is associated with hyponatremia and hypokalemia.

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## ADDRESSING THE NUANCES OF NEONATAL GIRAFFE

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### Abstract

Veterinary care of neonatal giraffe poses many unique challenges. Preparation typically requires time consuming investigation and discussion. The goal of this presentation is to summarize these issues and offer a reference for potential solutions and planning, based on previous literature and unpublished experience of zoo professionals.

Some of the key concerns include: preparation for birth (housing, substrate, separation, socialization and intervention plan); dystocia (when and how to intervene); maternal neglect (when to hand rear); failure of passive transfer (identifying and addressing the lack of antibody protection with colostrum and/ or various types of plasma); providing nutrition (milk replacers and parenteral nutrition); supportive care (intravenous fluids, blood monitoring, antibiotics and other drugs); bottle feeding (tactics, equipment and positioning techniques).

While many attempts at giraffe hand-rearing have failed, a wide variation of techniques has resulted in viable hand-reared giraffe calves. Patterns and consistencies have emerged that appear to increase the chances of success. Pre-emptive consideration of these issues should allow for advance planning that may make the difference in the outcome of newborn giraffe.

### ACKNOWLEDGMENTS

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## ESTABLISHING METRICS FOR FETAL DEVELOPMENT IN *Tamandua tetradactyla* USING SERIAL ULTRASOUND

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### Abstract

Little is published about the gestation and fetal development in southern tamanduas (*Tamandua tetradactyla*). From 2012-2013, nine pregnancies were monitored in five tamanduas housed at the Minnesota Zoological Garden, the Nashville Zoo at Grassmere, and the Reid Park Zoo. Clinicians recorded sonographic measurements to establish fetal growth curves using thoracic and skull landmarks described for giant anteaters.<sup>1</sup> Gestational measurements can be compared to the growth curve and used to predict parturition dates, providing information for population management.

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## A RETROSPECTIVE STUDY OF END-STAGE RENAL DISEASE IN CAPTIVE POLAR BEARS (*Ursus maritimus*)

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### Abstract

Eleven cases of end-stage renal disease (ESRD) were identified in captive polar bears (*Ursus maritimus*) from eight zoological institutions across the United States and Canada. The index case was identified by submission of a polar bear to Tufts Pathology Service for necropsy, and the remaining cases by searching two databases: annual reports of the Species Survival Plan Taxon Advisory Group and necropsy records from a private diagnostic institution. Ten bears were female, one was male, and the mean age at the time of death was 24 yr. The most common clinical signs were lethargy, inappetence, and polyuria/polydypsia. Biochemical findings included azotemia, anemia, hyperphosphatemia, and isosthenuria. Histological examination commonly showed glomerulonephropathies and interstitial fibrosis.

The prevalence of ESRD in captive polar bears in the United States was estimated by searching the database of a private diagnostic institution for all polar bears that were submitted for cause of death determination between 1995 and 2011. Thirty four polar bears were identified, seven of which died from ESRD. ESRD was the most common cause of death or reason for euthanasia with an estimated prevalence of over 20%. Further research is needed to discern the etiology of this apparently common disease of captive polar bears.

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## RETROSPECTIVE REVIEW OF LEG FRACTURE CASES IN ESTRILDIDAE FINCHES IN A FREE-FLIGHT EXHIBIT

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### Abstract

The National Aquarium, Baltimore maintains a collection of 10 Estrildidae finch species in a 6,000 m<sup>2</sup> (65,000 sq ft) mixed-species, walk-through exhibit. A retrospective review of femur, tibiotarsal, and tarsometatarsal fracture cases was conducted to evaluate the species most affected and success rate (defined as a return to exhibit). External coaptation (EC) and amputation were compared for case duration (defined as time from initial exam to exhibit release) and time on exhibit post-release.

Between January 1, 2005 and April 1, 2012, there were 40 cases in a cumulative population of 769 birds (5.2% prevalence). Gouldian finches (*Erythrura gouldiae*) were most affected representing 35% (14/40) of the cases and the highest individual species prevalence at 16.3% (14/86), but were only the third most common species in the population at 11.2% (86/769).

Of the 40 cases, two were seen on exhibit and healed without intervention, 12 were found dead/euthanized within 48 hr, and 26 were caught and managed with EC (n = 17) or amputation (n = 9). For this subset, success rate was 76.9% (20/26). For these 20 cases, the mean case duration was 43.9 d for EC and 32.0 d for amputation cases. The mean time on exhibit post-release was 732.7 d for EC and 387.9 d for amputation cases.

Both treatment strategies resulted in release of birds back to exhibit with a mean time on exhibit post-release over 1 yr. If distal limb viability is questionable or joint involvement is extensive, amputation is a viable treatment for exhibit birds.

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## PHARMACOKINETICS OF SELAMECTIN IN HELMETED GUINEA FOWL (*Numida meleagris*) AFTER TOPICAL ADMINISTRATION

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### Abstract

Parasitism is a common cause of morbidity in captive-held avian species. Treatment can be difficult with some parasite species due to lack of efficacious and therapeutically safe medications. Twenty one healthy helmeted guinea fowl (*Numida meleagris*) housed at the Oklahoma City Zoo were used to evaluate the pharmacokinetic parameters of topical selamectin. Study birds were divided randomly into 3 groups of 7 birds. On day 1 all birds received one dose of 20mg/kg selamectin applied to the dorsal cervical skin. Blood was collected from group 1 at 12 hrs and days 3, 7 and 14; from group 2 at 24 hrs and days 4, 7, and 21; from group 3 at days 2, 5, 7, and 28. Selamectin concentrations were determined in plasma using liquid chromatography with mass spectrometry. Mean terminal half-life and maximum plasma concentrations of selamectin were 5.8 days and 16.1 ng/mL, respectively with maximum plasma levels reached at 3 days. No adverse effects were detected. Therapeutic levels of selamectin are unknown for avian species. However, the birds in this study did reach and maintain plasma levels reported as therapeutic in other species for 19 days.<sup>1</sup> Based on these results selamectin may be a valuable antiparasiticide in some avian species although efficacy studies are needed.

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## DIAGNOSIS AND SUCCESSFUL TREATMENT OF TRICHOMONOSIS IN A CAPTIVE FINCH POPULATION

Kathryn E. Seeley, DVM,<sup>1\*</sup> Vincent Baldanza, VMD,<sup>2</sup> Leigh A. Clayton, DVM, Dipl ABVP (Avian and Reptile/Amphibian),<sup>1</sup> and Catherine A. Hadfield, MA, Vet MB, MRCVS, Dipl ACZM<sup>1</sup>

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### Abstract

*Trichomonas gallinae* infection has been associated with disease in pigeons and doves, raptors, budgerigars (*Melopsittacus undulatus*) and wild finches, but there are few reports in captive finch collections.<sup>1,3-6</sup> Twenty-five gouldian finches (*Erythrura gouldiae*), 20 double-barred finches (*Taeniopygia bichenovii*), and 25 star finches (*Neochmia ruficauda*), all captive bred, started quarantine in April 2011. Routine quarantine testing was unremarkable. *Trichomonas* was identified in the oropharynx and esophagus on histology of a double-barred finch that died on day 12 and on crop wet mount of a gouldian finch that died on day 38.

Group treatment was initiated on day 38 with metronidazole<sup>a</sup> in the drinking water at 500 mg/L SID x 21 d. Four thin gouldian finches were administered metronidazole<sup>b</sup> at 30 mg/kg PO BID x 5 d, in addition to the group treatment. Cleaning and disinfection of food and water bowls was increased.

There were four mortalities (3 gouldian finches, 1 star finch) between days 42 and 71. All were negative for trichomonosis on wet mount and histology. However histology showed avian gastric yeast (*Macrorhabdus ornithogaster*) and splenic and/or hepatic amyloidosis, which are reported with trichomonosis.<sup>2,3</sup> Oral swabs collected on days 71, 92, and 120 were negative for *T. gallinae* based on culture and direct microscopy.<sup>c</sup>

The birds cleared quarantine on day 126 and were introduced into a large mixed-species aviary exhibit. There has been no recurrence or evidence of transmission to exhibit Columbiformes since introduction. This case illustrates successful treatment of trichomonosis in a captive finch population.

**Products Mentioned in the Text:** <sup>a</sup>Teva pharmaceuticals, Sellersville, PA 18960 USA. <sup>b</sup>Compounded 25 mg/ml, BCP Veterinary Pharmacy, Houston, TX 77003 USA. <sup>c</sup>University of Florida Veterinary Diagnostic Lab, Gainesville, FL 32608 USA.

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## EVALUATION OF THE THERMAL ANTINOCICEPTIVE EFFECTS OF BUPRENORPHINE HYDROCHLORIDE IN AMERICAN KESTRELS (*Falco sparverius*)

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### Abstract

Recent studies have shown differences in the antinociceptive properties of opioid drugs in American kestrels.<sup>1,2</sup> Hydromorphone, a pure mu-opioid agonist, had a dose responsive thermal antinociceptive effect suggestive of analgesic properties.<sup>1</sup> In contrast, butorphanol, did not have a significant thermal antinociceptive effect at the dosages evaluated.<sup>2</sup> The current study evaluated buprenorphine hydrochloride, known as a slow-onset, long acting, partial mu agonist with poorly defined kappa receptor activity in American kestrels.<sup>3-7</sup> A masked randomized complete crossover study using foot withdrawal threshold to a noxious thermal stimulus was performed to evaluate antinociceptive effects and duration of action of buprenorphine hydrochloride. Buprenorphine hydrochloride (0.1, 0.3 and 0.6 mg/kg IM, (Buprenex®, 0.3mg/ml, Reckitt Benckiser Healthcare Ltd. Dansom Lane Hull, England HU8 7DS) and saline solution (0.9%NaCl, Hospira Inc., Lake Forest, IL 60045 USA) were evaluated in 12 kestrels. Baseline thermal withdrawal threshold data were generated prior to drug administration followed by foot withdrawal threshold measurements at 0.5, 1.5, 3, and 6 hr after buprenorphine or saline administration. Kestrels were assigned an agitation-sedation score and monitored throughout the testing period for adverse effects. Buprenorphine hydrochloride caused a significant dose dependent thermal antinociceptive response in American kestrels. The increase in mean withdrawal threshold was suggestive of analgesia. Further studies with other types of stimulations, formulations, dosages, and routes of administration in kestrels and other avian species are needed to fully evaluate the analgesic and adverse effects of buprenorphine and its clinical relevance.

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## AN OUTBREAK OF *Pasteurella multocida* ASSOCIATED WITH AN ACUTE MORTALITY EVENT IN CAPTIVE WILD TURKEYS (*Meleagris gallopavo*)

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### Abstract

The Calgary Zoo maintains a 2-acre partially wooded exhibit adjacent to the Bow River that houses a flock of wild turkeys (*Meleagris gallopavo*), six mule deer (*Odocoileus hemionus*) and a pair of Sandhill cranes (*Grus canadensis*). On March 21, 2012, 1 of 15 wild turkeys was found deceased, and within a 48-hr period an additional five birds died. All birds were well fleshed and demonstrated no premonitory signs.

Necropsies identified mucous in the oral cavity, cranial erythema, gastrointestinal hemorrhage, mild cloacal prolapse, and miliary white foci throughout the livers. The coeloms and airsacs contained small amounts of serosanguinous fluid, and the lungs and spleens were congested. Prime differentials included Fowl cholera (*Pasteurella multocida*), Fowl typhoid (*Salmonella enterica* subs *enterica* serovar gallinarum), viscerotropic velogenic Newcastle disease (VVND Avian paramyxovirus type 1), and Fowl plague (highly pathogenic Avian Influenza virus). Birds were quarantined and stringent biosecurity safeguards were implemented.

Histopathology revealed multifocal necrosuppurative hepatitis necrohemorrhagic pneumonia and large numbers of intralesional coccobacilli. Air sacculitis, enterocolitis and necroerosive proventriculitis were noted. Aerobic culture of lungs and livers yielded heavy growth of *Pasteurella multocida*.

The remaining birds were treated with trimethoprim-sulfadiazine (20 mg/kg q12 hrs p.o.).<sup>a</sup> All remaining birds remained free of clinical signs, and activity levels in the flock increased over the next two days. After the initial outbreak, no additional wild turkey or other waterfowl mortalities were reported. Fowl cholera has been reported in some winters within the waterways of Calgary, occasionally causing profound mortality in overwintering waterfowl.<sup>1</sup> Feeding practices in the exhibit have been altered to decrease the accessibility of both turkey and deer feed to wild birds and to assist in prevention of further outbreaks of this bacterial disease.

**Products Mentioned in the Text:** <sup>a</sup>Uniprim® powder; MacLeod Pharmaceuticals Inc, Fort Collins, CO.

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## MALIGNANT MELANOMA IN THE PENGUIN: CHARACTERIZATION OF THE CLINICAL, HISTOLOGIC AND IMMUNOHISTOCHEMICAL FEATURES OF MALIGNANT MELANOMA IN TEN INDIVIDUALS FROM THREE SPECIES OF PENGUIN

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### Abstract

Malignant melanomas are aggressive neoplasms that are relatively common in humans, dogs and horses, and rare in avian species.<sup>1,2</sup> Melanocytic neoplasms in 5 macaroni penguins (*Eudyptes chrysolophus*), 3 rock hopper penguins (*Eudyptes chrysocome*) and 2 Humboldt penguins (*Spheniscus humboldti*) are described. The average age of onset was 20.3 yr and the most common sites were the skin of the foot/ankle and near the edge of the beak. Tumors started as smooth, flat lesions and became raised and ulcerated over time. Two tumors less than 17 mm in greatest dimension were cured by complete surgical excision, while tumors larger than 20 mm were associated with metastatic disease. The liver was the most common site of metastasis, and was often largely effaced by neoplastic cells; despite this, serum AST values only became elevated weeks to days prior to death. Neoplastic cells varied from round to polygonal to spindloid, contained abundant intracytoplasmic melanin, and were arranged in clusters or nests, often within the overlying epithelium. Cellular indices of malignancy included anisokaryosis, multiple prominent nucleoli, intranuclear pseudoinclusions, and multinucleated cells. Immunohistochemical labeling with four common melanocytic markers (PNL2, Melan-A, S-100, and HMB-45) was performed on tissues from 8 of 10 affected penguins and included both primary and metastatic tumors. None of the tumors labeled with Melan-A or S-100. Four of 6 primary tumors showed positive labeling for HMB-45 and all showed positive labeling for PNL-2. Three different molecular diagnostic testing methods were performed to look for viral particles and results were negative.

### ACKNOWLEDGMENTS

Case materials were shared by three institutions including the Philadelphia Zoo, Louisville Zoo and Indianapolis Zoo. I'd also like to thank the Veterinary Staff and Penguin Staff at the Detroit Zoo.

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**PHARMACOKINETICS OF LEVETIRACETAM IN HEALTHY HISPANIAN  
AMAZON PARROTS (*Amazona ventralis*) FOLLOWING ORAL  
ADMINISTRATION OF A SINGLE DOSE**

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**Abstract**

Avian long-term anticonvulsive treatments have been poorly described and few pharmacokinetics studies have been performed, with mixed results. Levetiracetam, a new anticonvulsive drug, has shown good efficacy for monotherapy or adjunctive treatment of seizures in both human and veterinary medicine. Pharmacokinetic studies in domestic animals have shown it to have a favorable profile with rapid oral absorption, absence of hepatic metabolism, and a high safety margin.

The aim of this study was to determine the pharmacokinetics of oral levetiracetam in Hispanian amazon parrots, *Amazona ventralis*. Twenty healthy individuals were divided into two treatment groups and were administered either a 50 or a 100 mg/kg dose. Blood samples were collected at baseline and intermittently for 16 hrs. No animals showed any adverse behavioral effects to either dosing. Levetiracetam was quantitated in serum using an ARK Diagnostic Levetiracetam® Immunoassay (Sunnyvale, CA) on a Siemens Dimension Xpand (New York, NY) general chemistry analyzer.

Mean pharmacokinetic parameters were estimated using a non-compartmental analysis. The concentration time profiles resembled characteristic absorption, with maximum plasma concentrations ( $C_{max}$ ) of 56.5 and 93.9 mg/L ( $T_{max}$ ) at 30 to 60 min; terminal half-lives ( $t_{1/2}$ ) at 2.38 and 2.37 hrs; volumes of distribution ( $V_d$ ) at 0.807 and 0.773 L/kg; areas under the curve (AUC) of 14,125 and 28,182 mg•min/L, and clearance rates (Cl) at 3.65 and 3.60 mL/min/kg for 50 mg/kg and 100 mg/kg respectively. Plasma concentrations were greater than 5 mg/L for up to 9.4 and 12 hrs suggesting an 8hr and 12hr oral dosing at 50 and 100 mg/kg respectively would be sufficient to maintain systemic drug levels at or above what has been found to be clinically therapeutic in humans.

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**COMPARISON OF AIR SAC AND LUNG VOLUME IN AWAKE AND ANESTHETIZED HUMBOLDT PENGUINS (*Spheniscus humboldti*) POSITIONED IN VENTRAL, DORSAL, AND RIGHT LATERAL RECUMBENCY USING COMPUTED TOMOGRAPHY**

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**Abstract**

Captive penguins are commonly affected with respiratory disease and are especially prone to fungal disease (e.g. aspergillosis), which may be difficult to detect and treat. In this prospective study, computed tomography (CT) was used to compare air sac and lung volumes in 25 Humboldt penguins (*Spheniscus humboldti*) in four different positions: awake ventral (V), anesthetized ventral (AV), anesthetized dorsal (AD), and anesthetized right lateral (ARL). This was performed to determine which position allowed for the greatest air sac and lung volumes, providing information on the most appropriate position for this species under anesthesia. Physiologic parameters under anesthesia were recorded in each position. Air sac and lung volumes were calculated from helical CT images using designated image analysis software. A repeated measures ANOVA was used to determine if air sac or lung volumes differed by position while controlling for recumbency order and sex. There was a significant difference in air sac volume by position; however, recumbency order and sex were not found to influence the results. Differences in air sac volume were found between V and AV, V and AD, AV and ARL, and AD and ARL. There was no difference between V and ARL air sac volumes. There was no significant difference in lung volume by position, and sex and recumbency order were not found to have a significant interaction. In conclusion, lung volumes were not affected by position; however dorsal recumbency resulted in the lowest air sac volumes under anesthesia in spontaneous breathing Humboldt penguins.

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## THE BIG PICTURE: EVALUATION AND MANAGEMENT OF AGGRESSIVE BEHAVIOR IN CAPTIVE ANIMALS

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### Abstract

Aggressive behaviors are one major reason that zoo staff seeks assistance from veterinarians. A wide range of behaviors may be considered aggressive and assistance is often sought only after the frequency, duration, or intensity of the relevant behaviors have become intolerable to husbandry staff or are considered significantly atypical for the species. In many cases, the behavior has escalated to the point where the animal is consistently injuring itself or other animals, or threatening human safety.

Behavior, and why behavior develops or changes, can be examined and understood with a variety of sciences or models (e.g., neuroendocrine, genetic, physiology, etc.). These different approaches lead to different hypotheses about why the behavior is happening and different treatment or management strategies. Three major models directly relevant to clinical veterinarians include medical, ethological (natural history), and applied behavior analysis. These approaches are not mutually exclusive and when utilized together enhance the development and success of humane behavior change strategies.

Veterinarians are trained to consider medical causes for behavior. For example, increased aggression (e.g., biting when approached) can be a sign of disease such as pain, a brain tumor, or abnormal neurochemistry. Neurophysiologic research has demonstrated that some problem behaviors may be due to differences in brain activity at a molecular level.<sup>1,5</sup> The development of some problem behaviors is likely a combination of both biological predisposition and development events. For instance, animals raised in barren environments or under a great deal of stress during the early weeks, months or years of development have fewer neurons in the brain, decreased dendritic branching and spine density, and reduced synaptic connectivity compared to animals raised in enriched environments.<sup>1,5</sup> Many captive animals are reared in environments dissimilar to natural environments and this may contribute to neurophysiologic abnormalities, though this is currently speculative.

Whatever the cause, it is likely that some portion of a given population has neurophysiologic dysfunction that limits the individual's ability to learn and behave normally in common environmental situations. In addition, some animals may be normal but less able to cope with the physical, mental, and behavioral limitations of a given captive environment, either temporarily or long term. In these cases, using appropriate psycho-pharmaceutical medications such as serotonin reuptake inhibitors (e.g., fluoxetine) or benzodiazepines (e.g. alprazolam, diazepam) may be effective in reducing



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aggression.<sup>2</sup> Antipsychotics, such as haloperidol, produce inconsistent results for the treatment of aggression and may increase aggression in some cases. Antipsychotics lead to overall suppression of behavior and their high incidence of side effects make them inappropriate for long term therapy.<sup>2</sup> The effectiveness of any psychotropic medication can be challenging to predict. In addition, many of these medications take a long time to show efficacy. This needs to be taken into account when effectively planning interventions. It is inappropriate to expect medication to compensate for a poor environment. In addition to medications, increasing the animal's behavioral control and choice through appropriate enrichment and effective positive reinforcement training is generally critical to successful management plans, including those in which medication is called for and effective.

In the zoological field, we often consider ethological correlates for behavior and behavior change, i.e., the behavioral adaptations that have evolved to fit the animals' ecological niche. For example, increased aggression can be understood as an inherited modal action pattern such as a territorial defense chain elicited by breeding season cues, or secondary to social, hierarchical influences within a group. To complicate matters, many captive animals have not been reared in normal social groups and may not have learned to respond to other animals' cues typically, leading to increased or inappropriate levels of aggression.

Animals may display aggressive behaviors which are typical for the species, but which are disruptive to captive management goals or displayed at a greater intensity than desired for success in captivity.<sup>7</sup> These behaviors are often relevant to the reproductive cycle. In these cases, medications that specifically reduce reproductive hormones may reduce aggressive behaviors; examples include GnRH agonists (e.g., deslorelin or leuprolide), or progestins to suppress ovarian cycling (e.g., melengestrol acetate, megestrol acetate, medroxyprogesterone acetate). In addition, a thorough review of the individual (and where applicable, the group) behavior pattern is appropriate to better understand and document social interactions and other environmental stimuli that are associated with aggressive behaviors. For example, a subordinate (versus the more easily identified aggressor) may be precipitating aggressive events through inappropriate responses to social cues. Understanding the natural history of a species is important when conducting a thorough evaluation of the animal's environment, as well as ensuring that staff expectations for behavior are appropriate.

In addition to these more familiar models, behavior analysis is critical for understanding how a specific behavior emitted by an individual animal is learned and maintained, due to interaction with the environment in which it occurs.<sup>4</sup> Behavior analysis is a trans-species science that investigates the universal laws of behavior change due to experience, i.e., learning. Applied behavior analysis (ABA), the behavior change technology derived from behavior analysis, takes the individual animal's learning history and current environmental conditions into account and investigates the purpose (i.e., function) the behavior serves for the animal. In the above example, we can hypothesize that the increased aggressive behavior is the result of learning, i.e., the behavior was reinforced in the past. Even complex, severe aggressive behaviors are responsive to this approach.<sup>7</sup>

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From the ABA perspective, understanding and changing behavior results from identifying the discriminative stimuli that set the occasion for the behavior (i.e., setting events, motivating operations, and discriminative stimuli), and the consequences that give the behavior strength (frequency, duration, intensity, etc.). The focus of the behavior change plan is to modify the environment to set the occasion for appropriate alternative behaviors and reinforce them when they occur. With ABA, we change the environment to change the animal's behavior.

Reviews of behavior analysis science for veterinarians exist and a functional assessment and intervention design (FAID) worksheet for evaluating problem behavior and developing appropriate behavior support plans is also available.<sup>3,4,6,7</sup> The FAID worksheet provides a standardized approach to cases (similar to a SOAP format) and prompts a complete, individualized evaluation of the problem behavior situation and development of a plan specific to that individual in that environment. A summary of five major questions related to behavior is provided (Table 1). This model provides a powerful, systematic method of behavior evaluation and intervention. The ABA approach is under-utilized by most veterinarians and husbandry staff due to lack of training and general under-estimation of the importance of prior learning and current conditions as a major factor governing actual behavior in individual animals.

Veterinarians should utilize all three models when diagnosing and managing aggressive behavior displayed by animals so that relevant medical conditions, ethological, and learning variables are all evaluated.

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**Table 1.** Summary questions to prompt investigation of the environmental factors related to problem behavior.

Question	Purpose of Question	Example
What does the behavior look like?	Reduce use of labels and focus on actual, observable behaviors in preparation for identifying relevant environmental stimuli that predict and maintain the behaviors. If multiple disruptive behaviors are present, each should be evaluated individually.	Labeling the animal: The animal is aggressive. Operationalized behavior: Behavior A: The animal lunges at the door and bites the air. Behavior B: The animal pushes into the other male and bites his neck.
What conditions predict when the behavior will occur (when is the behavior most likely)?	Identify the relevant environmental stimuli that cue or set the stage for the behavior.	Behavior A happens when keepers approach the door with food. Behavior B happens when browse has been added to the enclosure.
What does the animal get from or get away from by doing the behavior.	Identify the relevant environmental stimuli that are reinforcing (maintaining) the behavior.	After behavior A happens, food is left in the enclosure and the keeper leaves the area. After behavior B happens, the other animal leaves the area and the first animal has access to the browse.
Under what conditions does the animal not exhibit the behavior (when is the behavior least likely)?	Identify the environment when the animal is most successful. This step helps staff realize there are environments in which the animal is successful and the problem behavior is not occurring.	Behavior A is least likely to happen if a keeper approaches without food. Behavior B is least likely to happen when the females, as well as other male, are present or the two males are fed hay.
What can the animal do instead?	Identify another behavior the animal can do in place of the problem behavior. These are behaviors that can be trained (reinforced). In some cases, reinforcing another behavior is not possible and management of environmental stimuli to not cue the problem behavior is appropriate.	Instead of doing behavior A, the animal can stand with his head near the water bowl when food is added to the enclosure. Instead of doing behavior B, the animal can eat browse in a separate part of the exhibit, or the two males are not fed browse when they are being housed without the females.

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**RELATIONSHIPS OF DOMINANCE AND TRACTABILITY WITH SERUM TESTOSTERONE AMONG A BACHELOR GROUP OF AFRICAN ELEPHANTS (*Loxodonta africana*)**

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**Abstract**

The Birmingham Zoo is working toward managing four bull African elephants as a single bachelor group. This had not been attempted previously in the United States, and it was unknown if competition or a lack thereof would influence antagonistic behavior or serum testosterone concentrations. Keeper safety also was a concern, and methods for anticipating behavioral and endocrine changes in the bulls were desired. To measure dominance, the bulls were each observed for a total of 9.5-17.9 hrs while on exhibit in pairs or trios. Dominance was determined using the percentage of the total antagonistic interactions that resulted in a “win” for each bull in every dyad, which then was organized in a dominance matrix. To measure tractability, keepers rated the bulls’ performance during daily training exercises. Blood was collected weekly, and testosterone was measured by enzyme immunoassay. A dominance hierarchy evolved in which the largest bull is most dominant, but he is followed by the second smallest bull, followed by the second largest and the smallest bulls together at the base. This result is different from wild, non-musth bulls whose dominance is correlated with size. Mean testosterone concentrations differed between only the oldest bull and the youngest bull ( $p < 0.05$ ), and therefore appear not to be related to dominance. There was no correlation ( $p > 0.05$ ) between testosterone concentrations and daily ratings of tractability. Testosterone levels that might be considered indicative of musth were not observed, so the possibility for tractability to diminish in association with testosterone fluctuations of that magnitude still exists.

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## COMPARATIVE ASSESSMENT OF ADRENOCORTICAL ACTIVITY AND TEMPERAMENT IN THREE CHEETAH POPULATIONS MAINTAINED FOR EDUCATION, EXHIBITRY AND PROPAGATION AT THE CINCINNATI ZOO & BOTANICAL GARDEN

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### Abstract

Fecal corticoid levels are frequently monitored as an indirect indicator of “stress” in wildlife species, including cheetahs (*Acinonyx jubatus*). In zoos, cheetahs often serve as education ambassadors but the relative effect of these activities on adrenocortical function is unknown. In this study, our goal was to assess and compare fecal corticoid levels among the three separate cheetah populations maintained for educational, exhibitry, and propagation purposes at the Cincinnati Zoo & Botanical Garden. Our specific objectives are to 1) compare variations of basal and peak fecal corticoid levels among these cheetah populations; and 2) assess the correlation between corticoid levels and each individual’s temperament. Fecal samples were collected (3-7 samples/week) for 10 wk from 12 cheetahs within the 3 groups (education, 3.2 cats; exhibitry, 0.2 cats; propagation, 3.2 cats). Cat keepers (2-3/population) also completed a temperament survey for each subject animal to allow interpretation of fecal corticoid levels relative to specific personality traits. For analysis, fecal samples (n=428) were freeze-dried and extracted for measurement of corticoid levels using a validated corticosterone enzyme-immunoassay. Overall, basal corticoid levels were lower ( $P < 0.01$ ) in cheetahs in the education program (mean  $\pm$  SEM;  $176 \pm 6$  ng/gm feces) and on public exhibit ( $409 \pm 71$  ng/gm) than for cheetahs in the propagation program ( $885 \pm 103$  ng/gm). Peak corticoid levels also were higher ( $P < 0.01$ ) in breeding cheetahs ( $2092 \pm 134$  ng/gm) compared to education cheetahs ( $484 \pm 41$  ng/gm) and cheetahs on exhibit ( $707 \pm 216$  ng/gm). Across populations, individuals that were tense, fearful of people or showed aggression to humans had higher ( $P < 0.05$ ) peak corticoid levels, whereas cheetahs that vocalized frequently or were friendly to people showed lower ( $P < 0.01$ ) peak values. These findings indicate that fecal corticoid concentrations may show substantial variation among cheetahs, depending on their temperament, daily stimuli and life histories. Notably, adrenocortical activity in cheetahs that serve as education ambassadors was similar to that of cheetahs maintained on public exhibit, and reduced relative to cheetahs managed for propagation at an off-exhibit breeding facility.

### ACKNOWLEDGMENTS

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## THE IMPORTANCE OF REVERSAL DATA IN MAKING CONTRACEPTION RECOMMENDATIONS

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### Abstract

The mission of the AZA Wildlife Contraception Center (WCC) is to provide information and recommendations about contraceptive products that are safe, effective, and reversible. The WCC relies on feedback from the zoo community to integrate these three mission pillars into frequently updated recommendations. While communication about safety and efficacy between the WCC and zoo professionals has always been strong, collecting reversal data has proven a challenge. The lack of reversal data has grown especially concerning in recent years due to the long suppression times associated with deslorelin (Suprelorin<sup>®</sup>, Virbac Animal Health, Carros cedex, France) use. Animals treated with Suprelorin<sup>®</sup> are often suppressed much longer than the minimum durations of effect suggested by the implant manufacturer. Consequently, timing breeding recommendations has grown increasingly difficult as some individuals do not reproduce for many years after treatment or may even remain permanently suppressed.

There are several challenges associated with collecting reversal data, challenges that make calculating times to reversal extremely complicated. At the most basic level is the question of whether an individual ever had an opportunity to breed after treatment. Without feedback from the housing institution, it is impossible to categorize an animal as one that never returned to cycling or one that simply was treated and was never given mate access. Another challenge is a byproduct of the nature of master planning, which frequently involves transferring individuals for breeding. Once an animal leaves the institution that administered contraception, the institution is no longer responsible for providing updates on that animal. Similarly, the receiving institution may not provide reversal data to the WCC since the animal was contracepted at a different location. Thus, critically important reversal data fall through the cracks. Another scenario that leads to loss of reversal data occurs when an animal is treated with contraception and then has a planned or unplanned pregnancy many years later. Because the contraceptive was not administered recently, the reversal data may not be reported to the WCC.

Since lions have been treated with Suprelorin<sup>®</sup> more than most other species, the WCC formed a formal relationship with the AZA Lion SSP to develop a model for improving the efficiency of reversal data collection. Thanks to excellent institutional feedback and support from the AZA Lion SSP Coordinator, we know more about reproductive recovery in lions than in any other species. Evidence is accumulating that the pituitary hormone that stimulates follicle growth (FSH) and estrogen production (which can stimulate estrous behavior) may recover before the hormone necessary for ovulation (LH). And, with seven recorded reversals to date, the average time from implant placement to conception can be calculated. For all African lions given the 9.4mg

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Suprelorin<sup>®</sup> formulation (n = 57), four lions reversed, conceiving between 2.3 and 3.6 yr later. For all lions treated with the 4.7 mg formulation (n = 17), two lions gave birth, conceiving 4.4 and 3.5 yr after implant placement. One of these females had an extensive contraceptive history, which may have affected recovery time. In contrast, all of the females who reversed after being given 9.4 mg implants had no prior contraceptive history. Finally, when either an unknown formulation or a 6mg implant was used (n = 10), one animal given the 6mg implant conceived 2.27 yr later.

Statistical figures enter a gray area when attempting to calculate the average time that has passed since treatment for lions that have breeding recommendations, but have failed to conceive. Despite having relatively complete records for this species, there are many factors complicating our ability to predict when an animal may reverse. Life history traits such as previous parity and age affect fertility whether or not a female has received contraception. Cumulative effects of multiple contraceptive treatments may delay return to fertility. Moreover, preliminary data from lions show that recovery is complex and that some points in the pituitary-gonad axis are restored before others. So, while birth of live offspring is the gold standard for reversal, feedback concerning return of estrous behaviors and hormone monitoring is invaluable as well.

In addition to continuing routine collection of information about safety and reliability of contraceptives, the WCC is increasing efforts to collect reversal data, but to do so more efficiently. Our goal is to be able to provide more accurate estimates to veterinary staff and managers about durations of contraceptives, especially Suprelorin<sup>®</sup>. To accomplish this goal, we have identified plans of action targeted at different communities within AZA. One recent development that will positively impact AAZV members emerged from the partnership formed between the WCC and the European Group for Zoo Animal Contraception (EGZAC). Our annual contraception survey will transition from paper and electronic reports to online data entry; the interface will provide an easy-to-navigate, one-stop site for institutions to view the entire contraceptive histories of their animals. Online survey entry will be available 24/7 so that contraceptive information can be added when administered rather than through the tedious process of checking records to complete the survey once per year. Thus, this technology will make data submission easier as well as help fulfill the WCC's need to flag records that are missing reversal information. This project is currently in the test phase and is expected to be rolled out to institutions in 2014.

While the WCC has identified and started implementing strategies to improve reversal feedback, suggestions and input from the AAZV are encouraged and welcomed. There should always be an open line of communication between the Center and zoo veterinarians so that we can address any concerns that arise as well as provide the most current recommendations. As institutional feedback increases, we hope to collect enough data for proper analysis to serve as the basis of improved reversibility statistics to the zoo community, strengthening the foundation of the mission of the Center.

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## THE EFFECTS OF CONTRACEPTIVES ON THE REPRODUCTIVE TRACT OF FEMALE OTTERS

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### Abstract

Otters are popular exhibit animals that present a reproduction management challenge; on one hand, otters are often kept in groups, necessitating contraception. However, specific individuals may be required to breed, but have low reproductive success. In other carnivore taxa such as canids and felids, subclinical histopathological lesions of the reproductive tract may be a significant cause for infertility or subfertility. Lesions in the endometrium are frequently associated with exposure to endogenous and/or exogenous ovarian hormones in canids and felids.<sup>1-2</sup> However, the literature on reproductive endocrinology and reproductive histopathology for otters is scarce.

The goal of this study is to evaluate the reproductive tracts of several species of otters, both contracepted and non-contracepted, in order to determine if contraception status plays a role in reproductive pathology in female otters. Preliminary results suggest that otters as a group may have a propensity for endometrial hyperplasia regardless of contraception status, as 80% of the otters currently in this study (16/20) have some degree of endometrial hyperplasia. Age may also be a factor, as 56% of otters currently in this study with moderate to severe hyperplastic change (9/16) were geriatric ( $\geq 10$  yrs). A better understanding of naturally occurring lesions, as well as the lesions associated with exogenous hormones, will allow managers to improve breeding success, overall health, and safety and potential reversibility of contraception.

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## NEW TOOLS FOR EARLY DIAGNOSIS OF ENDOMETRIAL DISEASE IN CAPTIVE WILDLIFE

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### Abstract

With limited space for individual species among zoological institutions, reproduction must often be controlled and maintaining non-reproductive females is common. This is achieved either through separation from males, pharmacological contraception, or surgical sterilization; however, preventing pregnancy may have serious unintended consequences. Individual cases of cystic endometrial hyperplasia (CEH) and life-threatening pyometra in different wild canid females have been reported.<sup>1,6,9,11,12</sup> CEH and pyometra are common conditions in older, nulliparous, intact female domestic dogs.<sup>15</sup> A review of medical records and pathology reports of the seven managed canid species in North American zoos showed that African painted dogs (*Lycaon pictus*) and red wolves (*Canis rufus*) are at especially high risk for development of CEH and pyometra.<sup>4</sup> Occurrence of these conditions was positively correlated with non-reproductive years and the use of deslorelin (Suprelorin<sup>®</sup>, Virbac Animal Health, Carros cedex, France) as a contraceptive agent. Suppressing the initial surge in gonadotropins after deslorelin implant placement by administering megestrol acetate, however, reduces the risk of CEH and pyometra with deslorelin use.<sup>3</sup> Clearly, the chronic, non-pregnant state predisposes the endometrium to disease in many canid species. Observations suggest that this may also be true in other taxa, such as stingray, bats, felids, elephants, rhinoceros, wildebeest, and equids.<sup>2,8,13,14</sup>

Ultrasound has shown some promise for diagnosis of more advanced cases of CEH,<sup>5</sup> but lacks the sensitivity to detect early stages of CEH, requires high-quality imaging, a skilled operator, and cannot detect fibrosis or inflammation, which may also cause significant subfertility in domestic canids.<sup>10</sup> Transcervical endometrial biopsy (TCEB) in domestic dogs can provide histopathological diagnoses of fibrosis, inflammation, and early stages of CEH which correlate well with full-thickness biopsy sample results from the same uteri.<sup>7</sup> In canids, it is recommended to perform TCEB outside of diestrus to avoid potentially inducing pyometra.<sup>7</sup> A similar technique is standard practice in fertility evaluations of domestic horses.<sup>16</sup> TCEB also requires specialized equipment and skill, but avoids surgery and can diagnose different types of uterine pathology at early stages of disease. This promising diagnostic tool may help identify individual zoo animals in at-risk categories that have endometrial disease before obvious clinical signs are present. Targeted treatments for infectious or inflammatory conditions include systemic and local

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(transcervical) antibiotics and anti-inflammatory medications. Treatment for cystic endometrial hyperplasia includes suppression of estrus/diestrus, or prolongation of anestrus, followed by breeding for pregnancy on the next estrous cycle. Early diagnosis and targeted treatment of these animals may save lives and prevent more serious infertility.

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## TREATMENT OF DIABETES MELLITUS IN VETERINARY PATIENTS

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### Abstract

With constantly changing information and availability, it can be difficult to make the best choices for insulin in veterinary patients. The characteristics of available insulin products, including NPH, lente, PZI, glargine, and detemir, as well as the rapid-acting insulins lispro, aspart, and glulisine, are reviewed in this article. The new human insulin analog, degludec, will be introduced briefly. Strategies for monitoring insulin therapy will also be reviewed. Oral medications used for treatment of type 2 diabetes will also be reviewed.

### Types of Insulin

There are many different types of insulin that vary with species of origin and with chemical modifications and formulations that affect onset and duration of action (Table 1). Porcine insulin, which is identical to canine insulin in its amino acid structure, is available for use in dogs in some countries, but, unfortunately, no specific feline insulin formulation is currently available. Human, bovine, or porcine insulins are used in treating both diabetic cats and dogs. Data concerning the pharmacokinetics and pharmacodynamics of insulin in dogs and cats are difficult to interpret. Most published studies have been conducted in normal animals, and some have been done in animals with diabetes. In either case, it is difficult to determine the effects of endogenous versus exogenous insulin. Determinations of potency, time to peak activity, and duration of activity— factors that influence choice of doses and dosing intervals— vary widely from animal to animal. In fact, there is no reasonable way to predict the kinetics of a given insulin preparation in any given patient.

In cats, neutralprotamine Hagedorn (NPH) insulin (Humulin-N, Eli Lilly; Novalin N (Novo Nordisk), porcine lente insulin (Vetsulin), and protamine zinc insulin (ProZinc, PZI) have been used commonly to treat diabetes mellitus. In recent years, insulin glargine (Lantus) has probably become the most commonly used insulin preparation in cats, despite relatively little published evidence supporting its use. Recently, another insulin analog, insulin detemir (Levemir), has received some attention among veterinary researchers and feline practitioners. In dogs, porcine lente insulin and NPH are used most commonly.

### NPH and Lente

NPH is considered an intermediate-acting insulin and is available as a human recombinant product. NPH is used commonly in animals with diabetes, and is typically

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given subcutaneously twice daily. Lente insulin uses zinc as a positively charged ion on which to base insulin polymerization. Polymers are absorbed and metabolized slowly so that the onset and duration of lente insulin are extended beyond those of regular insulin. Human recombinant lente insulin has been removed from the United States market and is no longer available for use. Porcine lente insulin, however, has gained in popularity in some countries and is currently marketed and labeled for use in cats. The only currently available veterinary product is Vetsulin® (the tradename is Caninsulin in other countries). This insulin product was withdrawn from the U.S. Market, but has recently become available again. While identical to canine insulin, porcine insulin is dissimilar in amino acid sequence when compared with feline insulin, but it is no more divergent (by three amino acids) than is human insulin. Lente is typically given twice daily by subcutaneous injection, and studies show it is a reasonable choice for treating diabetic cats.<sup>15</sup> A recent study suggested the duration of porcine lente is shorter than either PZI or glargine in cats.<sup>8</sup>

### **Protamine Zinc Insulin**

PZI has been used extensively in feline diabetes. It is typically given subcutaneously twice daily, with a starting dose of 1-3 U/cat. This insulin preparation was widely available, but was largely removed from the human market in the 1990s. Recently, PZI preparations marketed for use in cats have once again become available. A human recombinant protamine zinc insulin product approved for feline diabetics was introduced in 2009. This insulin was the subject of a multi-center clinical trial reported by Nelson et al.<sup>11</sup> In that study of 133 diabetic cats, “good” glycemic control was defined as an average blood glucose concentration below 200 mg/dL during a 9-hr blood glucose curve. A glucose nadir of less than 150 mg/dL was also considered good, as was a serum fructosamine concentration lower than 450 µmol/L. In that study, after 45 days of twice-daily treatment with PZI, 60% of cats exhibited good glycemic control based on the glucose nadir. Seventy-five percent of cat owners reported improved polydipsia and 79% reported improved polyuria.

### **Glargine and Detemir**

Insulin glargine is a genetically engineered insulin analog that has hormonal action identical to native insulin, has no known immunogenicity, and achieves long-lasting glycemic control while minimizing fluctuations in blood glucose concentration in many human diabetics. Glargine is based on human recombinant insulin with a few amino acid substitutions. Glycine is substituted for an asparagine residue at the amino terminal of the A chain, and two arginine residues are added to the end of the B chain. The result is a shift in the isoelectric point of the insulin molecule so that it is completely soluble at a low pH (around pH 4). The pH of interstitial fluid is approximately 7.4, and when glargine is injected into a patient, the insulin precipitates into hexamers that are inactive. These insulin hexamers are slowly broken down in the body to form active insulin monomers. The result is that the onset is gentle and the duration is long-lived. Because of the difference in pH, glargine cannot be mixed with other insulin formulations. Experience with using glargine in cats is growing,<sup>15, 21</sup> and many clinicians have had

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good success with its use. Glargine is best used twice daily subcutaneously. A study of glargine use in dogs was reported recently.<sup>4</sup> In that study 12 client-owned diabetic dogs were treated with insulin glargine BID for 24 weeks, and treatment resulted in significantly lower mean blood glucose concentrations and improvement in polyuria/polydipsia in 91 percent of dogs.

Insulin detemir has been used in Europe for several years and was just recently approved for use in the US. Rather than having amino acid substitutions (like insulin glargine), insulin detemir is acylated with myristic acid, which still allows some hexamers to form at neutral pH, but, more importantly, allows the insulin to bind to albumin. This results in a very slow, smooth delivery of insulin such that once-daily delivery is all that is needed in many human patients.

We have done some work comparing the pharmacodynamics of glargine, detemir, and regular insulin in cats.<sup>5</sup> In the cats we have studied we have found detemir to be relatively “peakless” and its duration of action is similar to that of glargine. The effect of detemir seems to be more predictable and consistent between cats, based on our studies, but this bears further investigation. The mean duration of action of insulin detemir was 13.5 hr, while glargine’s mean duration of action was 11.3 hr. The difference between the two was not significant, and it was concluded that either analog might be useful as a once-a-day insulin treatment in a given cat, while others would need more frequent dosing.

Another recent study has demonstrated similar effects of glargine and detemir in cats, although the dose of detemir needed to achieve the same clinical effect was less than with glargine.<sup>17</sup> One advantage of detemir might be that, because it is bound to albumin, it reaches higher concentrations in organs with fenestrated capillaries, especially the liver. This more closely mimics circulation of insulin in normal physiology. We have observed no toxic effects of detemir in cats we have studied, and it seems reasonable that detemir could be tried if adequate glycemic control is not achieved with other types of insulin.

There are very little clinical data available to guide the use of glargine and/or detemir in dogs. We have used both insulin preparations in dogs, however, especially in situations in which we suspect short duration of action of NPH as an underlying reason for poor glycemic control. Glargine can be dosed similarly to other insulin types, but not detemir. Detemir contains four times more insulin per unit than other preparations because of its lower affinity for the human insulin receptor, an affinity that may be greater in dogs, and that would increase the risk of hypoglycemia. In fact, one study showed a very high potency of detemir in dogs compared with other insulins.<sup>18</sup> Detemir should be used when the patient is large enough that dosing at 0.1 to 0.2 U/kg is possible using U-100 insulin, when there is evidence that NPH is very short-acting, if other insulin preparations have been tried without success, or any combination of these factors. Detemir can be used once a day in some dogs and may be needed twice a day at starting doses of 0.1 to 0.2 U/kg/dose.

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## **Lispro, Aspart, and Glulisine**

Historically, a combination of regular insulin and an intermediate-acting insulin was used to replace postprandial insulin in human diabetic patients. The action profile of regular insulin after subcutaneous (SC) injection may be inadequate, however, for the treatment of diabetes because its absorption is relatively slow and the duration of action is too long (about 5–8 hr in people, about 5 hr in cats and dogs).<sup>14,16</sup> Insulin lispro was the first rapid-acting analog to be approved for use in people.<sup>20</sup> The amino-acid sequence of insulin lispro consists of a reversal of proline at the B28 position and lysine at the B29 position. This small change greatly decreases the tendency for association and enhances the rate of absorption. In insulin aspart the proline residue at B28 is replaced with an aspartic acid residue. In insulin glulisine, lysine at B29 is replaced by glutamic acid and on position B3 asparagine is replaced by lysine. Insulin aspart and insulin glulisine have pharmacokinetic and pharmacodynamic profiles similar to insulin lispro.<sup>14</sup>

There are no reports on the use of rapid-acting analogs in the chronic treatment of diabetes in cats. Insulin lispro has been successfully used in dogs to treat diabetic ketoacidosis (DKA).<sup>19</sup> In that study insulin lispro was administered intravenously and had similar efficacy as the traditionally used regular insulin. No adverse reactions were seen. There is no clear rationale for preferring insulin lispro over regular insulin for use in constant-rate intravenous insulin infusions. The biochemical alteration in insulin lispro confers greater dissociation and faster absorption of insulin injected subcutaneously, but both insulin lispro and regular insulin should dissociate immediately when delivered intravenously.

## **Degludec**

Insulin degludec is the newest insulin analog to be garnering attention in the clinical diabetes community. Degludec is an ultra-long-acting human insulin analog in which the insulin molecule is conjugated to a hexadecanedioic acid residue linked to a gamma-L – glutamyl spacer at the end of the B-chain. This product is currently not available commercially in the United States, but its manufacturer reportedly plans to begin marketing the product within the next 2 yr. In people, this insulin analog has an extremely long half-life, and is being evaluated for a three-times-weekly dosing scheme.<sup>7</sup> Should this treatment regimen be successful in other species, it could potentially revolutionize insulin therapy for veterinary patients.

## **Monitoring Insulin Therapy**

Blood glucose curves have been used extensively to monitor insulin therapy in dogs and cats. Conventional wisdom has dictated that adjustments in insulin therapy, whether changes in dose, frequency, or type of insulin, only be made based on serial blood glucose measurements. The validity of blood glucose curves has been called into question by several recent studies. A European group of investigators studied the differences in blood glucose curves performed in-hospital vs. those performed by owners at home.<sup>1</sup> Owners were instructed in blood sample collection using a simple micro-lance

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technique, and blood glucose concentrations were determined using portable meters. When interpreting blood glucose curves in the hospital, veterinarians commonly consider that there may be a falsely elevated degree of hyperglycemia because of the stress of hospitalization. Surprisingly, Casella and colleagues found the opposite to be true. Following repeated curves in the same dogs multiple times at home and in the hospital, it was found that mean blood glucose concentrations were significantly higher when dogs were tested at home. Recommendations for changes in insulin therapy were different 42% of the time based on the at-home vs. in-hospital curves.

Another study of blood glucose curves found more startling results. Investigators in Australia performed repeated blood glucose curves in dogs two weeks apart with no change in insulin therapy protocols, and compared differences between the curves.<sup>3</sup> Minimum, mean, and maximum blood glucose concentrations, as well as morning and evening pre-insulin glucose concentrations, and the time to reach the nadir in blood glucose were compared. Variations in these measurements within the same dog were striking. For example, the coefficient of variation for the maximum glucose concentration was 100%, and for the time-to-nadir the CV was 98%. These results tell us that blood glucose curves may not be as helpful as we once thought. Certainly, changes in insulin regimens should be made conservatively when based on a glucose curve. Also, other methods of assessing glycemic control might be more important. Clinical signs of hyperglycemia (polyuria, polydipsia, appetite changes, weight loss, cataracts, etc.) and serum concentrations of fructosamine may be more useful indicators of glycemic control than blood glucose curves in many dogs.

How then are blood glucose curves useful? Veterinary academicians argue as to the existence of the Somogyi phenomenon, but whether some dogs experience true insulin-induced hyperglycemia (from a rebound effect in response to hypoglycemia), or whether it is a simple question of insulin kinetics, wide swings reminiscent of the Somogyi effect are possible. For example, a dog receiving insulin might respond quickly with profound hypoglycemia. If the dog experiences insulin-induced hyperglycemia or if the action of the insulin is short-lived, the dog can return quickly to a hyperglycemic state. In this instance, fructosamine would remain elevated (from persistent hyperglycemia), and clinical signs of hyperglycemia would persist. Without the information gained from a blood glucose curve, the dose of insulin might be mistakenly increased with disastrous consequences. Unfortunately, while they can only give a rough estimate of the duration of action of insulin or the appropriateness of a given dose, blood glucose curves may be the best means of identifying hypoglycemia, and they may have other uses as well. Still, the best strategy for monitoring insulin therapy may be by clinical response (resolution of polyuria/polydipsia, improvement in body condition and appetite, lack of signs of hypoglycemia).

### **Oral Hypoglycemic Drugs**

Oral medications have been available for many years for treatment of hyperglycemia in type 2 diabetic patients. It is important to note that oral hypoglycemic drugs are not useful for the treatment of type 1 diabetes, with the possible exception of acarbose (see

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below). Glipizide is the most commonly used of these drugs in veterinary medicine, but its use is limited to cats. Most oral hypoglycemic agents rely on a certain level of endogenous insulin-secreting ability in the patient, and they act either to increase the amount of insulin secreted, by sensitizing target cells to insulin, or by decreasing gluconeogenesis so that hyperglycemia is curbed. One exception is the drug acarbose.

Acarbose is an alpha-glucosidase inhibitor that inhibits digestion of complex carbohydrates at the small intestinal brush border. This action slows the absorption of small carbohydrates (such as glucose), and blunts the post-prandial rise in blood glucose seen in many diabetic patients. The major side effects of the drug are flatulence and diarrhea, although I have not observed these effects in several dogs treated with the drug. Dogs seem to tolerate the drug well, and it can effectively treat post-prandial hyperglycemia in some diabetic dogs. In one study of acarbose in 5 diabetic dogs, insulin therapy combined with acarbose resulted in better control of hyperglycemia than insulin alone, although diarrhea developed in 3 of the 5 dogs when treated with acarbose<sup>12</sup>. The use of acarbose has also been reported in cats.<sup>10</sup> In that study, diabetic cats were fed a low-carbohydrate diet and treated with acarbose in addition to insulin or glipizide. Diabetes went into “remission” in 11 of 18 cats, but the effect of acarbose was not studied per se, controls were not included, and glycemic control was not documented. No adverse effects were observed.

Glipizide (Glucotrol®), a sulfonylurea drug works by stimulating insulin secretion from the pancreatic beta cell, and it has been used fairly extensively in cats, usually in combination with a low-carbohydrate diet. In cats, it is given at a dose of 5 mg/kg BID, and reported side effects have included vomiting (15% of patients), hypoglycemia, and cholestatic liver disease.<sup>2</sup> Glyburide is another commonly prescribed sulfonylurea drug used in human diabetes (sold under several tradenames), but it has not been evaluated in veterinary diabetic patients.

Biguanide drugs work by decreasing hepatic glucose production. The prototypical biguanide, metformin (Glucophage®, Bristol-Myers Squibb), has been used extensively for decades in the treatment of human diabetes and pre-diabetes, but its use in veterinary medicine has been limited. Because the drug does not stimulate insulin secretion, hypoglycemia is a rare side effect, but vomiting and diarrhea can occur with the use of this drug. In one small study of 5 diabetic cats treated with metformin, hyperglycemia was controlled in only one, and one cat died shortly after starting treatment. Although no biochemical abnormalities were observed, side effects included lethargy, inappetence, vomiting, and weight loss. Based on this small study, metformin should be used with caution in veterinary patients.<sup>13</sup> In human diabetes patients, metformin is sometimes combined with other oral hypoglycemic drugs.

Thiazolidinediones have received considerable attention in treating type 2 diabetes in human patients, and these drugs act by increasing tissue sensitivity to insulin. This is accomplished by binding to the nuclear receptor peroxisome proliferator-activated receptor gamma, resulting in increased sensitivity to insulin in fat and muscle cells. Troglitazone (Rezulin®), the first thiazolidinedione approved for use in the U.S., was



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removed from the U.S. market in 2000 due to the increased risk of hepatotoxicity in people, but rosiglitazone (Avandia®, GlaxoSmithKline) and pioglitazone (Actos®, Takeda) are still available. The use of these drugs has also been associated with increased risk of heart disease and other adverse events, and they should be used with caution in veterinary patients, if at all. This class of drugs has not been evaluated in cats with diabetes, but one study showed improved insulin sensitivity in obese laboratory cats treated with darglitazone.<sup>6</sup>

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**Table 1.** Insulin products.

Type	Product	Product
Rapid-acting:		
Regular insulin <sup>a</sup>	Humulin R (Lilly)	U-100
Regular insulin <sup>a</sup>	Novalin R (Novo Nordisk)	U-100
Insulin lispro <sup>a</sup>	Humalog (Lilly)	U-100
Insulin aspart <sup>a</sup>	Novalog (Novo Nordisk)	U-100
Insulin glulisine <sup>a</sup>	Apidra (Sanofi-Aventis)	U-100
Intermediate-acting:		
NPH (isophane) <sup>a</sup>	Humulin N (Lilly)	U-100
NPH (isophane) <sup>a</sup>	Novalin N (Novo Nordisk)	U-100
Protamine zinc insulin <sup>a</sup>	ProZinc (Boehringer Ingelheim)	U-40
Porcine insulin zinc <sup>b</sup>	Vetsulin (Merck)	U-40
Long-acting:		
Insulin glargine <sup>a</sup>	Lantus (Sanofi-Aventis)	U-100
Insulin detemir <sup>a</sup>	Levemir (Novo Nordisk)	U-100

<sup>a</sup>human recombinant

<sup>b</sup>porcine origin

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## A RISK-BASED QUARANTINE PROGRAM: AN ALTERNATIVE TO ONE SIZE FITS ALL

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### Abstract

Zoo quarantine programs are typically based on laboratory animal models. Suggested programs have uniform quarantine periods for most importations (i.e., 30 days), a set battery of tests for broad taxonomic groups (i.e., psittacines), and strict animal separation protocols, imposed regardless of the imported animal's origin or the existing collection. These models are based on several assumptions: in-coming animals are medically novel (i.e., arriving without any medical history, such as an animal imported from the wild); all in-coming animals represent an equal risk to the collection; and the entire or the bulk of the collection is at equal risk to new additions.

These assumptions are, however, only occasionally true in today's zoological institutions. AZA institutions are now likely to acquire animals from other AZA-accredited institutions, where they have received good health care and frequently preshipment examination and testing. For example, there is, in our opinion, little reason to treat a captive-born gorilla, with a life-long medical history, identically quarantine-wise to a wild-caught, recently imported non-human primate. Additionally, due to the diverse nature of importations and zoo collections, most imported animals are risks to only a limited portion of the collection. For example, few imported reptiles represent any risks to a zoo's mammal or bird collection. Lastly, quarantine space is limited, and personnel time to care only for quarantine animals can be costly. In short, traditional quarantine programs can be a considerable expense to an institution, and this cost can be disproportionate to the amount of actual protection they might provide a collection.

A risk-based quarantine program designs quarantine requirements based on the in-coming animal's history (medical and husbandry), the species imported, and the collection at risk. New imports from the wild or an unknown source might undergo traditional quarantine, but animals with good medical histories and preshipment testing might undergo abbreviated acclimatization periods, without strict husbandry separation. Species or taxa completely new to the institution might undergo quarantine *in situ*. Our premise is risk-based quarantine programs can safeguard our animals, both those imported and those in the collection, without compromising animal health and with considerably less keeper and facility expense.

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## COMPUTER SIMULATION MODEL OF *Mycobacterium avium* SUBSP. *Paratuberculosis* CONTROL IN A ZOO: CAN WE IMPROVE OUR DISEASE SURVEILLANCE AND MANAGEMENT STRATEGIES?

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### Abstract

*Mycobacterium avium* subsp. *paratuberculosis* (*Map*) infection is a significant cause of morbidity and mortality in endangered wildlife and zoo animals.<sup>1,2</sup> The goal of this study was to use simulation to explore the impact of different *Map* surveillance strategies on infection incidence and detection for hoofed mammals housed in a zoo environment. Data from 20 yr of disease surveillance at San Diego Zoo Global facilities and scholarly literature were used to construct an animated, agent-based simulation model using Anylogic software<sup>a</sup>. The simulation was designed to capture important features of life history and disease biology, including aging, reproduction, infection status, shedding, and testing. Monte Carlo methods were used to predict incidence, proportion of infectious animals detected, and cost of testing over a 10-yr period for seven different scenarios. The model successfully simulated emergent behavior of an endemic disease with low incidence; the baseline strategy of annually testing all animals  $\geq 10$  mo of age yielded a mean 10-yr incidence rate of 2.1% (95% CI: 1.9-2.4). None of the scenarios evaluated completely eliminated *Map* infection. Several management strategies, such as waiting until animals reached 2 yr of age to start testing and testing only 50% of the population, decreased costs and did not significantly alter the incidence or proportion of animals detected. Keeping a test-positive animal for an additional year to breed prior to culling did not significantly affect incidence. Simulation offers an innovative and useful tool for zoos to evaluate different animal management and disease surveillance decision-making strategies.

**Products Mentioned in the Text:** <sup>a</sup>The Anylogic Company, St. Petersburg, Russian Federation.

### ACKNOWLEDGMENTS

This study was funded by the Ellen Browning Scripps Foundation. The authors would like to acknowledge Tristan Burgess (University of California, Davis) for assistance with population summaries and the many

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veterinarians, laboratory staff, animal managers, and keepers that contributed to data collection and processing during the past two decades.

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## COMPUTER SIMULATION MODEL OF *Mycobacterium avium* SUBSP. *Paratuberculosis* CONTROL IN A ZOO: CAN WE IMPROVE OUR DISEASE SURVEILLANCE AND MANAGEMENT STRATEGIES?

Carmel L. Witte, MS,<sup>1,2,3\*</sup> Nathaniel Osgood, MS, PhD,<sup>4</sup> Anthony Gamst, PhD,<sup>5</sup> Laura L. Hungerford, DVM, MPH, PhD,<sup>6</sup> and Bruce A. Rideout, DVM, PhD, Dipl ACVP<sup>1</sup>

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### Abstract

*Mycobacterium avium* subsp. *paratuberculosis* (*Map*) infection is a significant cause of morbidity and mortality in endangered wildlife and zoo animals.<sup>1,2</sup> The goal of this study was to use simulation to explore the impact of different *Map* surveillance strategies on infection incidence and detection for hoofed mammals housed in a zoo environment. Data from 20 yr of disease surveillance at San Diego Zoo Global facilities and scholarly literature were used to construct an animated, agent-based simulation model using Anylogic software<sup>a</sup>. The simulation was designed to capture important features of life history and disease biology, including aging, reproduction, infection status, shedding, and testing. Monte Carlo methods were used to predict incidence, proportion of infectious animals detected, and cost of testing over a 10-yr period for seven different scenarios. The model successfully simulated emergent behavior of an endemic disease with low incidence; the baseline strategy of annually testing all animals  $\geq 10$  mo of age yielded a mean 10-yr incidence rate of 2.1% (95% CI: 1.9-2.4). None of the scenarios evaluated completely eliminated *Map* infection. Several management strategies, such as waiting until animals reached 2 yr of age to start testing and testing only 50% of the population, decreased costs and did not significantly alter the incidence or proportion of animals detected. Keeping a test-positive animal for an additional year to breed prior to culling did not significantly affect incidence. Simulation offers an innovative and useful tool for zoos to evaluate different animal management and disease surveillance decision-making strategies.

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## **EMPLOYEE HEALTH AT THE MARYLAND ZOO IN BALTIMORE: COST/BENEFIT ANALYSIS FOR AN ANNUAL SCREENING PROGRAM**

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### **Abstract**

While the health of the collection is the first priority of zoo veterinarians, the health of employees working with these animals is certainly an additional topic of great importance. Not only are employees susceptible to transfer of zoonotic disease from collection animals, but employees are also a possible route of introduction of anthroponoses to the collection.<sup>1</sup> The risks of both of the possibilities may be mitigated by an effective employee health screening program.

At the Maryland Zoo in Baltimore, the annual Employee Health Fair comprises the following screening tests:

1. Intradermal tuberculin testing
2. Fecal submission for parasitology and enteric culture
3. Rabies vaccination and biannual titer
4. Serum banking
5. Recommendation to maintain appropriate vaccinations, especially influenza and tetanus

Institutional concerns for instating such a program not already in effect may include financial, administrative, and perceptual. Monetary costs for such a program can be significant. Collaboration with governmental public health, as opposed to private occupational health companies, may provide significant cost savings. Health Insurance Portability and Accountability Act (HIPAA) compliance and staff time commitment to organization of the event are substantial administrative concerns, but can be addressed via HIPAA training and creating a streamlined event preparation outline. Staff may be hesitant initially to participate in such a program, but with early education on the program's purpose, we have received excellent staff compliance with minimal complaints at our institution. In short, with community networking, careful planning, and forthright discussion with staff, such a program may be achievable by a large number of zoos and aquaria.

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## RECOGNITION OF AN IMPORTANT WATER QUALITY ISSUE AT ZOOS: PREVALENCE AND POTENTIAL THREAT OF TOXIC CYANOBACTERIA

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### Abstract

Cyanobacterial toxins have been implicated in numerous wildlife mortality events involving a wide array of animal species.<sup>1,2,3</sup> Zoo animals may be particularly vulnerable to water sources contaminated with cyanobacteria and their associated toxins given their non-voluntary close association with this resource. However, the prevalence and potential threat of toxic cyanobacteria to zoo moat water quality is unknown. Several otherwise unexplained turtle (*Trachemys scripta scripta*) deaths were documented in a moat with abloom of the toxic cyanobacterium, *Microcystis aeruginosa*. Furthermore, an extremely high and potentially lethal concentration of the hepatotoxin, microcystin (166 ng g<sup>-1</sup> dry mass), was found in the liver of one necropsied turtle that died in this moat. A subsequent 7-mo survey of water quality across 10 moats revealed detectable concentrations of microcystin in all moats (0.0001 to 7.5 µg L<sup>-1</sup>), with the concentrations in 3 of the moats being significantly higher than the threshold for safe drinking water (1 µg L<sup>-1</sup>) set by the World Health Organization.<sup>4</sup> These results demonstrate that, cyanobacterial blooms are an important water quality issue in zoos, and future research is necessary to identify potential associations between water quality and zoo animal health.

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## DISEASE RISK MANAGEMENT IN ASIAN ELEPHANTS (*Elephas maximus*) IN NORTH AMERICA: ESTABLISHING THE BASICS OF THE EPIDEMIOLOGY OF EEHV-1

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### Abstract

Elephant endotheliotropic herpesvirus (EEHV) causes acute and often fatal hemorrhagic disease in young Asian elephants (*Elephas maximus*), and is the single largest cause of death for Asian elephants born in North America since 1978.<sup>1</sup> The North American Asian elephant population is declining and is not sustainable. A recent population modeling study demonstrated that elimination of EEHV-related mortality, along with increasing breeding rates, gives this population its best chance of reaching a sustainable level.<sup>2</sup> There have been 21 EEHV-associated deaths in North American Asian elephants born in captivity since 1978. An additional 9 Asian elephants have become ill from EEHV infection but have survived with supportive care and antiviral treatment. The majority of EEHV-associated deaths occurred in elephants between 1 and 6 yr of age. A recently completed case controlled epidemiologic investigation of selected North American institutions found no evidence to support an association between EEHV occurrence in Asian elephants and exposure to African elephants. Additionally, there was no evidence in Asian elephants to support an association between other husbandry or individual risk factors with EEHV occurrence. Standardized classifications of EEHV-associated illness and fatalities are needed to facilitate the development of a universally accepted roster of EEHV cases.<sup>3</sup>

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## SEROLOGIC ASSESSMENT FOR HEPATITIS B VIRUS IN THE COMMON CHIMPANZEE (*Pan troglodytes*) SSP<sup>®</sup> POPULATION FOR PROSPECTIVE POPULATION MANAGEMENT

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### Abstract

In primate species, including humans, hepatitis B virus is a horizontally-transmitted infectious hepatopathy which can present as acute, chronic, or inapparent disease, and it may progress to hepatocellular carcinoma or carrier status.<sup>1,4,6,7</sup> Within the common chimpanzee (*Pan troglodytes*) SSP<sup>®</sup> population, individuals have been confirmed with positive hepatitis B serology or exposure, but the overall status for this disease is unknown in this population (n=259). Knowledge of dissimilar serologic status without a population context presents concerns for medical recommendations and complicates their management.

As of January 2013, this population was distributed across 35 institutions. For the preliminary assessment, two institutions with established current serologic status for hepatitis B virus were identified as negative (n=7) and positive (n=15) by both core antibody and antigen (VRL Laboratories, San Antonio, TX 78229, USA).<sup>2,3</sup> Two additional collections of unknown serologic status (n=6 and n=12) were analyzed from banked serum collected from 2008-2013, for analysis of 15% (n=40) of the target population. Signalment, origin, current health status, history of liver disease, and hepatitis B vaccination history was obtained for each animal.

This data was developed into a population-wide directive for hepatitis B virus to provide context for individual collections to develop management strategies in situations of variable status and vaccination recommendation, and determine treatment options.<sup>7</sup> Additionally, SSP<sup>®</sup> coordinators will use the analysis to make informed transfer recommendations, breeding plans, and determination of blood donors.<sup>5</sup>

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## DATA-DEFICIENT DISEASE RISK ASSESSMENTS: THE MOJAVE DESERT TORTOISE (*Gopherus agassizii*) AS A MODEL

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### Abstract

Disease risk analysis is a structured process for evaluating the likelihood and consequences of specific disease hazards occurring in a population as a result of a management action, such as a translocation. The process typically involves six steps: problem description, hazard identification, risk assessment, risk management, implementation and review, and risk communication.<sup>1,2</sup>

The hazard identification and risk assessment steps are data intensive, requiring detailed information on the prevalence of pathogens of concern in the target populations, and patterns of disease in both individuals and populations of affected species. This creates problems for most conservation programs where such data are lacking, but where management actions are still needed.

We recently conducted a comprehensive disease risk analysis for Mojave Desert tortoise translocations, despite significant data deficiencies. We took a combined approach, using traditional risk assessment procedures where data were available and a population risk matching approach where data were not available. The risk matching process involves matching relevant characteristics between the source and destination populations, such as population monitoring history, habitat characteristics, degree of human encroachment, and proximity to disease reservoirs (animal and environmental). Appropriate matching of characteristics helps to neutralize the risks associated with animal moves. Periodic program review and adaptive management are important when implementing any risk management strategy, but are particularly important when using risk matching in data deficient scenarios.

We believe this combined approach to risk analysis allows for sound, science-based decision-making and effective risk mitigation despite the data deficiencies typically encountered in conservation programs today.

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## MEDICAL AND HUSBANDRY RISK MANAGEMENT IN GIRAFFES: UPDATES ON IMPROVING GIRAFFE WELFARE

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### **Abstract**

From January 2011 through March 2013, there have been at least 41 giraffe deaths in the United States and Canada, 21 of which appeared to have been preventable. Giraffes are intolerant of cold and lack the ability to insulate themselves against cold weather the way other species of hoofed stock do by growing a thick coat or depositing fat. Damp weather and inadequate diets may exacerbate the problem.<sup>1</sup> Cold-related deaths were the most common preventable problem during this 2-yr period. There were ten or more deaths related to cold conditions or cold stress combined with feeding an inadequate diet. Many cold-related deaths occurred during a cold front in the more temperate regions of the southeastern and western United States. Insulated barns with a heat source should be available to all captive giraffes housed in areas where ambient temperatures below 50°F (10°C) might occur, however rare that might be.

There were eight exhibit or housing/husbandry-related deaths during this time period. Two were associated with entanglement, one where the ossicones were thought to have been entangled in the hotwire lining a pasture fence and the other where the giraffe put its head through the triangular support elements supporting a shade structure. Four giraffes died in barn fires. One slipped on ice in the exhibit and died shortly thereafter. One keeper fed oleander to the giraffe causing a death. Keepers and management staff should scrutinize all areas giraffes have access to, including areas outside of the enclosure that a giraffe might reach. Any opening a giraffe could put its head through is a potential hazard. All ropes and wires within reach of a giraffe should be carefully evaluated to ensure entrapment is not possible. Grass growing along fence lines where the fencing is constructed of materials with openings large enough for an ossicone to protrude (such as livestock wire fencing) should be killed or removed to eliminate the attraction. All heating elements should be checked routinely to ensure they are working, and are clean and free of flammable materials nearby such as nests. They must be installed and maintained properly.

Anesthesia is necessary for certain surgical procedures and one giraffe during this time period died as a result of an anesthesia required for a necessary course of action. However there were three anesthetic-related deaths for foot trim procedures. Giraffes have been trained to allow routine foot trims and other basic medical behaviors. Applying training-based methods to manage giraffes rather than anesthetizing them for similar procedures may save lives.

Solving thermoregulation-related problems using appropriate housing and inclement weather turn-out protocols, scrutinizing exhibits and any area a giraffe could reach for

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entrapment hazards, adequate keeper training, using training techniques to deal with overgrown hooves, and ensuring the specific nutritional requirements of giraffes are met would help to improve their quality of life and may help to decrease the alarming mortality rate seen in recent years.<sup>1,2</sup>

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## GROSS LESION RECOGNITION FOR THE ZOO CLINICIAN

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### **Abstract**

Zoo clinicians, by nature of their specialty, are skilled at necropsy technique, more so than clinicians in most other specialties. Gross lesion recognition (and photography), like other imaging modalities, is a bit of an art form. Vast zones of grey may confront the investigator when considering what the lesion is, what it could be, and what to do with it. And nobody is good at all of it: A seasoned livestock pathologist could possibly clear an entire days' necropsies without the help of a single histology slide. A bee keeper would recognize immediately the maggot stages that parasitize his colony. A dog-cat pathologist in a busy private laboratory or a companion animal clinician may not have a clue about gross lesions in livestock or bees. Historically, our attempts at accurately diagnosing disease by gross lesion recognition have been a humbling experience, and that has been the impetus for all further diagnostic specialties. Gross lesions, or lack thereof, are the first visual indication of what may be wrong with the patient (or cadaver, as the case may be). These are the lumps, the effusions, the asymmetrical oddities, the discolorations, the odiferous clues to disease that (hopefully) stimulate a "scientific" thought process culminating in a list of differential diagnoses. The purpose of this session is to present clear and not so clear images of common and not so common gross lesions in live and necropsy specimens, and in a participatory manner, establish a differential diagnosis and means for establishing a definitive diagnosis.



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## **POST-KATRINA MODIFICATIONS TO DISASTER PLANNING AND STORM RIDING AT AUDUBON NATURE INSTITUTE**

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### **Abstract**

Disaster planning is a crucial concern for all institutions that house and care for animals. With increasing impact from global warming and potential climate destabilization, planning for and dealing with major disruptions will be critically important to all zoos for the foreseeable future.

Additionally, the evaluation of existing plans and post-impact performance evaluations and subsequent recommendations for improvement are essential in preparing for future crises.

Hurricane Katrina was a major disaster for New Orleans and Audubon Nature Institute facilities. In the aftermath of the storm, many aspects of planning were revised and refined, and many changes made to improve an already heroic response of the team that rode out the storm and stayed through the long period of turmoil after it. These modifications were and continue to be based in many instances on storm team experiences and their recommendations for planning for and refining response to future storms.

Since Katrina, significant changes have evolved in storm team composition, communications (both internal and external), preparatory and post-event procedures, planned redundancy for vital equipment, interactions with external agencies at local and national levels, and in documentation and records for assessment and compensation.

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## **DISASTER RISK ASSESSMENT FOR CAPTIVE WILDLIFE FACILITIES: THE BUCK STARTS HERE**

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### **Abstract**

Every effective disaster risk plan must begin with situational risk analysis. Risk analysis evaluates potential threats unique to a defined geographic location, species-specific wildlife collection, and other outside factors beyond the perimeter boundaries and the control of an institution. Appropriate disaster training, planning, response, and recovery all begin with a complete and accurate risk assessment. Emergency risk assessment-based protocols are a small, but integral part of a disaster risk assessment (structural fire within an institution, animal escape, individual human health crisis, etc.). Emergencies occur within disasters and may be multiple and simultaneous (moat flooding allowing animal escapes, electrical fire with blocked transportation, etc.). A well-managed plan may prevent or mitigate an emergency within the disaster.

### **Introduction**

A disaster declaration is made by the local, state or federal government. Consult local, regional, state, and federal agencies, including the State Veterinary Office and the USDA, for their list of regional risks. Veterinary involvement and considerations are essential at captive animal facilities. Risk assessment must consider risk to the animals from the basic event, risk to the animals from secondary situations created by the event, risk from the animals to human health, and potential risk to the facility and animals from subsequent human activities.

### **Compounding Events and Threats**

When conducting risk assessment, consider the secondary and compounding risks. Disasters spur novel emergencies or disasters within. Hurricanes spin off multiple tornados. Seismic activity overlapping areas with nuclear plants provide a roadmap to dual disaster. Floods are not only destructive, but may disperse sewage, fertilizer, etc., thus perpetuating and expanding the impacted area.

Staff familial responsibilities impact them just as other disaster zone residents. Mandatory evacuation orders include facility staff and family too. One Texas zoo set up temporary employee childcare when the local scholastic infrastructure collapsed in the aftermath of a hurricane.

In worst case scenarios, the initial event creates more serious sequelae; these should be anticipated in risk assessment. Hurricane Katrina destroyed property and lives. Subsequent levee failure caused an ongoing flood crisis. In 1888, the “Great White

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Hurricane” blizzard in NYC was initiated by the Krakatoa eruption in Indonesia. The debris altered global weather patterns. Transportation halted, over 400 died and snow drifts approached 50 ft. The storm was the impetus for the elevated NY “subway” above the snow.

### **Transportation and Resources**

Transportation limitations are potential risks with almost any disaster. Transportation both into and out of an area may be simultaneously interrupted/altered. Analyze multiple escape/evacuation routes for risk from river crossings, urban congestion, etc. that may mean entrapment vs. escape.

Resource loss risk or unavailability of ongoing resources may require an alternative to sheltering in place even if the wildlife facility infrastructure is intact. Post-disaster events may lead to additional loss of resources. A New Orleans zoo’s diesel fuel was commandeered/confiscated for a local hospital after Katrina. Risk assessment must include resource interruption.

Administration of any facility is essential to continued function. Banks, fund transfers, animal and institutional data bases are all at risk. Institutional data back-up may be adequate, but banking, payroll and billing are routinely outside services. Consult with providers to assess risk of administrative jeopardy and alternatives.

### **Information Resources**

Risk assessments have already been created by many governmental agencies. Acquire and use them. Local Emergency Response Services (EMS) and State Emergency Management Agencies (EMA) may have the best basic geographic risk assessment. Risk assessment can start with geographically specific threats.

Many years of historical data assist future risk assessment. Hydrological risk factors have copious data utilized by insurance, Army Corp of Engineers, FEMA, etc. However, many locations that have not experienced flooding in several decades have become complacent and facility managers do not competently consider flood risk.

Nuclear accidents are rare; nevertheless they do occur. Thermonuclear plant locations are available online. Consider proximity, watershed and prevailing weather direction in risk assessment. Nuclear radiation can impact distant locations. The jet stream, thus plume effects and atmospheric shifts in North America generally flow west to east, and north to south.

Winter storms may encompass and isolate entire regions. As climate change occurs, volcanoes like Krakatoa erupt, and winter storms occur outside weather projections; more preparation may be required for heavy snow, ice or ultra-low temperatures.

Fire risk analysis, historical data and real time wildfire information are available through

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the US Forest and Fire Services. A massive explosion and fire in urban Kansas City, MO was triggered by a natural gas leak downtown in February 2013, placing thousands at risk.

Rail and highway hazardous materials transportation creates risks to facilities in close proximity. The State EMAs use haz-mat transportation information for response planning. Location-specific information can also be acquired through the Federal Railroad Administration and US Dept. of Transportation.

Terrorist events and the risk they pose are far more difficult to predict. Target cities such as NY and Washington DC are at highest risk. The Department of Homeland Security (DHS) may be the best source for risk assessment recommendations. It may require visiting a local DHS office, often located in major air terminals such as Atlanta's Hartsfield International Airport.

### **Infectious and Zoonotic Diseases**

The veterinarians' influence is strongest with infectious disease. The epidemiology of zoonotic diseases and wildlife may be complicated further by the outbreak response. Federal and State health officials' wildlife concerns may be overwhelmed by concern for agriculture and human health. A veterinarian may use this compelling argument for infectious disease facility resource allocation.

A complete infectious disease risk analysis must consider the animal collection with regard to susceptibility/reservoir species, transmission and vectors, enclosure design, chemical control agents, and medicinal stockpile stability. For example, standing water post-flooding can be a breeding ground for massive vector population explosions. Vectors can be a prime propellant for a secondary disaster.

USDA infectious disease educational information is available to assist facilities. Veterinary accreditation information is an exceptional risk evaluation reference. Take advantage of every risk assessment resource.

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## **BIG PROBLEMS IN A SMALL SETTING: FLASH FLOOD AT THE LAKE SUPERIOR ZOO**

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### **Abstract**

Environmental disasters present unpredictable challenges to zoological parks regardless of institutional size. In the past, facilities accredited by The Association of Zoos and Aquariums were required to complete written emergency plans and to conduct emergency drills.<sup>1</sup> As of this year, the United States Department of Agriculture requires its licensed facilities to produce emergency plans to cover any unexpected situation that interrupts normal animal care activities.<sup>2</sup> Many zoos and animal exhibiting facilities that did not have contingency plans are now faced with the legal requirement to be prepared for a major disaster despite their small size, small budgets, and limited staff.

Zoological facilities that have experienced and survived a disaster can serve as valuable resources to other institutions that are developing disaster plans, or recovering from a disaster.

The Lake Superior Zoo in Duluth, MN, is a 16-acre parcel perched at the base of a rocky hillside. A brook trout stream bisects the landscape. Earthquakes, tsunamis and hurricanes were not in the zoo's disaster plan. Neither was a flash flood.

In June 2012, a torrential downpour of at least 8 inches of rain fell on the Duluth area in less than 12 hr. Flash flood alerts were issued for low lying areas. Given the slope and elevation of the land, flooding was not considered to be a high risk. But what happens downstream can flow upstream.

A mucky torrent of water displacing boulders the size of refrigerators sped through the zoo. At about 2 a.m., the violent creek quickly became a swirling 14-foot-deep lake when a railroad culvert downstream from the zoo failed. The zoo's animal care director became aware of the problem after a passing motorist found one of the zoo's harbor seals sliding along the street next to the zoo.

Key animal care employees were summoned and began to work in the sodden darkness to respond to the unfolding disaster.

What followed in the next 20 hr included the successful darting and capture of the zoo's polar bear which had swum out of her enclosure and was meandering on the zoo grounds; the capture of two harbor seals that were able to swim off grounds; the on-site relocation of three lions and two brown bears; the relocation of three marine mammals to another facility; and the recovery of the bodies of 14 animals that perished in the flood.

A crisis of this magnitude will quickly reveal your organization's strengths. More

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importantly, it will starkly reveal your organization's weaknesses.

The aftermath of this disaster showed us that there were things we had done right and things we had done wrong. Some things were out of our control. And in some cases, we just got incredibly lucky.

Things we did correctly included:

- Writing contingency plans for weather emergencies
- Conducting multiple drills to deal with escaped animals, including dangerous carnivores
- Training animal care team members in firearm safety
- Replacing inadequate darting equipment with a long-range darting gun
- Stocking an emergency escape kit to be used for dangerous carnivore capture with an easy-to-use drug protocol

Things we did incorrectly included:

- Lack of 24-hr security
- Lack of a "telephone tree"
- No previous arrangements for off-site housing of animals
- No crisis communication plan
- Poor communication between zoo leadership and animal care team
- No previous involvement of public safety personnel in zoo emergency training

Things that were out of our control included:

- Continued rain after initial crisis
- Widespread flooding in the Minnesota/Wisconsin area "diluted" resources

Ways we got lucky included:

- Neighboring zoos initiated offers of help
- Local veterinary clinics immediately honored requests for controlled drugs and animal housing
- Dangerous large carnivore was safely contained, did not have to "shoot to kill"
- Polar bear didn't catch the veterinarian and didn't damage the fleeing police car
- Harbor seals didn't swim to Lake Superior
- Excellent community response with food, personal supplies, cleaning up, etc.
- Helpful press coverage
- Grief counseling services were donated

Well-thought-out contingency plans, drills, proper equipment and adequate facilities are important tangible parts of disaster response. Just as important, intangible assets such as flexible leaders who can think on their feet, the energy and stamina of youth, and the community's bond to a zoo play important roles in response and initial recovery from an unforeseen event.

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## **ACKNOWLEDGMENTS**

The Lake Superior Zoo graciously acknowledges the Como Park Zoo and Conservatory, The Wildcat Sanctuary, Northland Spay/Neuter, Superior Animal Hospital, Great Lakes Aquarium, Duluth Police and Fire Departments, hundreds of community volunteers, and many zoos across the country that donated to flood relief efforts.

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## **WE JUST WANT TO HELP! HOW ZOOLOGICAL ORGANIZATIONS CAN BEST PREPARE TO ASSIST DURING OIL SPILLS**

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### **Abstract**

Oil spills are a common occurrence in most developed coastal environments throughout the world. Ecological response to these incidents (in particular, organized recovery and rehabilitation of affected wildlife), however, is a relatively new science. Depending on the region, zoos and aquaria play varying roles during spill responses, ranging from no involvement to leading the management functions necessary to mount a large-scale effort. In many cases, organizations may wish to develop their capacity to assist in such disasters (especially if the species impacted match their collection and/or conservation focus), but have little knowledge of how best to do so. This fact was most recently evidenced during the 2010 Deepwater Horizon oil spill in the northern Gulf of Mexico, where a large number of AZA and AAZV-associated individuals and organizations wished to assist in the effort, but were not effectively incorporated in the response structure. The Oiled Wildlife Care Network, a program based at UC Davis, is committed to helping key zoological and wildlife veterinary organizations identify how they might assist in future spills both locally as well as nationally and internationally. This presentation will help define the actions necessary for developing facility and personnel readiness, thereby allowing groups to integrate into responses and perhaps even to provide leadership during spill response. Various aspects of readiness including facility design, equipment/supply caches, elements to an effective training program, and the means to integrate within local contingency planning efforts will be presented.



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## CONTINGENCY PLANNING FOR MOUNTAIN GORILLA DISEASE OUTBREAK RESPONSE

**Michael R. Cranfield DVM,<sup>1,2\*</sup> Kirsten Gilardi DVM, Dipl ACZM,<sup>1</sup> and Antoine Mudakikwa DVM<sup>3</sup>**

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### Abstract

The 2010 census of the Virunga Massif mountain gorilla (*Gorilla beringei beringei*) population counted 480 individuals, up from 250 gorillas in 1981.<sup>2</sup> The unhabituated portion of the population is declining at 0.7%/yr while the habituated portion (73%) of the population is growing at 4.1% annually; 40% of the annual increase in the habituated portion of the population is due to veterinary interventions.<sup>4</sup> Despite this conservation success, transmission of pathogens remains a threat to the sustainability of the mountain gorilla. The gorillas are susceptible to human pathogens,<sup>3</sup> but naïve to many, such as measles. The Virungas are surrounded by the highest densities of humans and livestock in Africa, but lack adequate infrastructure for implementing many public health measures to mitigate a disease outbreak. In 2012, over 28,000 tourists visited mountain gorillas in Rwanda alone, on the Democratic Republic of the Congo side, M23 rebels occupied the park; and in Uganda, Marburg and Ebola outbreaks in humans occurred within 200 miles of the Massif. Considering the intensity of the human/gorilla interface and the potential for a disease outbreak of serious proportions, a regional contingency plan has been developed to minimize the negative impact of an outbreak for the gorillas. Development of the contingency plan involved all stakeholders and the government conservation authorities of the three range countries, with Gorilla Doctors taking the lead on drafting the plan. The contingency plan defines risk categories for outbreak categorization and prioritizes activities necessary for outbreak response, including garnering local, regional and international participation.<sup>1</sup>

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## **UPDATE ON THE ANIMAL WELFARE ACT CONTINGENCY PLANNING REGULATION**

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### **Abstract**

In January 2013, additions to the Animal Welfare Act Regulations were published in the Federal Register. Information pertaining to these new regulations can be found at: [http://www.aphis.usda.gov/animal\\_welfare/awa\\_contingency\\_plan.shtml](http://www.aphis.usda.gov/animal_welfare/awa_contingency_plan.shtml). The following information is from the Tech Note on Contingency Planning found at this site.

### **Considerations When Making a Contingency Plan**

Dealers, exhibitors, research facilities, intermediate handlers, carriers and other entities regulated under the Animal Welfare Act are now required to take steps to be prepared for emergencies and disasters. An emergency plan, also referred to as a “contingency plan,” safeguards the animals should an emergency or disaster occur and supports the facility’s response to emergencies and recovery from emergencies most likely to happen to their facility. Employees and other personnel involved in animal care or business processes should be aware of the facility’s contingency plan and sufficiently trained on it.

This tech note provides general considerations a facility can take into account when designing its own contingency plan. There is *no* required format or length for a facility’s contingency plan. APHIS wants these plans to be useful for the facility and beneficial for the animals during potential disaster situations. Individual facilities have the freedom to design their plans to best suit their facility, location and needs. Dealers, exhibitors and research facilities housing animals at holding facilities need to include these holding facilities in their contingency plan if the holding facility does not have its own contingency plan. APHIS inspectors are available for assistance in reviewing and developing plans, as needed.

Specific details of the Animal Welfare Act regulations regarding contingency plans can be found here:

*9 CFR, Part 2, Subpart C, § 2.38(l)(1)(i-iv) and Subpart I, § 2.134(a)(1-4); Subpart H, § 2.102(a)(4) and (b)(3) 9 CFR, Part 2, Subpart C, § 2.38(i)(4).*

Based on the regulations, the following five basic elements should be addressed in the plan.

1. Common emergencies likely to happen to your facility: The terms “emergency” and “disaster” are not limited to major natural disasters, such as hurricanes, and should include consideration of localized events such as a fire, severe weather or any other

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unexpected situation that interrupts normal animal care activities. Some possible emergencies to consider include, but are not limited to:

- structural fire
- electrical outage
- disruption in clean water or feed supply
- disruption in access to facility (e.g. road closures)
- intentional attack on the facilities/animals/personnel
- hazardous materials situation
- employee absence
- unexpected change in ownership
- faulty heating, ventilation and air conditioning (HVAC) system
- animal escape
- animal disease outbreak
- flooding
- earthquake
- landslide/mudslide/avalanche
- hurricane
- tornado
- blizzard/ice storm
- extreme heat/humidity
- wildfire

2. Specific tasks to be taken during emergencies: Depending on the circumstances and type of emergency, a facility should decide for itself whether its animals and employees should be relocated. Facilities housing animals should also have a plan for animal escapes.

- Sheltering-in-place: Animals remain in primary enclosures during an emergency and employees stay on premises to provide animal care.
  - husbandry and care needs for the animals (e.g. food and water)
  - access to a veterinarian
  - staffing and housing options (including food and water) for the staff
  - environmental conditions (e.g. temperature, ventilation, lighting)
  - animal identification
  - records maintenance
- Evacuation. Animals are moved out of their primary enclosure to another location, either on the grounds of the facility or to a remote location.
  - transportation vehicle(s) and equipment/caging
  - alternate location(s) for housing animals: These locations should be included on the facility's contingency plan.
  - husbandry and care needs for the animals during transport and once animals are relocated
  - environmental conditions on transportation vehicle and at alternate location(s)

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- staffing during transport and at the alternative location
  - animal identification
  - records transfer
  - Escaped animal(s)
    - supplies needed (e.g. communication equipment, gloves, nets, drugs, darting equipment)
    - secondary barriers secured
    - employee/public safety procedures
    - notification of emergency response/security/law enforcement personnel
    - recovery/transport of animal(s) back to original enclosure

3. Chain-of-command for implementing the plan: Pre-assigning responsibility to personnel for the tasks identified above as part of the planning process can streamline your response during an emergency by establishing clear authority structures and lines of communication.

- Identify the person who has the authority to initiate the contingency plan.
- Identify the people (or positions) in charge of the tasks listed in the plan.
- Have an updated contact list distributed to all of the people involved in the plan.
- Identify the methods to be used to maintain communication.

4. Materials and resources required for response and recovery: Facilities have the freedom to decide which equipment and materials they will need to make possible the tasks identified in their contingency plans. Arrangements should be made for how the facility is going to obtain the equipment during an emergency if it is not on-hand, as well as determining if certification or other specialized training is required for the use of any equipment.

- Identify and list materials and resources necessary to implement the plan.
- Obtain the necessary materials or have a plan for obtaining the materials during an emergency

5. Employee training on the plan: Ensuring that employees are aware of the plan and understand their roles will result in more expedient and organized responses during an emergency. "Employees," in this sense, refers not only to paid staff but also to volunteers and others involved in animal care and/or integral to the business operation.

- On your contingency plan, document any and all training provided to your employees.
- Ensure that employees are aware of any changes to the plan or their assigned roles.

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## COMPUTED TOMOGRAPHY, MAGNETIC RESONANCE IMAGING AND GROSS CROSS-SECTIONAL VIEWS OF THE NORMAL ANATOMY OF THE KOALA (*Phascolarctos cinereus*) NASAL CAVITY AND PARANASAL SINUSES

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### Abstract

Knowledge of the normal anatomy of the nasal cavities and paranasal sinuses of the koala (*Phascolarctos cinereus*) is important in the diagnosis of nasal disease, including cryptococcosis, nasal polyps, and neoplasia.<sup>1</sup> Computed tomography (CT) and magnetic resonance imaging (MRI) are useful tools in diagnosing abnormalities of the nasal cavities and paranasal sinuses and for planning surgical intervention.<sup>2</sup> This study compared gross anatomic sections with CT and MRI images in order to describe the normal appearance of the koala nasal cavity and paranasal sinuses. Three deskinning koala skulls were transected at approximately 10 mm intervals in transverse, sagittal, and dorsal planes prior to being photographed. A clinically normal koala was anesthetized for acquisition of dorsal plane CT scan images of its nasal cavities and paranasal sinuses. Sagittal and transverse plane images were reformatted from the dorsal plane scans. A second koala underwent the same procedure for MRI images acquisition. The MRI and CT images obtained were matched with the corresponding gross anatomic images and the normal bone, tissues and airway passages were identified. The resulting comparative images can be used to aid diagnosis, plan surgical intervention, and monitor therapeutic responses of diseases of the nasal cavity and paranasal sinuses of the koala.

### ACKNOWLEDGMENTS

This study was approved by the San Diego Zoo Global Institutional Animal Care and Use Committee (Proposal #11-029). San Diego Zoo Global has an Animal Welfare Assurance (A3675-01) on file with the Office of Laboratory Animal Welfare.

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## GASTRIC PNEUMATOSIS IN A BENGAL SLOW LORIS (*Nycticebus coucang bengalensis*)

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### Abstract

An aged Bengal slow loris (*Nycticebus coucang bengalensis*) was examined for progressive weight loss. A firm but depressible mass was palpable in the left cranial abdominal quadrant in close association with the liver. Radiographs showed right liver enlargement, calcification in the area of the liver, and what was erroneously interpreted as air from aerophagia in the stomach delineating ingesta. A fine needle aspirate of the mass demonstrated mixed flora suggestive of gastrointestinal contents. Ultrasonography was non-diagnostic due to air interference. A subsequent contrast study and gastroscopy demonstrated the “mass” to be the stomach enlarged by gastric pneumatosis. The loris was euthanized due to clinical decline. At necropsy, the gastric wall was thickened by submucosal emphysema. Histologically, submucosal air bubbles were often surrounded by mild granulomatous inflammation. Additional findings included a hepatocellular carcinoma, a calcified leiomyosarcoma in the terminal esophagus, and unrelated geriatric changes.

Gastric pneumatosis is a rare problem in veterinary<sup>1</sup> and human medicine<sup>2</sup> where air is trapped within the layers of the stomach. More frequently reported is pneumatosis intestinalis or pneumatosis coli.<sup>3,4</sup> Any underlying condition that allows gas or gas-producing bacteria to enter the submucosa and dissect the tissue layers could initiate the process. Examples are necrotizing gastrointestinal disease, tumors, trauma, and gas trapping from bacterial growth after intestinal surgery. While there were both esophageal and hepatic cancers in this animal, no definitive underlying cause for the pneumatosis was identified.

This is the first known report in a loris and the second known report in a non-human primate.<sup>5</sup>

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## UNUSUAL TURNER SYNDROME MOSAIC WITH A TRIPLE X CELL LINE (47,X/49,XXX) IN A WESTERN LOWLAND GORILLA (*Gorilla gorilla gorilla*)

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### Abstract

A 29-yr-old female Western Lowland gorilla (*Gorilla gorilla gorilla*) was evaluated for low fertility and a mid-term abortion. Laboratory testing included karyotyping, which revealed an unusual mosaicism for Turner syndrome with Triple X (47X/49XXX). This appears to be the first report of Turner syndrome in a great ape. In humans, Turner syndrome occurs in approximately 1/3000 females, with half of those monosomic for the X chromosome. A small proportion is mosaic for a triple X cell line (3-4%).<sup>2</sup> In humans, Turner Syndrome is associated with characteristic phenotype including short stature, obesity, a broad chest with widely spaced nipples, webbing of the neck, and anteverted ears.<sup>1</sup> This individual gorilla is significantly shorter in stature than conspecifics and is obese despite normal caloric intake. Individuals with Turner syndrome should also be screened for common health issues including congenital heart defects, obesity, kidney abnormalities, hypertension, hypothyroidism, and diabetes mellitus. Animals with decreased fertility, multiple miscarriages, fetal losses, and/or unusual phenotypes should be evaluated for genetic abnormalities.

### ACKNOWLEDGMENTS

The authors thank Marlys Houck, CLSp (GC) from the San Diego Institute for Conservation Research for consultation regarding this case, Melanie Meyer from Pinon Perinatal for editing assistance, and to the ape keepers at the Rio Grande Zoo for their excellent care of the gorillas.

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## PHARMACOKINETICS OF SUBCUTANEOUS VERSUS INTRAMUSCULAR ADMINISTRATION OF CEFTIOFUR CRYSTALLINE-FREE ACID IN INLAND BEARDED DRAGONS (*Pogona vitticeps*)

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### Abstract

The objective of this study was to evaluate the pharmacokinetics of ceftiofur crystalline-free acid (CCFA)<sup>a</sup>, the long-acting formulation of ceftiofur, after subcutaneous (SC) and intramuscular (IM) administration in bearded dragons (*Pogona vitticeps*, n=6). Ceftiofur is a third-generation cephalosporin with broad-spectrum bactericidal activity.<sup>2</sup> A recent study of CCFA in ball pythons (*Python regius*) yielded promising results, but concluded that a higher dosage than was tested (15 mg/kg IM) might be more effective.<sup>1</sup> In the current study, each bearded dragon received a single dose of 30 mg/kg CCFA IM or SC; the experiment was then repeated after a 28-day wash-out period using the opposite route of administration for each animal in a complete crossover design. Blood samples were collected from each animal during each phase of the experiment at the following times: 0, 4, 12, 24, 48, 72, 120, 144, 192, and 288 hr post-injection. A target minimum-inhibitory concentration (MIC) of 1 µg/mL was selected based on previous MIC research of common bacterial isolates in turkey poults.<sup>3</sup> Results indicate that both routes of administration achieved plasma levels of ceftiofur equivalents above 1 µg/mL by 4 hr post-administration in all animals and remained above that target for at least 288 hr (12 days) on average. No negative effects were observed. The subcutaneous route of administration was preferred due to ease of administration. A single administration of CCFA at 30 mg/kg SC appears to be a safe long-acting antibiotic in bearded dragons.

**Products Mentioned in the Text:** <sup>a</sup>EXCEDE<sup>®</sup>, Zoetis, Madison, NJ 07940 USA.

### ACKNOWLEDGMENTS

This project was supported by the University of Wisconsin - School of Veterinary Medicine's Companion Animal Fund.

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## IDENTIFICATION OF A NOVEL ADENOVIRUS ASSOCIATED WITH VASCULITIS IN A NORTH AMERICAN RIVER OTTER (*Lontra canadensis*)

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### Abstract

A 4-mo-old North American river otter (*Lontra canadensis*) was unexpectedly found dead. Significant findings at necropsy included severe palmar/plantar ulceration, oral ulcers, interstitial pneumonia and pericardial and pleural effusion. Oral and skin lesions were the result of necrotizing vasculitis that was associated with endothelial intranuclear viral inclusions. DNA sequence was amplified by PCR from multiple tissues, including brain, liver, spleen, kidney and skin, using degenerate primers designed against adenoviral polymerase and hexon genes. Sequence analysis identified this as a novel adenovirus in the genus *Mastadenovirus*; California sea lion adenovirus-1 and bat adenovirus were identified as closest relatives, which shared 73% and 74% identity, respectively, to the novel otter adenovirus DNA polymerase gene. Ultrastructural analysis revealed icosahedral viral particles with a diameter of 60-80 nm, compatible with adenovirus, within the cytoplasm and nuclei of endothelial cells. In addition to the viral-associated vasculitis, there was diffuse alveolar damage with hyaline membrane formation in the lungs; viral inclusion bodies were not identified in these lesions. A source of the viral infection was not identified; conspecifics co-housed with the affected individual, including the dam and two littermates, were either negative for adenoviral DNA or remained asymptomatic.

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## PERIORBITAL ADENOCARCINOMA IN A BEARDED DRAGON (*Pogona vitticeps*)

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### Abstract

A 4.5-yr-old female bearded dragon (*Pogona vitticeps*) presented for periorbital swelling affecting the left eye, two weeks in duration. The swelling was non-responsive to ciprofloxacin<sup>a</sup> ophthalmic and oral enrofloxacin<sup>b</sup> medications. The lizard had been normal at home, but was recently inappetent and lethargic. Complete blood count performed one week prior to referral revealed a heterophilic leukocytosis, although absolute values of all white blood cells were within normal limits. Serum chemistry showed hypercalcemia and hyperphosphatemia.

On presentation, a 1 cm x 1 cm x 0.3 cm, soft, tan-yellow gelatinous mass was evident at the medial canthus. The mass appeared well-organized and hypoechoic on ocular ultrasound. Cytologic examination of a fine needle aspirate was suggestive of an inflammatory process; heterophilia and monocytosis with occasional spindle cells (presumed fibroblasts) were noted. Aerobic and anaerobic cultures were negative. The mass continued to grow despite adding ceftazidime<sup>c</sup> to the treatment regimen. An exenteration was performed. Histopathologically, the mass was composed of solid clusters or sheets of moderately pleomorphic, irregular polygonal shaped cells with a mitotic rate of 1–2/hpf. Interspersed areas of necrosis, hemorrhage, and necroheterophilic inflammation were noted. Immunohistochemical staining was also suggestive of a lacrimal gland adenocarcinoma, although a Harderian gland origin could not definitively be ruled out.

The owners reported that swelling at the surgical site recurred quickly and the dragon died 2 mo post-operatively. Periorbital adenocarcinoma arising from tear producing tissues may be under-reported in bearded dragons and can occur in iguanas and chameleons (Garner, M.M, personal communication, January, 2013).

**Products Mentioned in the Text:** <sup>a</sup>Ciprofloxacin ophthalmic solution (0.3%), Pack Pharmaceuticals, Buffalo Grove, Illinois, USA. <sup>b</sup>Enrofloxacin solution (50 mg/ml), Triad Compounding Pharmacy, Cerritos, California, USA. <sup>c</sup>Fortaz, GlaxoSmithKline, Research Triangle Park, North Carolina, USA.

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## ABDOMINAL ULTRASONOGRAPHY OF THE NORMAL VERVET MONKEY (*Chlorocebus sabaeus*)

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### Abstract

The vervet monkey is an important non-human primate species utilized for biomedical research. Abdominal ultrasonography is commonly used in small animal veterinary medicine and normal abdominal echoanatomy has been described in other primate species.<sup>2</sup> Thoracic radiography has also been described in the vervet monkey.<sup>3</sup>

The object of this research was to describe the normal abdominal echoanatomy and to provide reference values for selected abdominal organs in the vervet monkey.

Twenty clinically normal sexually mature vervet monkeys between 5 and 12 yr of age and weighing between 3.13 and 6.85 kg were evaluated with ultrasound. Individuals were randomly divided into one of two immobilization protocols and scanned at 18.0 MHz. Physical examination, hematology and serum biochemistry values were all within normal limits.

High-quality images of the liver, gallbladder, kidneys, urinary bladder, spleen, adrenal glands, and gastrointestinal tract were acquired. A dorsolateral approach was considered to be particularly useful in order to avoid the fecal/gas content of the colon. The prostate and trigone area of the urinary bladder were not visualized. Abdominal lymph nodes other than ileocolic, the pancreas, and the female reproductive tract were not evaluated. Gastric and duodenal motility were significantly different between immobilization protocols ( $P < 0.05$ ). Gender dimorphism in weight was observed. Species-specific findings included, but were not limited to, pyelectasia, prominent right liver, and iso- to hyperechoic renal cortices compared to spleen and liver.

In conclusion, ultrasonography provided an excellent non-invasive assessment of the vervet monkey's abdomen and normal size parameters for abdominal viscera were established.<sup>1</sup>

### ACKNOWLEDGMENT

Funding provided by an institutional grant from Ross University School of Veterinary Medicine. Abstract reprinted with permission from the Journal of Medical Primatology.

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## ARE FREE-RANGING BROWN BEARS (*Ursus arctos*) AN IMPORTANT RESERVOIR AND POTENTIAL SOURCE OF *Campylobacter* spp. INFECTIONS TO HUMANS?

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### Abstract

Campylobacteriosis is a zoonotic emerging disease with a broad range of asymptomatic animal hosts. In humans, *Campylobacter* species is the most commonly reported bacterial cause of gastroenteritis. The role of different wildlife species as reservoirs of *Campylobacter* spp. remains to be determined. The population of brown bears (*Ursus arctos*) in Sweden is approximately 3,500 individuals. During the legal bear hunt up to 300 bears are shot in the fall and the meat is consumed by humans. The aim of this study was to investigate if free-ranging brown bears host *Campylobacter* spp. in feces. Samples were collected by directly swabbing the rectum of anesthetized brown bears ( $n=69$ ), or by collecting feces from the colon of hunted brown bears ( $n=52$ ). Bacteriological analyses were performed by culture in selective media for *Campylobacter* spp. Six samples (5 %) were culture-positive. All isolates were *C. jejuni*, which is the species that causes most cases of human campylobacteriosis. The prevalence of *Campylobacter* spp. in free-ranging brown bears in this study was low, but brown bears may play a role in the epidemiology of pathogenic *Campylobacter* spp. Feces from wild bears could potentially contaminate surface water as well as bear meat which may be consumed by humans. In conclusion, the study results indicate that brown bears are probably not an important reservoir, but their feces can be a potential source of infection for humans.

### ACKNOWLEDGMENTS

We thank the rangers from Länsstyrelsen for sample collection from hunted brown bears, and Dr Andrea Miller and fieldworkers within the Scandinavian Brown Bear Research Project for sample collection from anesthetized brown bears. The study was performed in collaboration with the Scandinavian Brown Bear Research Project, which was supported by the Swedish Environmental Protection Agency, the Norwegian Directorate for Nature Management, the Research Council of Norway, and the World Wide Fund for Nature in Sweden.

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## EXPLORATION OF METHODS OF FIBRINOGEN MEASUREMENT IN AVIAN SPECIES

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### Abstract

Fibrinogen is an ancient protein found in all vertebrates.<sup>1</sup> It is a useful analyte because levels tend to correlate with systemic inflammation.<sup>2</sup> As an acute phase protein, levels increase demonstrably in the first five days of an inflammatory response. However, levels may remain elevated with chronic inflammation. Fibrinogen has not been well-studied in birds,<sup>3</sup> but in preliminary investigations of rehabilitated seabirds, fibrinogen was inversely correlated with post-release survival.<sup>4</sup> Quantitative measurement of fibrinogen in birds requires at least 0.5ml of plasma, which must be immediately frozen. A methodology that would require smaller volumes and not necessitate immediate freezing for transport would be valuable in a rehabilitation setting, where small birds are common and an in-house laboratory is not available. The Abaxis VSPRO, a point-of-care analyzer, was evaluated using a fibrinogen cartridge with blood from birds commonly seen in rehabilitation centers: brown pelicans (*Pelecanus occidentalis*) and African penguins (*Spheniscus demersus*). In addition, using penguins, a commercial human fibrinogen sandwich ELISA kit<sup>a</sup> was evaluated. This study also estimated fibrinogen by performing protein electrophoresis on plasma and serum by calculating the difference in the beta fractions. Despite reporting values within the expected range, results from the VSPRO were not correlated with values from the reference laboratory. The ELISA kit produced results much lower than expected values, and uncorrelated with reference values. Similarly, the estimated values were not correlated with reference values. Therefore, more research is needed to develop a low-volume, convenient, reliable methodology for determining avian fibrinogen.

**Products Mentioned in the Text:** <sup>a</sup>Genway Human Fibrinogen ELISA test kit.

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## MANAGEMENT OF SUSPECTED IVDD IN A GERIATRIC DWARF MONGOOSE (*Helogale parvula*) USING CONVENTIONAL AND COMPLEMENTARY THERAPIES: A CASE REPORT

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### Abstract

A female dwarf mongoose (*Helogale parvula*), 14 yr of age, who was living with two males, became acutely grade 1-2/5 ataxic. Radiographs indicated an area of narrowed disks in the T-L region, consistent with her neurologic signs. She was isolated from the males and started on meloxicam (Metacam®, 0.5 mg/ml oral suspension, Boehringer Ingelheim Vetmedica, Inc, St. Joseph, MO, USA) a non-steroidal antiinflammatory drug, and gabapentin (100 mg capsule, Camber Pharmaceuticals, Inc, Piscataway, NJ, USA), used for neuropathic pain, daily. After eight weeks, she continued to show evidence of grade 4/5 ataxia. At that time, acupuncture was added to the treatment plan. It has been demonstrated in canine patients that adding acupuncture to traditional Western medical treatment plans for intervertebral disk disease (IVDD) can help decrease recovery times and improve outcomes.<sup>1</sup> Another study has suggested that electro-acupuncture is more effective than decompressive surgery in canine patients with long-standing neurologic deficits due to thoracolumbar disk protrusion.<sup>2</sup> Once weekly for seven consecutive weeks, the dwarf mongoose was anesthetized via isoflurane induction. She was maintained on isoflurane varying from 20-40 minutes. While under anesthesia, body temperature, pulse oximetry, heart and respiratory rates were monitored.

The mongoose's acupuncture treatment was formulated from both Traditional Chinese Medicine (TCM) and Western medicine approaches. From a TCM standpoint, the goal is to move Qi and Blood to eliminate stagnation caused by injury, thus improving function and comfort.<sup>3</sup> Local points above and below the area of concern were treated along the Bladder meridian (the iliocostalis muscles). In addition, peripheral points on the extremities were also treated to try to benefit appetite and stress. Electro-acupuncture was used on a low-intensity, low-frequency setting during all of the sessions. Electrodes were attached to needles above and below the lesion, as well as to distal points. After each of her sessions, the patient recovered uneventfully from anesthesia and was relatively calm (less anxious) for a period of 1-2 days.

After three acupuncture treatments, the dwarf mongoose was re-introduced to her primary enclosure without the males present. She showed improvement by climbing deliberately, using her right hind leg well and the left better than she had used it previously. At this time, her private, secondary enclosure was modified to try to encourage her to climb easy obstacles and to increase her daily exercise. Unfortunately, given the social nature of the dwarf mongoose, she was not comfortable enough in the solitary, secondary enclosure to venture out much. Even with coaxing and food motivators (crickets and yogurt) the



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keepers were unable to get her to consistently come out of hiding.

After seven weeks of acupuncture, a physical therapy plan was instituted. Given her sedentary behavior, it was deemed necessary to help to rebuild her musculature before reintroducing her to the group and her primary habitat. A large, clear plastic exercise ball was introduced. The dwarf mongoose was surprisingly calm in the exercise ball even at the first introduction. She was started at a routine of 3-5 minutes twice daily. Between exercise sessions, she was still being isolated from the other dwarf mongooses. Because she was reluctant to move around her secondary enclosure, the exercise in the ball was the most efficient way to get her to use her front and hind legs. The ball turned out to be an excellent tool for a number of reasons. It required little handling of the patient and she was always visible in the ball, making it easy to ascertain that she was not in a compromised position. The inside of the ball has ridges which acted as Cavaletti rails to help develop proprioception and balance. Initially, she would fatigue and someone would have to help roll the ball for her to walk. Within a week, she was strong enough to propel the ball on her own. By the end of 4 weeks, she was walking in the ball over smooth terrain for a total of 15-20 minutes twice daily.

At this point in treatment, the dwarf mongoose was returned to her primary habitat. This decision was made for a variety of reasons, one being concern regarding the amount of time the animal had been in an isolated environment. The other two dwarf mongooses were removed from the primary habitat and the patient was given time alone to explore and climb. At this time, it was observed that her ataxia maintained at a grade 4/5. She was able to navigate the tree branches and tunnels, but still tumbled on occasion. She was given three days in the enclosure on her own. Then, due to concerns about her slow positive progress, safety and welfare in the habitat, and inability to return to a fully social status, humane euthanasia was elected.

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## BODY CONDITION SCORES FOR MANAGEMENT OF CAPTIVE FROG SPECIES

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### Abstract

Assessing body condition in amphibians can provide early signs of disease or can be used as part of ongoing population health monitoring. Amphibians represent diverse species that may preclude an easily applied and accurate scoring system, but amphibian body condition indexes have been used to identify reproductive activity, compare habitat types, and evaluate movement behaviors in wild amphibians.<sup>2,4,6</sup> Additionally, body condition has been shown to negatively correlate with physiological stress and decreases in condition have been linked to global warming trends.<sup>3,5</sup> Patterns of lipid deposition have been described in many amphibian species and this information can be applied to management of captive amphibian management.<sup>1</sup> This poster details application of body condition scores in the management of a large dendrobatid frog collection in a zoological setting.

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## PHARMACOKINETICS OF BUPRENORPHINE HYDROCHLORIDE IN AMERICAN KESTRELS (*Falco sparverius*) FOLLOWING IM AND IV ADMINISTRATION

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### Abstract

Opioids are the most effective class of analgesic drugs for management of moderate to severe pain. Based on the results of previous studies, *kappa* opioid receptor agonist drugs have been used predominantly in birds.<sup>4,5,7,8</sup> However, recent studies have shown that the *mu* agonists fentanyl and hydromorphone may have analgesic properties in raptors.<sup>2,3,6</sup> Buprenorphine is a semi-synthetic partial *mu* opioid receptor agonist; in mammals it has a long-lasting, moderate analgesic effect and high margin of safety.<sup>1</sup> Preliminary data using a thermal stimulus in American kestrels (*Falco sparverius*) suggests that buprenorphine has analgesic properties in this species (Guzman, unpublished data). In the study reported here, the pharmacokinetics of buprenorphine hydrochloride following IM and IV administration were determined in kestrels (n=16) in a complete crossover design, with a 2-week washout period. Blood was collected from 3 or 4 birds at each of 9 time points, from 5 min to 9 hr. Plasma buprenorphine concentrations were measured by liquid chromatography-mass spectrometry, and pharmacokinetic parameters were determined using non-compartmental analysis. After IV administration, the steady state volume of distribution was 4,024 mL/kg, with a clearance of 49 mL/kg/min and an elimination half-life of 105 min. After IM administration, bioavailability was 94.5%, with a maximum plasma concentration of 243 ng/mL at 5 min and an elimination half-life of 93 min. IM administration of buprenorphine hydrochloride to American kestrels results in rapid absorption, high bioavailability, and similar kinetics to IV administration.

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## CALVARIAL OSTEOMA WITH CRANIAL VAULT INVASION IN THE SKULL OF A FERRET (*Mustela putorius furo*)

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### Abstract

An osteoma is a mass of abnormally dense but otherwise normal bone, which generally arises from the periosteum of bones formed by intramembranous ossification.<sup>5</sup> An uncommon tumor found in various species,<sup>5</sup> osteomas are particularly rare or underreported in ferrets.<sup>1-4</sup> The 2-yr-old neutered male ferret (*Mustela putorius furo*) described in this case report was presented with an asymmetrical firm mass on the right dorsolateral skull. Radiographs and computed tomography revealed a well-marginated mineral-attenuating mass with a granular core that involved the dorsal right calvarium and was growing concentrically into the cranium. Surgical biopsy was pursued and the cytological diagnosis was a sarcoma with reactive osteoblasts and osteoclasts, while the histopathological diagnosis was an osteoma. It was suspected that only the outer bony rim of the mass was collected for histopathological evaluation, thereby adversely affecting the diagnostic quality of the sample. Consequently, based on interpretation of the combined imaging, cytological, and histopathological findings, a multilobular tumor of bone was highly suspected. Due to clinical decline, the animal was euthanized 4 mo after presentation, and post-mortem examination yielded a definitive diagnosis of osteoma. This is the first report of an osteoma with cranial vault invasion in a ferret, and one of very few reports of this tumor in this species. The lack of neurological signs in the patient, marked and extensive growth of the mass over several months, and the similar clinical appearance to other bony tumors, leading to diagnostic ambiguity, contribute to the distinctiveness of this case.

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## CONSERVATIVE MANAGEMENT OF HEMOABDOMEN IN A GREY SEAL (*Halichoerus grypus*)

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### Abstract

A 6-yr-old female grey seal (*Halichoerus grypus*) at the Indianapolis Zoo was presented with acute lethargy and tremors. Five weeks prior to presentation, this animal had a right rear limb amputation at the level of the stifle due to chronic self-mutilation of the rear flippers. Four weeks after surgery, the animal developed elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT), and anemia. Initial abdominal ultrasound showed a small amount of hypoechoic fluid near the right kidney. However, one week later, radiographs showed complete loss of abdominal serosal detail, with marked free fluid in the abdomen confirmed on ultrasound. Fluid aspirated from the abdomen was determined to be hemorrhagic effusion with a higher hematocrit than systemic blood. Since splenic fracture, hepatic fracture, or avulsed vasculature in the abdomen were suspected, the animal was kept in dry isolation to minimize movement or disruption of any clots. The animal was maintained on oral and subcutaneous fluids, hepatoprotectants, gastroprotectants, antibiotics, and antifungals. Five days later, recheck ultrasound showed markedly decreased abdominal fluid, although the liver edges appeared be rounded. In the subsequent days, the animal's attitude and appetite markedly improved, and serial blood work showed increasing hematocrits and normalizing liver enzyme levels. The animal was released back into the exhibit three weeks after initial evaluation. This report describes the clinical course and conservative management of hemoabdomen in a grey seal, with a discussion of possible causes of hemoabdomen in this patient.

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## **ELECTROCARDIOGRAPHIC PARAMETERS OF FREE LIVING RUSSELL'S VIPER (*Daboia russelii*) AND INDIAN SPECTACLED COBRA (*Naja naja*)**

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### **Abstract**

The aim of this study was to describe the normal electrocardiographic patterns in Russell's viper (*Daboia russelii*) and Indian spectacled cobra (*Naja naja*). Ten adult rescued vipers and ten adult rescued cobras were used for this study. The snake was restrained without anaesthesia, with the help of a restraining tube and held in dorso-ventral recumbency by two assistants. Traditional forelimb leads were connected two heart lengths cranial to the heart on the left side and rear limb leads were connected two heart lengths caudal to the heart on the right side, which yielded an ECG tracing similar to standard lead II as in case of dogs. The waveforms were analyzed in lead II at 25mm/sec at 10 mm = 1 mV to determine heart rate, rhythm, P, PR, QRS, ST and QT durations and P, net QRS complex and T amplitude. The polarity of each wave was tabulated in lead II. The P waves and QRS complex were predominantly positive in standard bipolar lead II. T waves were found to be both positive and negative. Establishment of normal electrocardiogram values will help to identify abnormal ECG patterns during procedures like anesthesia and for the future investigations of cardiovascular diseases.

### **Introduction**

Conservation of reptiles in general is of negligible importance in India since olden days. Snakes have always been known for their natural defenses. Hence the first reaction of any human being when they encounter a snake is to kill them, except a few of which are of mythological importance. Also research undertaken in this class of the taxa in India is very minimal thereby making the availability of diagnostic tools minimal. For successful conservation practices, understanding the biology and physiology, availability of diagnostic tools, and treatment and management of diseases are important factors. Standardization of electrocardiographic parameters may serve as a valuable diagnostic tool for various research investigations and surgical procedures.

Electrocardiography is useful for assessing cardiac rhythm and chamber size and for diagnosing conduction abnormalities.<sup>1</sup> The electrocardiogram (ECG) can provide relevant information about electrical activity and cardiac remodeling; however, abnormal parameters suggest, but do not identify structural cardiac diseases. The ECG is a useful tool for diagnosing most arrhythmias and can provide information on the myocardial status because the deflections and intervals recorded are often altered by either pathologic or physiologic factors.<sup>2,3</sup> It can also be used as an indicator of cardiac chamber



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enlargement and electrolytes imbalances.<sup>3</sup>

The ECG can be an invaluable tool for monitoring cardiac function in reptiles. Unfortunately, this diagnostic test is used sparingly by many veterinarians because of limited understanding regarding its interpretation. Because the heart rate is tied to environmental temperature, it is important to maintain a reptile within its preferred optimal temperature range when performing this test. The reptilian ECG has many of the same characteristics of a mammalian ECG.<sup>4</sup> The present study was undertaken to establish the normal electrocardiographic parameters using a safe physical restraint. The ECG was recorded without using any anesthetic agents for the snake. Thus the cardiac activity remained uninfluenced by chemical immobilization and values closer to the physiologic values could be recorded.

## **Materials and Methods**

Ten rescued adult Russell's vipers and ten rescued Indian spectacled cobras were selected for the study. All the snakes used for the study were rescued from populated areas in and around Bangalore. Necessary permissions for acquisition were procured from Principle chief conservator of Forest (Wildlife), Karnataka Forest Department. As the snakes would have undergone significant stress during the rescue activities, they were housed for two days to allow the animal to stabilize and the stressors on them to reduce. During this period the snakes were observed for their behavior and movements. Those with normal behavior and activities were selected for the study.

The snakes were restrained with the help of standard snake hooks and restraining tubes (Midwest<sup>®</sup>). Post-restraint a thorough physical examination was carried out for each specimen. Snakes with any injuries or physical abnormalities were not selected for the study. Five out of ten Russell's vipers were females and five were males. Five out of ten Indian spectacled cobras were males and five were females. The length and weight of each individual snake is tabulated in Table 1.

## **Recording of ECG**

Electrocardiograms (BPL Cardiac 9108, 2011 with Simultaneous 12 Channel ECG Recording and 12 lead simultaneous acquisitions) of all 20 snakes were recorded under manual restraint without usage of any anesthetic agents. All snakes were held in dorso-ventral recumbency on a wooden table for ECG. The tracing was recorded at a paper speed of 25 mm/second at the sensitivity of 1 with the voltage of 1 mv per 10 mm. ECGs were recorded in standard bipolar limb leads (I, II, III), and augmented unipolar limb leads (aVR, aVL, aVF); however, for calculation of various parameters, standard lead II was considered. The electrodes were attached to the skin using alligator clips after application of coupling gel (BPL Cardijelly<sup>®</sup>) liberally. The gel applied was more than the amounts applied while recording ECG of mammals. Larger quantities of gel were used to attain excellent contact as the skin of snakes is keratinized and hard. The traditional leads were placed approximately two heart lengths cranial and caudal to the heart.<sup>4,5</sup> The cranial leads included RA (right arm) followed by LA (left arm) and the

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caudal leads included RL (right leg) followed by LL (left leg). The cranial leads were placed on the left lateral side of the snake where the dorsal scales and ventral scales meet while the caudal leads were placed on the right lateral side of the snake<sup>5</sup> (Fig 1.).

## Results

Typical ECG tracings of snakes in the standard bipolar limb leads and augmented unipolar limb leads are shown in Figures 1 and 2. The mean heart rate was determined to be  $66.64 \pm 10.89$  beats per minute (BPM) in Russell's vipers and  $68.48 \pm 10.72$  BPM in spectacled cobras.

The various ECG parameters measured in standard bipolar lead (lead II), viz. amplitude and duration of 'P', 'QRS' and 'T' waves; the PQ, PR, ST and QT segments of each individual snake is summarized in Table 2a (Russell's viper) and 2b (Indian spectacled cobra). The bottom two rows of the table comprises the mean and standard deviation for each particular parameter.

The QRS complexes of Indian spectacled cobra were of lesser amplitude in comparison with the QRS complexes of Russell's viper. The mean value of the QRS amplitude in Russell's viper was 1.27 mV whereas in Indian spectacled cobra it was 0.82 mV. 1.55 mV was the maximum recording in viper while in cobras the R wave did not exceed 0.9 mV. Other parameters of the two species did not show much difference. There were no evident differences in the ECG of males and females within the species.

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In one Russell's viper, a phenomenon similar to sinus arrhythmia in mammals was noted wherein clusters of QRS complexes were evident with extended periods of time gap between them. An approximate time duration of 2.16 seconds was recorded between the last T wave of one cluster and first P wave of the consecutive next wave. No electrical activity was recorded on the ECG during this time gap.

## Discussion

The interpretation of ECG of snakes can be difficult because of limited reference material available presently for comparison. In addition, the ECG can be influenced by various environmental factors. For example, the heart rate is dependent on the body temperature, and the intervals such as the P-R and Q-T segments are influenced by the heart rate. To reduce the likelihood of misclassifying the results of an ECG, it is important to perform these tests under optimal conditions.<sup>4</sup> In the present study the ECG was recorded with the environmental temperature within the POTZ. However, there are no established

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POTZ values for both the species. As both these species are generally found in tropical countries,<sup>6,7</sup> the temperature range maintained during this study was 26 – 28 °C. Hence, the variations in ECG that could be attributed to temperature were reduced.

Reptiles have a tremendous capacity for anaerobic metabolism and can hold their breath for extended periods of time<sup>8</sup>. This was more commonly observed in Russell's viper than Indian spectacled cobra during this study. As seen in one of the snakes, the phenomenon similar to sinus arrhythmia could be because of prolonged breath holding. Absence of any electrical activity was witnessed during this period.

The various ECG parameters recorded in the study were found to be almost similar to the ECG recordings of canines.<sup>3</sup> One exception seen in this study was prolonged ST interval which may be suggestive of slower depolarization of ventricle in these species.

The anatomical location of the heart in snakes is usually in the first one third to one fourth of the body.<sup>4,5,9</sup> In the present study it was found that the Russell's viper heart is located almost midway through the body. In contrast, the spectacled cobra heart was located just after the base of the hood.

To standardize the normal ECG in these species, a larger sample size must be taken into consideration so that individual variations and human errors can be nullified. There is scope for further research to be done before ECG for snakes can be standardized any further.

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**Table 1.** Length, weight, and sex of each snake.

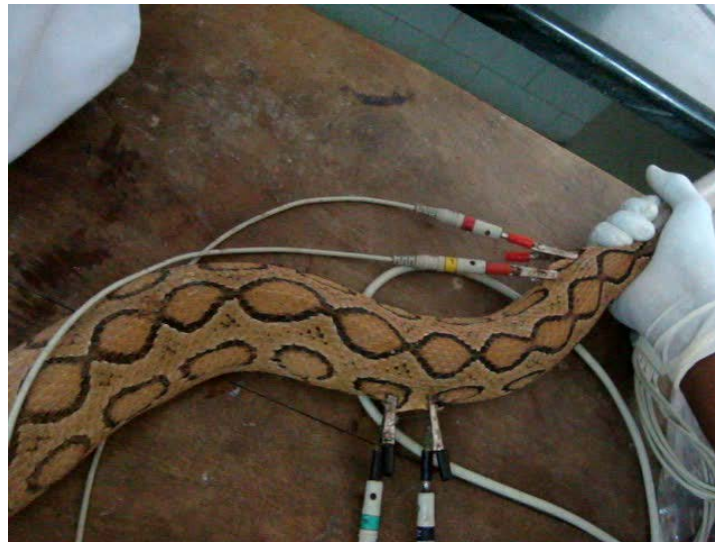
Specimen No.	Russell's viper				Indian spectacled cobra			
	Length		Weight (g)	Sex	Length		Weight (g)	Sex
	(cm) HV <sup>a</sup>	VT <sup>b</sup>			(cm) HV <sup>a</sup>	VT <sup>b</sup>		
1	110	22	1320	M	150	20	1240	F
2	115	21	1440	M	157	21	1320	F
3	120	14	1650	F	140	32	1180	M
4	98	18	920	M	135	18	1070	F
5	118	15	1840	F	155	35	1350	M
6	102	13	940	F	160	36	1450	M
7	115	15	1240	F	162	31	1790	M
8	119	22	1380	M	148	21	1580	F
9	123	21	1410	M	152	20	1450	F
10	121	16	1520	F	158	32	1570	M

<sup>a</sup>Head to vent length<sup>b</sup>Vent to tail length**Table 2a.** ECG parameters of Russell's viper with mean and standard deviation.

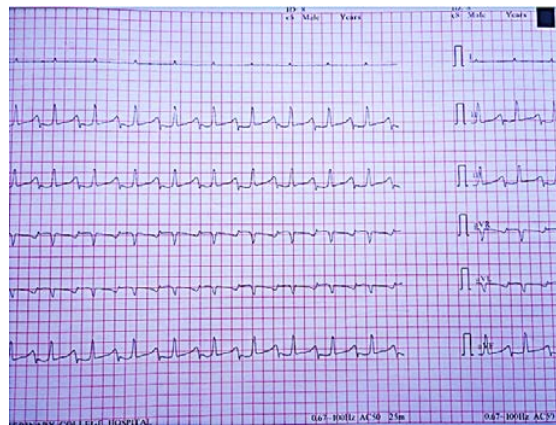
Sl. No.	P		PQ	PR	QRS		ST	QT	T		HR
	mV	sec	sec	Sec	mV	sec	sec	sec	Sec	mV	BPM
1	0.1	0.04	0.12	0.19	1.2	0.07	0.21	0.26	0.08	0.5	57.69
2	0.1	0.03	0.11	0.15	1.55	0.08	0.19	0.22	0.06	0.25	68.18
3	0.15	0.03	0.1	0.13	1.1	0.06	0.26	0.32	0.11	0.9	59.17
4	0.1	0.03	0.09	0.13	1.25	0.07	0.19	0.22	0.06	0.15	78.94
5	0.1	0.02	0.13	0.15	1.1	0.04	0.27	0.31	0.04	0.25	41
6	0.15	0.04	0.1	0.13	1.1	0.05	0.17	0.22	0.04	0.2	75
7	0.1	0.04	0.13	0.16	1.2	0.05	0.2	0.25	0.05	0.4	69.34
8	0.15	0.03	0.11	0.14	1.25	0.06	0.21	0.26	0.06	0.5	71.36
9	0.2	0.05	0.09	0.12	1.5	0.05	0.22	0.27	0.06	0.4	78.45
10	0.1	0.05	0.14	0.18	1.4	0.08	0.21	0.29	0.08	0.6	67.23
Mean	0.125	0.036	0.112	0.148	1.265	0.061	0.213	0.262	0.064	0.415	66.636
SD	0.03354	0.00917	0.01661	0.02182	0.15661	0.013	0.02934	0.03458	0.0201	0.21337	10.89

**Table 2b.** ECG parameters of Indian spectacled cobra with mean and standard deviation.

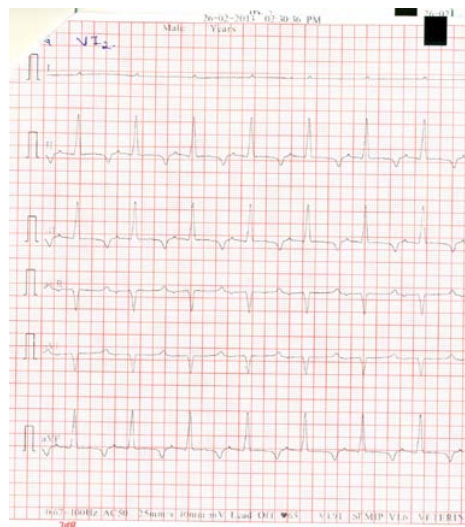
Sl. No.	P		PQ	PR	QRS		ST	QT	T	HR	
	mV	sec	sec	sec	mV	sec	sec	sec	sec	mV	BPM
1	0.2	0.04	0.1	0.13	0.7	0.05	0.2	0.24	0.04	0.2	83
2	0.15	0.04	0.12	0.16	0.85	0.04	0.2	0.24	0.07	0.25	65.22
3	0.2	0.05	0.12	0.15	0.9	0.05	0.2	0.25	0.07	0.3	51.74
4	0.2	0.04	0.12	0.14	0.75	0.05	0.2	0.25	0.08	0.3	83.33
5	0.15	0.04	0.11	0.16	0.8	0.05	0.18	0.23	0.05	0.3	78.24
6	0.2	0.05	0.12	0.14	0.9	0.05	0.19	0.24	0.05	0.3	71.23
7	0.25	0.045	0.11	0.13	0.85	0.04	0.19	0.23	0.06	0.25	68
8	0.2	0.05	0.11	0.14	0.85	0.04	0.2	0.24	0.07	0.2	71.46
9	0.3	0.05	0.12	0.13	0.7	0.06	0.21	0.27	0.06	0.3	53.27
10	0.2	0.04	0.13	0.15	0.9	0.05	0.2	0.25	0.08	0.25	59.35
Mean	0.205	0.0445	0.116	0.143	0.82	0.048	0.197	0.244	0.063	0.265	68.484
SD	0.04153	0.00472	0.008	0.011	0.07483	0.006	0.00781	0.01114	0.01269	0.03905	10.72



**Figure 1.** Attachment of standard bipolar limb leads. Image reproduced with permission from M. Karthik.



**Figure 2.** ECG recording from Indian spectacled cobra showing the bipolar limb leads and augmented unipolar limb leads. Image reproduced with permission from M. Karthik.



**Figure 3.** ECG recording from Russell's viper showing the bipolar limb leads and augmented unipolar limb leads(\*Note the negative T waves). Image reproduced with permission from M. Karthik.



**Figure 4.** Magnified image of Lead II of Russell's viper. Image reproduced with permission from M. Karthik.

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## RETROSPECTIVE REVIEW OF THE PREVALENCE OF MYELOLIPOMAS IN GOELDI'S MONKEYS (*Callimico goeldii*) AND IMPLICATIONS FOR FUTURE STUDY

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### Abstract

Myelolipomas are rare mesenchymal tumors composed of adipose and hematopoietic tissue.<sup>3</sup> They have been reported in many species, including domestic dogs and cats, cattle, birds, wild felids, and non-human primates.<sup>1-8</sup> Myelolipomas in Callitrichidae have been reported at necropsy and rarely antemortem.<sup>2-8</sup> Multiple cases of hepatic myelolipomas in a captive collection of Goeldi's monkeys (*Callicimio goeldii*) prompted a retrospective study to determine the prevalence and investigate factors associated with this condition. A total of 816 necropsy reports (1965-2013) collected from multiple captive collections were reviewed. Myelolipomas were present in 16.8% (n=137) of pathology reports, with a non-significant (p=0.095) trend toward female cases (54.7% of diagnosed cases). There was a significantly older mean age at death in affected animals (131.78 mo) compared to unaffected animals (80.32 mo)(p<0.001). Correlation of inbreeding (p=0.252) and generations removed from the wild (p=0.994) didn't differ compared to non-affected animals. All myelolipomas were diagnosed either focally (n=68, 49.6%) or multifocally (n=69, 50.4%) in the liver, and less commonly in the adrenal glands, spleen, or mediastinum. The continent of diagnosis was not evenly distributed (p<0.0001), with the majority of cases occurring in North America. Future studies are merited to examine the prevalence in living collections. Utilization of modern imaging modalities for accurate age of diagnosis, staging, and monitoring progression of the disease is warranted. Examining family genealogies, links to other disease processes, as well as examination of clinical and husbandry trends in affected animals may help to determine predisposing factors of myelolipoma in this species.

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## **HYPOADRENOCORTICISM (ADDISON'S DISEASE) IN A HOFFMAN'S TWO-TOED SLOTH (*Choloepus hoffmanni*)**

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### **Abstract**

A 22-yr-old, captive-born, presumed female Hoffmann's two-toed sloth (*Choloepus hoffmanni*) presented in respiratory distress with severe dehydration, hypotension, and a palpably distended stomach. During treatment, dysphagia was noted and oral examination revealed enlarged palatine tonsils and mucosal plaques. Bloodwork showed a low normal sodium:potassium ratio, a decreased baseline cortisol, and a decreased ACTH-response test. All values were compared to a healthy male Hoffmann's two-toed sloth at the same facility. Despite aggressive medical management and treatment for hypoadrenocorticism, the sloth was found deceased in her hospital enclosure. Necropsy revealed abdominal effusion, multifocal yeast-like plaques throughout the upper gastrointestinal tract, subcutaneous edema, pulmonary effusion, and testes. Histopathology revealed marked adrenal cortical atrophy and intranuclear mucosal inclusions. Advanced molecular techniques were unable to uncover any viral etiologies – it is unknown whether the yeast-like organisms or viral disease confounded the diagnosis of Addison's disease. This is the first reported case of hypoadrenocorticism in a Hoffman's two-toed sloth.

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## STANDARDIZATION OF ULTRASONOGRAPHIC ANATOMY OF COELOMIC ORGANS IN INDIAN SPECTACLED COBRA (*Naja naja*) AND RUSSELL'S VIPER (*Daboia russelii*)

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### Abstract

There is scarcity of reports on ultrasonographic studies of the coelomic cavity in Indian spectacled cobra (*Naja naja*) and Russell viper (*Daboia russelii*). A total of ten adult Indian spectacled cobras and six adult Russell vipers were used for this study. Ultrasonography was performed using a Doppler ultrasound unit equipped with a probe of 6.0 to 10 MHz frequency. The snakes were restrained without anaesthesia, with help of a restraining tube and held in ventro-dorsal recumbency by two assistants. A thick layer of acoustic coupling gel was applied ventrally to ensure optimal contact between the probe and the snake's scales. Cardiac mode of the ultrasound was used for better visualisation of coelomic organs. The snakes were scanned across the ventral body wall methodically from a rostral to caudal direction using transverse and sagittal scanning planes to enable all organs to be identified. The liver was elongated; echogenicity of the liver being homogenous, and vena cava appeared as hyper-echogenic line dividing the liver into two halves on transverse plane. A large central hepatic vein was evident on examination. Gall bladder appeared as an anechoic focal area well caudal to the liver. Spleen appeared as a small regular sphere slightly more hyperechoic than the liver. Colon appeared anechoic with hyperechoic floating urates. Echogenicity of the kidney was similar to that of liver, plurilobulated in nature. Such knowledge of the normal ultrasonographic anatomy of coelomic organs of individual species is important for accurate diagnosis and interpretation of routine health examinations.

### Introduction

Abdominal ultrasonography is a non-invasive technique that can be used for diagnosis of abdominal disorders and pregnancy.<sup>17</sup> The normal ultrasonographic anatomy of visceral organs in domestic small animal species and some wild mammals are well documented,<sup>3,4,11,13,16,18,19</sup> but there are very few established reports on ultrasound study in reptiles.<sup>1,2,6,12,14,15,21-25</sup> The Indian spectacled cobra is native to the Indian subcontinent which includes present day Nepal, Pakistan, India, Bangladesh and Sri Lanka. Its distribution ranges from sea-level up to 2,000m (6,560ft) above sea-level.<sup>26</sup> Russell's viper is found in India, Pakistan, Sri Lanka, Bangladesh, Nepal and in some Southeast Asian countries like Myanmar and Thailand. Its distribution ranges from sea-level up to 2,756m (9040ft) above sea-level.<sup>27</sup>

Both the species are often found in highly urbanized areas and settlements in the

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countryside, the attraction being the rodents commensal with man. Because of this, they are commonly involved in human - animal conflict. Many cases are presented commonly to wildlife veterinarians and wildlife rehabilitation centers with resultant injuries from such conflicts (Krishna et al. 2013). Captive populations of both species are found almost in all the zoos in India and are also kept as exotic pets in many western countries. The aim of this present work was to study the normal echogenicity and location of visceral organs in the coelomic cavity of these two species so that it can be used as future reference. Such knowledge of the normal ultrasonographic anatomy of visceral organs of individual species is also important for accurate diagnosis and interpretation at routine health examinations.

## **Materials and Methods**

The study was carried out in the Department of Veterinary Medicine, Veterinary College, Bengaluru, India. A total of ten adult Indian spectacled cobras and six adult Russell's vipers were used for this study. All the snakes used for the study were rescued from populated areas in and around Bangalore. Necessary permissions were procured from Principle chief conservator of Forest (Wildlife), Karnataka Forest Department. Snakes selected for the study appeared to be healthy and clinically stable based on activity level, physical examinations, and body condition scores, although some had minor superficial wounds. Only those snakes weighing more than 500g were selected for the study. spectacled cobras used for study had average length of  $\pm 1.43$  meters and Russell's vipers had average length of  $\pm 0.97$  meters.

The ultrasonographic examination was done using a 6 to 10 MHz Curvilinear –Array Sector Doppler probe (GE Logic Book XP). Each snake was restrained without anaesthesia with help of a restraining tube (Midwest<sup>®</sup>) and held in ventro-dorsal recumbency by two assistants. Ventral scales were cleaned thoroughly with an alcohol swab, dried and a thick layer of acoustic coupling gel was applied ventrally, at least 5 minutes before application of the transducer to ensure optimal contact between the probe and the snake's scales.<sup>24</sup> Cardiac mode of the ultrasound was used for better visualisation of organs. The snakes were scanned across the ventral body wall methodically from a rostral to caudal direction using transverse and sagittal scanning planes to enable all organs to be identified. Apex of the heart was used as a landmark at the rostral end.<sup>15</sup> The probe was then moved toward the cloacal opening, which was used as a landmark at the caudal end. Anatomical position and echogenicity of visible visceral organs were recorded and analyzed in each snake.

## **Results**

The liver was visible in all the snakes. It was elongated and had a homogenous echotexture throughout its length. Echogenicity of the liver ranged from hypo to hyperechoic and the vena cava appeared as a hyper-echogenic line dividing the liver into two halves on transverse plane (Fig 1). A large central hepatic vein, anechoic in nature was evident on examination (Fig 2). Anatomically, the liver was located in the mid-third

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of the body, from apex of the heart and ended well cranial to the gall bladder.

Gall bladder appeared as an anechoic focal area well caudal to the liver. It was visible in all the snakes (Fig 3). When the snake was anorectic for a period of time, the gall bladder was often greatly enlarged, filling approximately one third of the diameter. Spleen, located close to gallbladder appeared as a small regular sphere slightly more hyperechoic than the liver and was visible only in three specimens.

Colon appeared anechoic. It was visualized in snakes having digested prey, hyperechoic floating urate crystals, air or fluid (Fig 4). Anatomically, the colon was located in the caudal third of the body, close to the kidney. Evaluation of the kidney was done by identifying each kidney in the caudal third of the body, dorsolateral to the colon. Echogenicity of the kidney was similar to that of liver. The kidneys showed plurilobulation (Fig 5), with the right kidney larger and positioned anteriorly compared to the left. Both the kidneys were visualised in all the snakes. Ultrasonographically, detailed structures of kidneys were not distinguishable.

Testes could not be identified in any of the snakes. Although ovaries could not be identified in any of the snakes, one Russell's viper (ovoviviparous species) showed skeleton and movements of embryos (Fig 6).

## **Discussion**

The 6 to 10 MHz Curvilinear –Array Sector Doppler probe (GE Logic Book XP) used for ultrasonographic examination could differentiate between echogenicity of different coelomic organs in both the species used for the study. Compared to other modes, cardiac mode of the ultrasound gave better view of organs. The use of copious amounts of acoustic coupling gel gave satisfactory results, with minimal artifacts.

Studies have demonstrated that the liver was the most evident structure among the viscera of the coelomic cavity in aquatic snakes.<sup>5</sup> Earlier studies have also explained that serpents have single lobed cylindrical, tubular liver which begin just behind the heart and extended to the middle of the stomach.<sup>24</sup> Observations in this present study also correlate with above studies. In all, the liver was located in the mid-third of the body, from the apex of the heart to a bit cranial to the gall bladder covering a large area in the coelomic cavity.

Some of the authors in their ultrasonographic studies have described findings regarding the image of large vessels in snakes during ultrasound scanning.<sup>25</sup> There is also an opinion that snakes have a typical large hepatic vein, visualized as an anechoic stripe that helps differentiate the liver from fat bodies.<sup>24</sup> This study also confirmed the presence of two large vessels, caudal vena cava and hepatic portal vein. Vena cava appeared as hyperechoic line dividing the liver into two halves on transverse plane. A large central hepatic vein, anechoic in nature was evident on examination in all the snakes. This investigation also showed that it is possible to calculate portal vein velocity using standard 6 to 10 MHz probe.

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The gall bladder in reticulated python, unlike in domestic animals, is not associated nor attached directly to the hepatic parenchyma. There may be difficulty in imaging of the gall bladder in Reticulated pythons due to the obstruction brought about by the presence of numerous adipose tissues and/or ovarian follicles.<sup>1</sup> In this present study the gall bladder appeared as an anechoic focal area well caudal to the liver which showed that it was not directly attached to liver. However there was no difficulty in visualizing the gall bladder in both species. The reason behind this may be less accumulation of adipose tissue in both species in study compared to Reticulated Pythons or boids in general.

Majority of snake species have the right kidney positioned anteriorly compared to that of the left.<sup>8-10,20</sup> Present studies also had similar findings, where right kidney was larger and positioned anteriorly compared to the left kidney in both Indian spectacled cobra and Russell's viper. Some studies have demonstrated that the kidney was plurilobulated in snakes.<sup>24</sup> Certain studies have also explained the gross anatomy of the kidney in Indian spectacled cobra, demonstrating lobulations in it through post mortem examinations (Sridevu et al. 2013). This study also confirmed the presence of multiple lobes in each kidney in both the species.

Even though adult snakes were used for the study, testes could not be visualized in any of the snakes. Testes are slender and fusiform in shape, making them difficult to image in snakes.<sup>24</sup> Difficulty in visualizing testes in this study can also be attributed to the relative small size and slender shape of both the species.

According to some authors the inactive ovaries are anechogenic and small in snakes. Eggs, if present in the oviducts, appear as two distinguishable layers – a superficial layer formed of anechogenic albumen and the deeper layer formed of more echogenic vitellus. Around the circumference of the eggs, egg shell is found whose echogenicity depends on the degree of calcification. In ovoviviparous species, skeletons and movements of embryos are easily visualized.<sup>12,14,15,24</sup> In this study no such structures were visualized except for one Russell's viper where movement of embryos was visualized and recorded.

## Conclusions

Ultrasound examination can be used as a good diagnostic tool in reptile medicine to provide information regarding organ location, pathologic changes, pregnancy and confirmation of sex. The results of this study can serve as baseline information for the ultrasonographic examination of coelomic organs in Indian spectacled cobra and Russell's viper in future.

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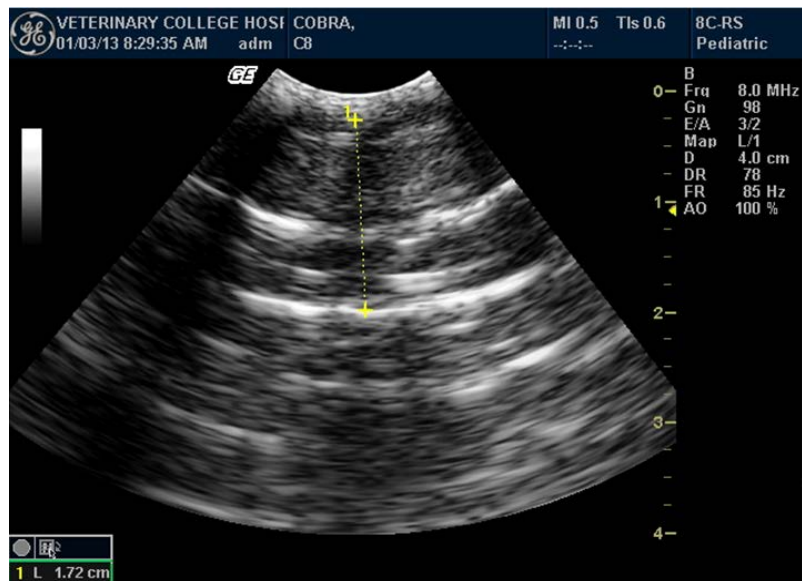
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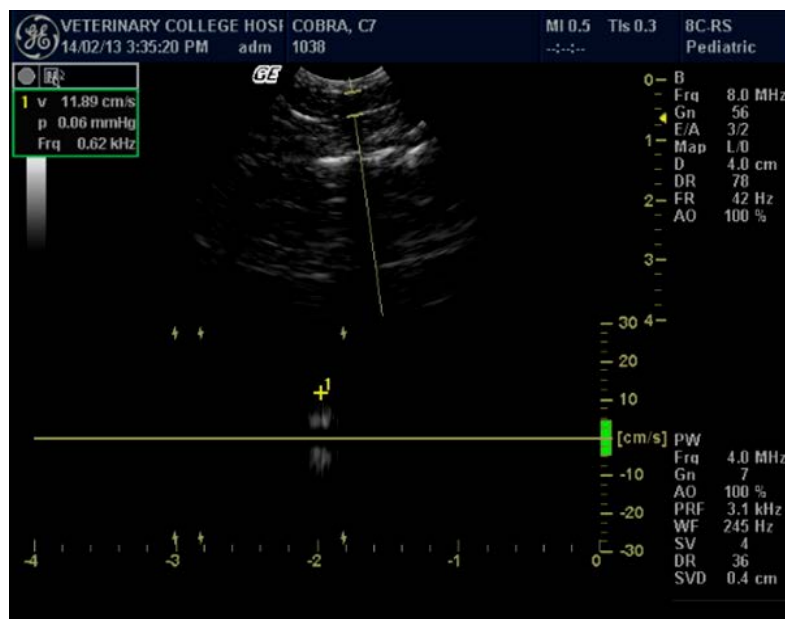
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**Table 1.** Ultrasonographic measurement of liver thickness in spectacled cobra and Russell's viper.

Species	Range (cm)	Mean $\pm$ SD (cm)
Indian spectacled cobra (n=10)	0.69-1.72	1.101 $\pm$ 0.11
Russell's viper (n=6)	0.51-1.73	1.04 $\pm$ 0.18

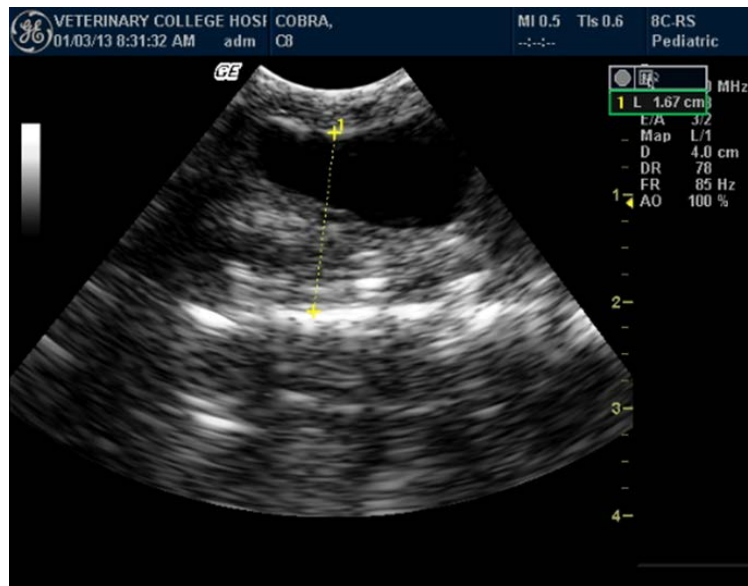


**Figure 1.** Homogenous liver and vena cava appearing as hyper echogenic line dividing the liver into equal halves on transverse plane in a spectacled cobra.

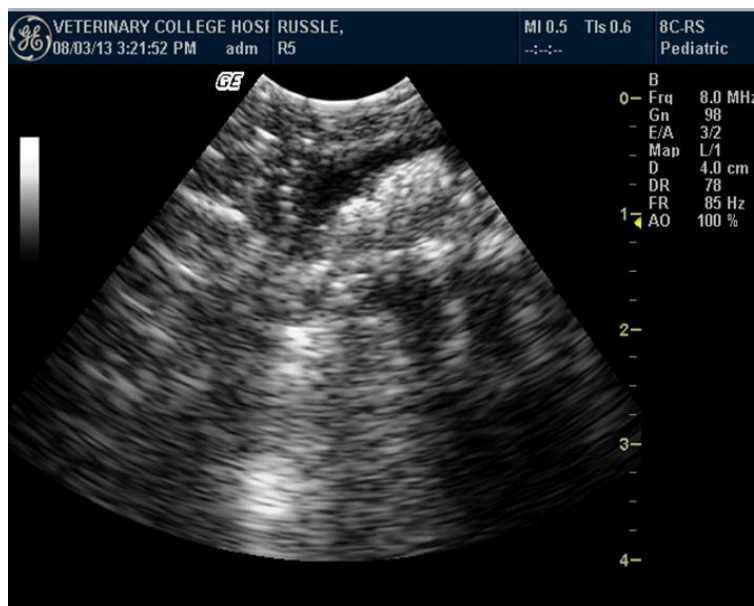


**Figure 2.** Portal vein and calculation of its velocity in Indian spectacled cobra.

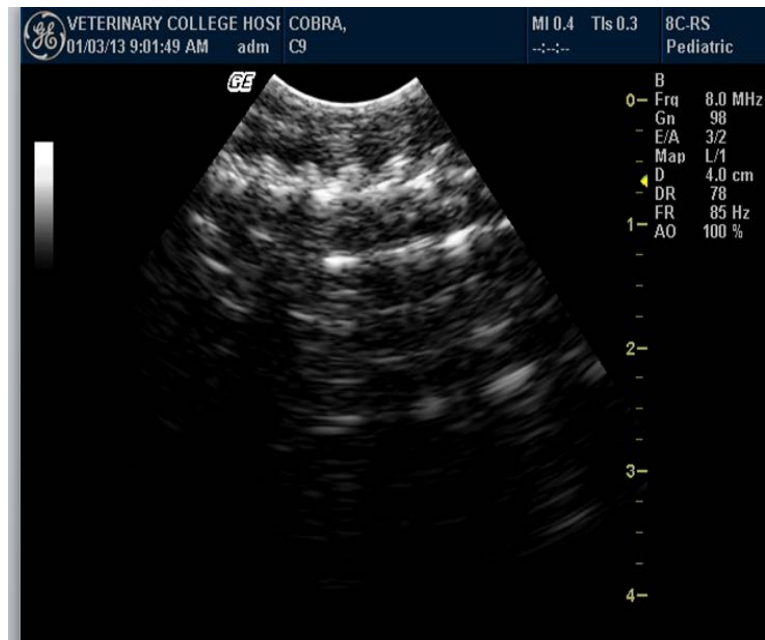




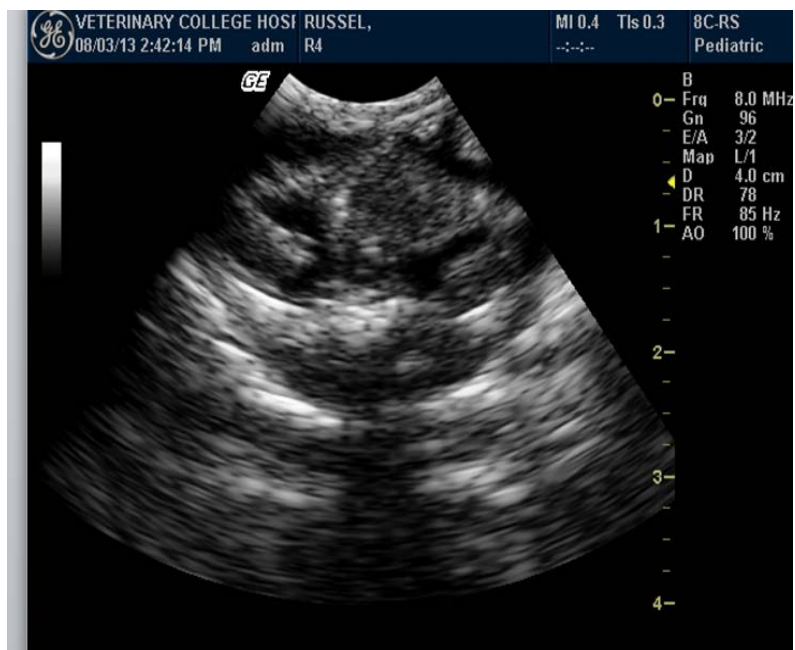
**Figure 3.** Enlarged gall bladder in Indian spectacled cobra.



**Figure 4.** Anechoic Colon of Russell's viper with hyperechoic floating urates.



**Figure 5.** Appearance of normal kidney in Indian spectacled cobra.



**Figure 6.** Skeletons and movements of embryos in Russell's viper.

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## ISIS NORMAL VALUES FOR TIGERS (*Panthera tigris*): ARE THEY BETTER THAN DOMESTIC CAT REFERENCE INTERVALS?

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### Abstract

ISIS clinical pathology data are commonly used by zoo clinicians in interpreting their patients' clinical pathology values. However, ISIS values have several potential sources of error, including: multiple reports from some individual animals; possible inclusion of values from individuals with subclinical disease(s); no exclusion of outlying values; and inclusion of analyses performed at multiple laboratories, using multiple analytical techniques. Additionally, ISIS values are described by parametric methods (means and standard deviations); however, data were not determined to be normally distributed. A robust, accepted technique for determining clinical pathology reference intervals was applied to tiger (*Panthera tigris*) blood samples (n = 42) evaluated at The University of Tennessee's College of Veterinary Medicine's Clinical Pathology Laboratory, and in-house tiger reference intervals calculated (Tables 1 & 2).<sup>1</sup> These were compared to two sets of intervals calculated from ISIS data (ISIS #1 intervals = mean  $\pm$  1 standard deviation; ISIS #2 intervals = mean  $\pm$  2 standard deviations) and to in-house domestic cat reference intervals in an effort to determine which set most accurately reflected tiger reference intervals.<sup>2</sup> Very few clinically important differences were identified amongst the four sets of intervals. Of the 18 clinical chemistry analytes evaluated, 10 domestic cat reference intervals were the closest fit to tiger intervals, more than either ISIS #1 or ISIS #2 intervals. Hematology values were also not more closely associated with ISIS intervals than with the domestic cat intervals. These data suggest domestic cat reference intervals are as useful for interpreting tiger clinical pathology as ISIS data.

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**Table 1.** Tiger and domestic cat hematological reference intervals (RI) derived from ISIS data<sup>1</sup> and determined by the University of Tennessee's College of Veterinary Medicine's (UTCVM) Clinical Pathology Laboratory. Reference intervals with asterisk were deemed most similar to UTCVM tiger reference intervals.

Analyte (units)	ISIS #1 RI (mean $\pm$ 1 SD)	ISIS #2 RI (mean $\pm$ 2 SD)	UTCVM Tiger RI	UTCVM Domestic cat RI
White blood cells ( $\times 10^3/\mu\text{L}$ )	8.2 - 15.3	4.7 - 18.9*	5.1 - 21.1	4.7 - 15.3
Red blood cells ( $\times 10^6/\mu\text{L}$ )	5.7 - 7.6	4.7 - 8.6*	5.6 - 8.8	7.5 - 11.7
Hemoglobin (g/dL)	11.0 - 14.8	9.1 - 16.7	11.2 - 16.4	11.5 - 15.9*
Hematocrit (%)	33.3 - 44.1	27.9 - 49.5	31.8 - 49.2	34 - 48*
Mean cell volume (fL)	53.3 - 64.3*	47.8 - 69.8	50.7 - 62.7	36 - 46
Mean cell hemoglobin (Hb) (pg)	18.0 - 21.4	16.3 - 23.1*	8.5 - 32.0	12.5 - 16.4
Mean cell Hb conc. (g/dL)	30.8 - 36.2	28.1 - 38.9	31.3 - 37.1	32.2 - 36.8*
Total plasma protein (g/dL)	6.5 - 7.7	5.9 - 8.3	6.7 - 9.0	6.5 - 8.6*
Neutrophils ( $\times 10^3/\mu\text{L}$ )	4.5 - 11.7	0.9 - 15.2	2.0 - 21.0	2 - 9.2*
Bands ( $\times 10^3/\mu\text{L}$ )	0.0 - 2.6*	0.0 - 4.0	0.0 - 1.7	na <sup>a</sup>
Lymphocytes ( $\times 10^3/\mu\text{L}$ )	0.5 - 2.8 *	0.0 - 4.0	0.0 - 2.8	1.05 - 8
Monocytes ( $\times 10^3/\mu\text{L}$ )	0.1 - 0.6	0.0 - 0.9*	0.0 - 0.9	0.1 - 0.3
Eosinophils ( $\times 10^3/\mu\text{L}$ )	0.0 - 0.5	0.0 - 0.7	0.0 - 1.1	0.2 - 1.1*
Basophils ( $\times 10^3/\mu\text{L}$ )	0.0 - 0.1	0.0 - 0.2*	0.0 - 0.2	Rare
Platelet count ( $\times 10^3/\mu\text{L}$ )	134 - 400*	1 - 533	105 - 433	169 - 480

<sup>a</sup>Not available.

**Table 2.** Tiger and domestic feline clinical chemistry reference intervals (RI) derived from ISIS data<sup>1</sup> and determined by the University of Tennessee's College of Veterinary Medicine's (UTCVM) Clinical Pathology Laboratory. Reference intervals with asterisk were deemed most similar to UTCVM tiger reference intervals.

Analyte (units)	ISIS #1 RI (mean $\pm$ 1 SD)	ISIS #2 RI (mean $\pm$ 2 SD)	UTCVM Tiger RI	UTCVM Domestic cat RI
Blood urea nitrogen (mg/dL)	20 - 34*	13 - 41	19 - 34	19 - 39
Creatinine (mg/dL)	1.9 - 3.7	1.0 - 4.6	0.8 - 2.2	0.9 - 2.0*
Total protein (g/dL)	6.5 - 7.7	5.9 - 8.3	6.6 - 8.8	6.7 - 8.3*
Albumin (g/dL)	3.3 - 4.1	2.9 - 4.5*	2.9 - 4.3	2.9 - 4.0
Globulin	2.8 - 4.0	2.2 - 4.6	2.8 - 5.3	2.8 - 4.8*
Glucose (mg/dL)	90 - 178	46 - 222*	31 - 213	88 - 183
Calcium (mg/dL)	9.4 - 10.8*	8.7 - 11.5	9.4 - 11.8	9.5 - 11.2
Phosphorus (mg/dL)	4.5 - 6.9	3.3 - 8.1	2.1 - 5.7	2.2 - 5.5 *
Alkaline phosphatase (U/L)	0 - 74	0 - 111	3 - 57	13 - 71*
Alanine aminotransferase (U/L)	26 - 94*	0 - 128	18.8 - 98.7	32 - 110
Aspartate aminotransferase (U/L)	12 - 44*	0 - 60	14.5 - 43.0	12 - 50
Sodium (mEq/L)	146 - 154	142 - 158	151 - 159	148 - 155*
Potassium (mEq/L)	3.8 - 4.6	3.4 - 5.0	3.0 - 4.5	2.8 - 4.8*
Chloride (mEq/L)	116 - 124	112 - 128	113 - 123	113 - 123*
Bicarbonate (mEq/L)	13.1 - 18.1*	10.6 - 20.6	13.1 - 20.8	11 - 19
Anion gap (mEq/L)	13.3 - 18.9	10.5 - 21.7	16.4 - 27.3	17 - 25*
Total bilirubin ( mg/dL)	0 - 0.4*	0 - 0.6	0.0 - 0.4	0.1 - 0.6
Creatine kinase (U/L)	0 - 646	0 - 989*	0 - 1043	69 - 893
Cholesterol ( mg/dL)	177 - 289	121 - 345	54 - 263	77 - 253*

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## INTRAOCULAR PRESSURE IN CAPTIVE AMERICAN FLAMINGO (*Phoenicopterus ruber*) AS MEASURED BY REBOUND TONOMETERY

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### Abstract

Intraocular pressure as measured by applanation and rebound tonometry has been reported in several avian species, but differences exist between species and device used.<sup>1-4</sup>

No reference ranges have been published for any member of the *Phenicopteridae* family. Flamingos are common in captivity and have the potential to develop ocular diseases that alter normal intraocular pressures, including glaucoma and uveitis. In this study, intraocular pressure was measured via rebound tonometry (Tono Vet<sup>a</sup>) in manually restrained, healthy adult male and female American flamingo (*Phoenicopterus ruber*, n=29) with the head in normal upright standing position and lowered in a feeding position. Mean (+/- SD and range) intraocular pressure in all birds while the head is in a normal upright position was OD = 10.9 (+/- 1.8 and 7 - 15 mmHg) and OS = 11.2 (+/- 2.3 and 8 - 21 mmHg). This was less than when the head was in the feeding position with OD = 14.2 (+/- 2.4 and 10 - 22 mmHg), OS = 14.3 (+/- 2.6 and 11 - 24 mmHg). This difference was statistically significant. These results establish an intraocular reference range for the American flamingo using rebound tonometry.

**Products Mentioned in the Text:** <sup>a</sup>Tono Vet, Jorgensen Laboratories, Loveland, Colorado 80538, USA.

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## DIAGNOSIS OF INFLAMMATORY BOWEL DISEASE IN A SERVAL (*Leptailurus serval*) AND TREATMENT WITH BUDESONIDE

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### Abstract

Inflammatory bowel disease (IBD) is defined as gastrointestinal signs, with incomplete response to dietary management and anthelmintics, histological injuries with intestinal mucosa inflammation, and response to immunomodulatory therapies.<sup>1,2</sup> This report is about an adult, female, serval (*Leptailurus serval*), with a history of intermittent episodes of vomiting, diarrhea and hematochezia for 4 yr. Clinical signs: thickened intestines, macrocytic hypochromic anemia, hypoproteinemia, low serum levels of folate and cobalamin, low infection by ascarids, and growth of *Campylobacter* spp. in rectal swab culture. Treatment: fenbendazole 50 mg/kg PO SID for five days and repeated after 15 days, erythromycin 12 mg/kg PO BID for ten days, and dietary trial with novel proteins. After treatment coproparasitological and *Campylobacter* spp. culture were negative, but there was no remission of clinical signs nor any significant differences in the ultrasonography and blood tests. To define between IBD and gastrointestinal lymphoma, endoscopic guided mucosal biopsy samples of stomach and duodenum were collected and mesenteric lymph node and full thickness biopsy samples of the jejunum and ileum were collected through laparoscopic guided laparotomy. Histopathology concluded the diagnosis of IBD and it was initiated treatment with budesonide 1 mg PO SID, a locally active steroid with minimal systemic effects.<sup>3</sup> There was complete remission of the clinical signs, significant reduction in the thickening of the bowel, and the blood tests were clinically normal. Chronic treatment with budesonide was maintained and after 1 yr the clinical condition remains stable. In conclusion, budesonide was effective in treating IBD in this animal.

### ACKNOWLEDGMENTS

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## BLOOD PRESSURE MEASUREMENTS IN CAPTIVE AFRICAN LIONS IMMOBILIZED WITH XYLAZINE-KETAMINE COMBINATION

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### Abstract

Blood pressure measurement remains an extremely valuable diagnostic modality in the evaluation of the cardiovascular system of living animals. In this study, Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and Mean Arterial Pressure (MAP) measurements from 5 male African Lions kept at the Zoological Garden, University of Ibadan, Nigeria were recorded from three different sites: fore limb, hind limb and the tail. Immobilization was done with a combination Ketamine hydrochloride (10mg/kg) and Xylazine (3mg/kg). Blood pressure measurements were recorded as mean  $\pm$  standard error of mean. Fore limb measurements for SBP, DBP and MAP were  $177.60 \pm 6.8$  mmHg,  $157.20 \pm 5.9$  mmHg and  $168.60 \pm 5.2$  mmHg respectively. Hind limb measurements for the SBP, DBP and MAP were  $135.40 \pm 9.5$  mmHg,  $120.60 \pm 5.9$  mmHg and  $123.00 \pm 6.8$  mmHg respectively while the tail measurements for the SBP, DBP and MAP were  $149.60 \pm 8.3$  mmHg,  $132.80 \pm 5.9$  mmHg and  $137.20 \pm 5.8$  mmHg respectively. The means of the readings obtained from each site were compared using ANOVA at a 5% level of significance. Statistically significant ( $p < 0.05$ ) differences were found in the SBP, DBP and MAP recorded from each of the sites.

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## MORTALITY PATTERNS OF SRI LANKAN LEOPARDS (*Panthera pardus kotiya*) IN THE SOUTH AND UVA WILDLIFE REGIONS IN SRI LANKA

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### Abstract

Among the 91 species of native mammals found in Sri Lanka there are 14 species in the order Carnivora. Of these, four are members of the Felid family: the Sri Lankan leopard (*Panthera pardus kotiya*), the jungle cat (*Felis chaus*), the fishing cat (*Prionailurus viverrinus*) and the rusty-spotted cat (*Prionailurus rubiginosus*). The Sri Lankan leopard is the largest of four wild cat species recorded on the island of Sri Lanka. In this study, the causes of death were investigated in leopards that died at the Uva and Southern wildlife regions from January 2011 to March 2013.

Thirteen leopard necropsies were conducted: three of them from the Ratnapura District belonging to the southern wildlife region and the rest from areas associated with the Yala National Park, belonging to the Uva wildlife region. During the post mortems, all the morphological features and measurements were recorded along with the data of gastrointestinal content as well as internal and external parasites. Animals were weighed and age estimations were done based on the size and weight of the animal, morphological features, dentition and tooth wear characteristics.<sup>2,3</sup> The animals ranged from 5 mo to over 10 yr of age. The sex ratio was 11:2 male to female. The three animals from Ratnapura were adult males weighing over 60kg that lived near the tea plantations. Causes of mortality for them were gunshot wounds, poisoning and internal organ damage from snares, respectively. The sex ratio of animals that died in the Yala National Park is 8:2, male to female. Out of these animals, 8 deaths occurred in the Yala Block 1, one in the Block 4 and the other one in the Babawa area; 1 km outside the Yala Block 1. The animals that died in block 1 ranged from 5 mo to 10 yr of age. The causes of mortality included one from a vehicular accident, three from intra-species attack, one from an explosion in the oral cavity from a gun powder trap, and one each for starvation, wild boar attack, and snare trap. The animal, in block 4, was a pregnant animal with 3 fetuses that died due to a vehicular accident. The animal that died in Babawa was due to poisoning.

In this study, the mortality rate for males was higher than for females. This sex dependent mortality finding has been seen in other studies.<sup>1</sup> The Sri Lankan leopard lives both within and outside the protected areas. Those outside are under even more pressure from human interference. Likewise, this study shows that the majority of leopard deaths were due to human interference and appears to be the leading cause of the diminishing leopard population outside the protected areas. Intra-species aggression accounted for the second

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largest number of deaths after human-related mortality. As leopards are known to defend their territories from same-sex intruders, this may be considered a natural process at places like Yala Block 1, where there is a high population density of leopards.

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## **INTRAVENOUS CARBOPLATIN CHEMOTHERAPY AND SURGICAL RESECTION FOR THE TREATMENT OF UTERINE ADENOCARCINOMA IN A JAGUAR (*Pantehra onca*)**

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### **Abstract**

A 13-yr-old, female jaguar, *Panthera onca*, who was implanted with multiple melengestrol acetate implants for approximately 7 yr, was diagnosed with a uterine mass based on ultrasonographic finding after a history of hematuria, anorexia, and weight loss. An ovariohysterectomy was performed and histology revealed a uterine adenocarcinoma with severe diffuse pyometra and multifocal metritis. Based upon the invasive nature of the tumor on histopathology and previous reports of metastasis documented in domestic and wild felids, it was presumed that this tumor had the potential for metastasis, warranting the administration of chemotherapy. Prior to starting treatment, a complete blood count, serum biochemistry, thoracic radiographs, abdominal ultrasound and echocardiogram were performed evaluating the overall health and suitability of chemotherapy for this patient.

The protocol chosen for the jaguar was based on the one set forth by Kissiberth using carboplatin in tumor-bearing cats.<sup>1</sup> Carboplatin chemotherapy (TEVA pharmaceuticals, Sellersville, Pennsylvania, 18960, USA) was initiated at a dosage of 180 mg/m<sup>2</sup> IV at 4-5 week intervals for 5 treatments. Side effects were graded as mild to moderate using the (VCOG-CTCAE) as a guideline.

Approximately 20 mo from diagnosis, the animal was found dead. On necropsy, animal's death was presumed to have been due to liver failure, which appears to be unrelated to the chemotherapeutic protocol. Neoplasia was not detected on gross or histopathology, indicating complete remission. Carboplatin at a dosage of 180 mg/m<sup>2</sup> IV at 4-5 week intervals for 5 treatments appears to be an effective and well-tolerated protocol in jaguars.

### **ACKNOWLEDGMENTS**

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## COMPARISON OF THAWING TECHNIQUES FOR OPTIMIZING POST THAW SPERM VIABILITY IN THE SOMALI WILD ASS (*Equus africanus somaliensis*)

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### Abstract

The Somali wild ass (*Equus africanus*) is a critically endangered species that would benefit from biological resource banking. Development of sperm cryopreservation protocols often focus on freezing, but not thawing rates. The objective of this study was to compare post-thaw motility and viability of sperm frozen in either an egg yolk-based or milk based extender, and thawed in a water bath at a control temp (37°C) or a higher temp (50°C). Cryopreserved semen from two adult animals was frozen at a concentration of  $25 - 50 \times 10^6$  sperm/ml in 0.5 ml straws in equine extender EQ (20% egg yolk, 4% glycerol) or INRA96 (5% egg yolk, 6% glycerol) and was thawed at 37°C for 55 seconds or 50°C for 20 seconds. Sperm motility (% of total and progressive motile) and sperm viability (% of viable sperm) using Live/Dead stain were assessed. Significant post-thaw differences between INRA and EQ diluents were observed for total motility (EQ 0hr = 45.5±5.6% 1hr = 27.1±5.6% 2hr = 12.3±2.7% and INRA 0hr = 28.5±3.7% 1hr = 5.3±1.1% 2hr = 0.9±0.3%;  $P < 0.001$ ) and viability (EQ 0hr = 58.2±5.64% 1hr = 57.0±5.87% 2hr = 49.1±5.72 4hr = 53.9±5.24% and INRA 0hr = 48.4±3.85% 1hr = 51.9±3.92% 2hr = 45.2±3.63% 4hr = 43.1±3.37%;  $P < 0.001$ ), but not for thawing temperature at time 0, 1, 2 and 4 h ( $P > 0.05$ ). This study indicates that sperm tolerate fast thawing rates (~700°C/min) at 50°C vs. 37°C (~230°C/min) as long as the straw internal temperature does not exceed 37°C, potentially allowing greater flexibility for field procedures.

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## ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV) INFECTION IN AN ASIAN ELEPHANT (*Elephas maximus*) IN NEPAL

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### Abstract

Elephant endotheliotropic herpes virus (EEHV), a fatal herpes viral infection in young Asian elephant calves has become a concern in elephant camps and breeding centers. EEHV has a very rapid onset and progression and often leads to a fatal outcome. It was first reported from North American Zoos; later many cases were reported throughout the world.<sup>6</sup> In Nepal, five sporadic cases have been reported since 2002 from which four of the affected calves have died. Only clinical signs and postmortem lesions were the basis for diagnosing herpes in the past. For the EEHV-affected female elephant calf presented in 2012 at the breeding center, recent diagnostic molecular tests were performed. The calf died despite the early supportive treatment with Aciclovir (Ovir-800, XL Laboratories Pvt. Ltd., Rajasthan, India; 10 mg/kg p.o., every 8 hr) because famciclovir or ganciclovir were not available. Typical signs and symptoms of classical EEHV infection (lethargy, facial edema, cyanosis of the tongue) were observed before her death. Blood samples collected before death revealed a severe lymphocytopenia, monocytopenia and thrombocytopenia. Histopathologic examination showed presence of basophilic intranuclear inclusion bodies in capillary endothelial cells of various tissues. DNA extracted from tissue samples was analyzed by conventional PCR methods and DNA sequencing confirmed EEHV type 1A. This is the first confirmed herpesvirus case in Nepal.

### Introduction

Elephant endotheliotropic herpes virus (EEHV) has been a problem in young elephants throughout the world. This disease is highly pathogenic to juveniles from 1-4 yr of age and lethal in most of the cases.<sup>5</sup> The number of reports of this disease has crossed over 60 with mortality of 85% and death within 24-48 hr after the onset of first clinical symptoms of lethargy, facial edema and tongue cyanosis.<sup>9</sup> Thus EEHV poses one of the biggest threats in captive elephant breeding programs.<sup>4,9,14,20</sup>

Herpes virus in elephants was first reported in the early 1970s in an African elephant (*Loxodonta africana*) as inclusion bodies in ultrastructural findings from pulmonary nodules.<sup>1,10</sup> Later, the virus was also detected as intranuclear inclusion bodies from cutaneous skin lesions<sup>7</sup> and the virus was also identified in the tissue samples from the

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dead Asian elephant (*Elephas maximus*).<sup>11</sup> The first index case for the Herpesvirus infection was from the National Zoo, Washington, D.C. in 1995 which was studied extensively and then reported in 1996.<sup>13</sup> After that, several cases from all over the world were investigated to understand this novel disease. In 1999, this virus was suggested to be specific for elephants, different from other B-herpesvirus and then termed as elephant endotheliotrophic herpes virus (EEHV).<sup>14</sup>

Phylogenetically, EEHV is classified into family Herpesviridae under the genus *Proboscivirus*.<sup>2</sup> To date, eight different strains of EEHV are known. These include two chimeric variants of EEHV-1 (i.e. EEHV-1A and EEHV-1B), EEHV-2, EEHV-3, EEHV-4, EEHV-5, EEHV-6 and EEHV-7. Their range of pathogenicity and distribution is also variable. For example, the wild African elephants frequently carry four viruses- EEHV-2, EEHV-3, EEHV-6 and EEHV-7 in benign lung nodules and cutaneous nodules and these elephants are less susceptible to disease severity. On the other hand, EEHV-1 is the most commonly isolated strain in the Asian elephant and most pathogenic both in the wild and captivity.<sup>4,5,19</sup>

Understanding disease dynamics in EEHV has been a challenge. Laboratory findings of this virus in asymptomatic elephants even for the fatal strain<sup>4,16,18</sup> suggests the latency and reactivation characteristics as described for Herpesviridae family viruses<sup>8</sup>, but EEHV mechanisms of latency and pathogenesis are expected to be different from other herpesviruses like cytomegaloviruses and roseoloviruses.<sup>5</sup> Continued inability of the virus to grow in the cell culture has limited the understanding of its pathophysiology.<sup>15</sup> Sporadic detection of EEHV-1 gene in nasal secretions, conjunctival swabs and blood from clinically healthy Asian elephants,<sup>4,16,17</sup> wide range of genetic diversity among Indian EEHV-1 cases<sup>19</sup> and identification of the first EEHV-4 case in Thailand<sup>15</sup> corroborate the ideas of the Asian elephant as a natural host species and the virus could be commonly distributed in host species causing sporadic clinical infection in the young calves. Recent advances in molecular methods have led to a better detection and understanding of EEHV strains. Still the important epidemiological feature like prevalence, exposure and pathogenesis are complex to understand. A study on EEHV-1 viral kinetics and genetics<sup>16</sup> has also provided new information on further understanding this disease. A single elephant can be infected with multiple EEHV strains though simultaneous infection with both strains at the same time has not been found. Sterilizing immunity against one subtype does not confer sterilizing immunity against subsequent infection with other strains.

There is no treatment for the latent herpesvirus infection in animals or even in humans. Fanciclovir and Ganciclovir are used in clinical cases believing that these can suppress viral replication and cellular damage while the virus is circulating and thus an early detection of EEHV following early treatment could lead to treatment success.<sup>6</sup> Recent study on EEHV-1 cases<sup>16</sup> showed that viral DNA (i.e. viremia) is detected in elephants before the onset of clinical illness. The viral DNA in blood precedes the detection in trunk washes. Regular monitoring of the herd for viremia and virus in trunk washes could help in making the decision for early treatment before the clinical symptoms appear, especially in young elephants in which the disease is often fatal. Even though these drugs

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might suppress viremia and widespread organ infection, it probably could not prevent the viral localization in specific organs without any clinical manifestation. So it was found in these studies<sup>16,18</sup> that even after viremia was detected in negligible amounts after successful treatment, viral DNA remained detected in trunk washes in higher level for much longer period and there were also cases where DNA was detected in trunk washes at the point where no viremia was detected in the blood.

Various studies have been done in captive elephants in North American and European zoos, but with fewer studies being done on these viruses in the Asian elephant range countries this limits the understanding about these viruses in these areas. The purpose of this article is to report a case of EEHV infection in a captive-born Asian elephant, which is the first confirmed case of EEHV in Nepal.

### **Case Study**

The following case describes EEHV in a juvenile Asian elephant in Nepal. This female calf was born from a captive dam and wild sire on 9 May 2011 and was a herd member (herd consisted of mature cycling females, gravid females and juveniles with their mother) in the elephant breeding center. She was healthy and playful and had normal body condition and health before she showed herpesvirus symptoms. Mahouts reported her sluggish behavior in the evening on 21 November 2012. The next day in the morning she was accessed for a health examination in which she was inactive, sluggish and reluctant to move and had developed cyanotic spots on the tongue. She was highly suspicious for EEHV infection. An attempt to measure her body weight was not successful so she was estimated by her age at 500 kg. Since Famciclovir and Ganciclovir were not available, treatment was initiated with Aciclovir as the only alternative, the therapeutic efficiency of which is questionable.<sup>6</sup>

Treatment was started on 22 November 2012 with Aciclovir (Ovir-800, XL Laboratories Pvt. Ltd., Rajasthan, India; 10 mg/kg PO every 8 hr), Meloxicam (Melonex Power, Intas Pharmaceuticals Ltd; 0.4 mg/kg IM once daily) and Ampicillin-Dicloxacillin (Moxel-D, Alembic; 5 mg/kg IM once daily) and was continued until she died. Acyclovir were administered per os, putting the tablets inside bananas, three times a day; 10:00 am, 6:00 pm and 2:00 am and she was accepting the treatments. On 23 November, facial, trunk and limb edema was pronounced and she was hypothermic (95.9 °F). Heart sounds were not easily audible and pulse was very feeble. The day before she died, 24 November, severe body trembling and hypothermia (94.2°F) were pronounced at the time she was examined (10:00 a.m.). She was given intravenous fluids after warming them in lukewarm water. 500 ml Rintose (Wockhardt Ltd., India), 500 ml Normal saline and 500 ml Ringers Lactate were given by placing a 21-gauge IV catheter in her aural vein, glued to the skin. In the evening, she was weak and lying in sternal and lateral recumbency. After some struggling, she could get up and walk, but there was trunk trembling and she moved with difficulty. From 6:00 pm onwards, she was very weak unable to accept the Aciclovir. She died on 25 November at 4:00 am.

Hematology examination was performed from a blood sample collected before death.



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Manual blood cell differentiation and platelet count was performed. The tests revealed low hemoglobin levels (10.7 g/dl), lymphocytopenia ( $2.88 \times 10^3/\mu\text{l}$ ), monocytopenia ( $0.45 \times 10^3/\mu\text{l}$ ) and severe thrombocytopenia ( $53 \times 10^3/\mu\text{l}$ ). Postmortem examination was performed on the day of her death. The tongue had raised, white nodules over the surface and was cyanotic. Extensive hemorrhages were observed in the subcutaneous blood vessels. Generalized serosal hemorrhages were also observed over the peritoneum and muscles. Kidneys were enlarged and severe hemorrhage were observed in intestinal serosa, and uterus. The heart had a burnt appearance with severe involvement of the epicardium. Endocardium was also affected and there was severe edema over the papillary muscles. Histopathologic examination was performed on the formalin fixed tissue with identification of inclusion bodies in the heart, liver and tongue. Fresh tissue samples were collected in cold box and then deep frozen until further analyzed.

Molecular tests were performed with DNA extracted from frozen whole blood and minced liver tissue using DNeasy Blood & Tissue Kit (Qiagen) as per the manufacturer's recommended protocol. Conventional PCR was performed and platinum blue supermix (Invitrogen) was used to provide necessary reagents for PCR. Pan EEHV POL and EEHV1-specific U38 POL primers used in this study were given by Dr. Gary Hayward (Johns Hopkins School of Medicine, Baltimore, MD) for the Herpes project of Emily Picciotto (student, Tufts Cummings School of Veterinary Medicine). Pan EEHV POL PCR was performed with forward primer (L1 LGH6710) and reverse primer (R1 LGH6711) while EEHV-1 Specific U38 Pol PCR was performed with forward primer (L1 LGH7446) and reverse primer (R1 LGH7445). The thermocycler program used was as follows: 95°C for 2 minutes, then 45 cycles of 95°C for 40 seconds, 50°C for 45 seconds, 73°C for 1 minute, then final 72°C for 7 minutes.

Laboratory tests showed positive results from both PCR with PAN POL and EEHV-1 specific U38 POL primers. Specific DNA bands could be visualized after gel electrophoresis on 1.5% agarose gel. No second round nested PCR were performed since there were adequate DNA levels for visualization in the first round PCR. Bands detected of the appropriate length were excised using a clean scalpel and DNA fragments were extracted using a Qiaex II Gel Extraction Kit (Qiagen). DNA sequencing confirmed that EEHV-1A was responsible for the death of the calf.

## **Discussion**

Nepal has a captive elephant population of more than 215 individuals distributed in five conservation areas of terai belt. They are involved in tourism activities as well as in the management of protected areas. In addition to elephant tuberculosis as the most problematic disease in captive populations, herpesvirus has now been identified causing fatal infection in juveniles.

EEHV was first confirmed in 2006 to be present in Asia<sup>12</sup> and since then, other herpesvirus cases from the Asian elephant range countries have also been reported. Still, studies in these range countries have not been done in as much detail as in the North American and European zoos. Thus the exact status of Herpesvirus in these range

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countries is still largely unexplored. The recent finding of the EEHV-1 vast genetic diversity in South Indian EEHV cases encompasses almost all of the overall genetic range of EEHV-1 variants from North America and Europe.<sup>19</sup> Still, the North-West population of elephants (captive as well as wild that are closely related to the elephants of Nepal) are not accessed for Herpesvirus diagnostics. When considering wild tamed native elephants, elephants gifted from other range countries (e.g. Thailand, Burma, India) and wild bulls involved in captive breeding, these conditions suggest that there could be a vast EEHV diversity with the possibility of EEHV strains in other range countries that enter and get established in the Nepalese elephant population. This situation could be further complicated with superinfection<sup>16</sup> of EEHV strains.

Wild as well as wild-born, but captive-raised juveniles were confirmed with the EEHV in South India<sup>19</sup> which suggests that this virus could affect the survivability of juveniles in the wild population. EEHV could be one of the contributing factors for decreasing the range of endangered Asian elephants in most of its range countries and for the crossing of the critical endangered threshold level for this species in Sumatra and Vietnam,<sup>3</sup> though the correlation between these two has not yet been made. EEHV spread in range countries could also be aggravated by the trans-boundary migration of elephants; more in countries like Nepal, Bhutan, Bangladesh and Indonesia where the trans-boundary migration population consists of a major fraction of the wild population.

The history of herpesvirus in captive elephants in Nepal dates only a few years back with the first report of herpesvirus symptoms on 6 November 2002. To date, there are five reports of herpesvirus infection in juvenile elephants and only one could be saved by the aggressive use of Famciclovir. No such information on sudden death of elephant calves with herpesvirus symptoms was available previously and so was thought to be a novel disease in Asian elephants. The finding of Indian EEHV-1 cases as native to the Asian elephant population<sup>19</sup> was in contrast to what was assumed earlier. This is further supported by the report of EEHV-4 in Thailand.<sup>15</sup> It is uncertain whether modern diagnostic capability improvements have increased disease identification or if several interacting environmental agents and various other co-factors could be responsible for increasing the susceptibility to infection and the number of cases in juveniles. The first EEHV-1A confirmed case in Nepal showed that this strain could be prevalent, but still it is not known whether the calf had acquired herpesvirus from her mother or from other herd members. It is also uncertain whether the same strains were responsible for the previous herpesvirus case or that completely different strains may be involved. The impact of EEHV infection on the wild population is also unknown. Research in the future should be directed toward exploring these questions. Routine evaluation for herpes viremia in captive herd populations and further investigation in the wild will help to explore the diversity of EEHV in Nepal.

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**CESTODE INFESTATION IN TWO CLOSED COLONIES OF DAMARALAND MOLE RATS (*Cryptomys damarensis*)**

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**Abstract**

Two distantly related Damaraland mole rat (*Cryptomys damarensis*) colonies became infested with cestodes. Colony one had approximately 10 out of 24 animals develop soft subcutaneous (SQ) swellings. One animal was euthanized for necropsy and histopathology. Histopathology and parasitological analysis suggested an infection with a *Taenia* sp. Colony one was treated with oral praziquantal at approximately 30 mg/kg body weight every other day for 10 doses and has shown a reduction in the size of the swellings as well as increased activity. Colony two had only one individual develop a soft SQ swelling. This animal had several surgeries for mass debridement and was treated parentally and orally with praziquantal and orally with albendazole. Parasitological examination of the mass contents suggested infestation with a *Mesocostode* sp. No source for the cestode infestation has been identified.

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## FUNGAL INFECTIONS IN ZOO ANIMALS OF THE ROYAL ZOOLOGICAL SOCIETY OF ANTWERP

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### Abstract

Virology, bacteriology and parasitology appear to dominate modern veterinary clinical practice. Coles lists 78 viral, 29 bacterial, 49 parasitic and 7 mycotic infectious avian diseases, the latter being very important in the differential diagnosis, specifically in birds where aspergillosis is frequently diagnosed.<sup>1,2</sup> Trying to evaluate the impact of fungal infections on zoo animals, the present retrospective study was carried out in the Royal Zoological Society of Antwerp. During the last 34 yr, approximately 42,000 animals were born in the zoo or were brought in from other zoos i.e. 4,000 reptiles, 4,000 fishes, 4,000 amphibia, 10,000 mammals and 20,000 birds. Fungal infections causing dermatological or systemic disease in all 5 animal orders were demonstrated in a total of 675 samples, corresponding with an overall prevalence of 1.6%. These samples were from: 5 amphibia, 26 fishes, 49 reptiles, 150 (22%) mammals and 445 (66%) birds, in total 169 (25%) from living animals and 506 (75%) from dead animals. Fungal infection was 50% more prevalent in birds (2.2%) than in mammals (1.5%). More than half (56%) of all infections were due to *Aspergillus* species (predominantly *A. fumigatus*), of which 93% occurred in birds (i.a. 24% gallinaceous birds, 15% geese and ducks, 14% penguins, 9% songbirds, 8% parrots, 5% turacos and 4% birds of prey). Although the overall prevalence may not seem very high, mortality due to fungi is important bearing in mind that these samples came from 25% live animals and 75% dead animals.

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## THE ROLE OF NON-GOVERNMENTAL ORGANIZATIONS IN ASSISTING ZOOS AND AQUARIA DURING DISASTERS

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### Abstract

Although there is still controversy about climate change, reports and research describe the probability of an increase in the severity and intensity of extreme weather events (severe storms, droughts, flooding). The frequency and intensity of violent events such as tornadoes or hurricanes will become more difficult to reliably predict. Zoos and aquariums may find themselves in situations where these intense weather events may place their animals in jeopardy and therefore must be moved or require the assistance from volunteers to aid with injured or displaced animals. One source of trained volunteers could be found within non-governmental organizations (NGOs) that have experience working with wild animals through various research programs. NGOs that have assisted with the collection of physiological data from wild marine mammals (dolphins, manatee, and seals) are an untapped source of volunteers who have hundreds of hours of valuable experience in handling and caring for wild species. Also, because these wild animals must often be sedated for the collection of data, these volunteers also have experience with anesthesia and the care of sedated wild animals. For example, a collaborative effort has been ongoing for 6 yr between the United States Geological Survey (USGS) and the authors' NGO (Oceanographic Environmental Research Society - OERS) where volunteers have been sent to assist with the capture/care of wild manatees for the collection of samples/data. The benefits of initiating collaborative efforts between an NGO and a zoo or aquarium would create a huge resource of experienced volunteers during times of disaster or crisis.

### Introduction

There still much controversy about climate change and possible effects. However, it would seem that there is credible climate change research describing the probability of increasing numbers of extreme weather events (large storms, droughts, floods, etc) that will be more severe and of higher intensity.<sup>9,11</sup> As well, the frequency and intensity of violent events such as tornadoes or hurricanes will become more difficult to reliably predict.<sup>9</sup> It has also been reported that regional geographical differences such as being near mountains, located on a river, or situated along the coast may place more cities at risk as it is harder to predict extreme weather events within these smaller areas.<sup>3,9,11</sup> This was clearly seen during Hurricane Katrina where some areas were either totally devastated due to a storm surge or affected by high winds (Figures 1,2).

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Most of the earth's population lives along some coastal region and as a result, eight of the world's largest cities can be affected by earthquakes and six of the world's largest cities are vulnerable to storm surge and tsunami waves.<sup>4</sup> In the United States, depending on the criteria used to describe coastal counties, population numbers living on or near a coastal zone varies between 37% to 49%.<sup>3</sup> Most zoos and aquariums are built near the larger population centers (e.g. Aquarium of the Americas in New Orleans) or in disaster-prone areas (e.g. Monterey Aquarium, California). Therefore they are also at a higher risk of being affected by some sort of natural disaster or strong storm-like event.

Unfortunately since many zoos or aquariums are located within or near these large populated areas, they find themselves in situations where these intense weather events may place their animals and facility in jeopardy (Figure 3). This may require moving some of their animals to areas of safety or even relocation as was seen with the Aquarium of the Americas in New Orleans during and following Hurricane Katrina in 2005 when they had to relocate their entire penguin colony to the Monterey Aquarium in California.<sup>9</sup> The New York Aquarium was seriously affected when Hurricane Sandy devastated the facility with over \$65 million dollars in damage. It took 7 mo for it to re-open, and required the dedicated aquarium staff to work courageously to save every animal in their care except for 150 freshwater koi.<sup>8</sup>

### **Zoos, Aquariums and Disaster Planning**

In December 2012, the United States Department of Agriculture Animal and Plant Health Inspection Service announced that all licensed and registered facilities must have an emergency plan in place to better protect their animals during times of disaster.<sup>10</sup> The Zoological Best Practices Working Group for Disaster Preparedness and Contingency Planning (ZBPWG) has written an excellent guide to assist the various groups who care for and deal with wildlife which includes zoos and aquariums.<sup>13</sup> It is a very complete guide for facilities of any size, individuals, or professional associations within the United States. However, it is only a guide and the mission statement of the ZBPWG is to "promote a culture of 'all hazards contingency planning and preparedness' for the managed wildlife community. To that end, the group will research, prepare, review and disseminate documents to assist facilities in drafting their own contingency plans. The Working Group will encourage facilities to work with first responders, local emergency management and other stakeholders to draft useful plans that are integrated into their jurisdictional emergency management infrastructure."<sup>14</sup> It leaves the details of the emergency plans to each facility or individual which are based on several factors or the resources available including the size of the facility, number of animals involved, and number of staff. The ZBPWG advises assembling a planning team comprised of stakeholders, experts and partners which could include key Non-Governmental Organizations (NGOs) such as American Association of Zoo Veterinarians (AAZV), Association of Zoos and Aquariums (AZA), American Association of Zoo Keepers (AAZK), and many others. The AAZV has organized a Disaster Preparedness Committee which supports its members to prepare for disasters through education, developing of disaster/response templates, web resources, a frequently asked questions (FAQ) sheet for



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veterinarians and disaster response, and initiate contact with several agencies.<sup>1</sup>

As far back as 2003, the AAZV has published several papers in their Proceedings or had posters presented at their annual meeting to address topics such as a veterinarian's role during a disaster or disaster planning for zoos or aquariums. In 2003 and 2008, Dr Mark Lloyd published papers describing the role of zoological veterinarians in various emergency scenarios and the need for an integrated national plan for zoos and aquariums to help mitigate the pain and suffering of their animals.<sup>6-7</sup> Wittnich and Belanger (2009) presented their study of how unprepared Canadian zoos and aquariums were and found that 6 out of 13 facilities did not have an emergency plan in place.<sup>13</sup> That same year, Dr. Lisa Done reviewed the need for zoos and aquariums to be prepared for disasters and the specific concerns and considerations of the many species (fish, avians, mammals) found within facilities<sup>4</sup> (Figure 4). However, there has been no mention in any plans or presentations of using NGOs during times of disaster. NGOs have at their disposal an excellent pool of trained volunteers who have experience in the handling and caring for wildlife while assisting with research in the wild.

### **NGOs and Disasters**

Following any type of disaster or emergency, there is a knee-jerk reaction on the part of the authorities to close off outside volunteer assistance until Incident Command has been firmly established. This was seen with Hurricane Katrina and the BP Gulf of Mexico oil spill where the response to assist with the care of pets or the rehabilitation of wildlife was controlled by authorities which allowed very few outside NGOs or volunteers to assist.<sup>12</sup> The lack of volunteer assistance might put enormous stress on the regular staff members who, during times of a disaster, will spend huge amounts of time performing life-saving daily activities to keep the animals alive, which was seen at the New York Aquarium after Hurricane Sandy.<sup>8</sup> Therefore, it is critical that zoos and aquariums develop a disaster response plan that includes NGO volunteers that they can call upon to assist. However, it is often very difficult to find qualified and trained volunteers who have experience with wildlife species found in a zoo or aquarium and it is very expensive and time consuming to train them. One source of trained volunteers could be found within NGOs that have volunteer members who have hands-on experience working with wild animals through various research programs. These NGOs have worked alongside scientists, biologists, and veterinarians assisting with the collection of physiological data from wild marine mammals (dolphins, manatee, and seals). These volunteers have hundreds of hours of valuable experience in handling and caring for wild species, often sedated, while collecting data.

A collaborative effort has been ongoing for 6 yr (2008-2013) between the USGS, Florida Fish and Wildlife Conservation Commission, and our NGO, the Oceanographic Environmental Research Society (OERS), where volunteers have been trained to assist with the capture and care of wild manatees during the collection of samples and data for health assessment programs (Figures 5,6). The collaboration between these various groups includes biologists, scientists, wildlife veterinarians, veterinary technicians, and NGO volunteers who are trained as teams for the collection of data to understand the

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health status of a large species of marine mammal- the manatee (*Trichechus manatus*)<sup>H</sup>. The manatee is a large marine mammal weighing up to 800 kilograms and has a total length of up to 335 centimeters.<sup>2</sup> This means that although a docile animal, the manatee may become very difficult and dangerous to handle if the team members are not properly trained or understand the anatomy, physiology, or behavior of this unique marine mammal species. Therefore the training and coordination of a large number of volunteers and the organizing of the logistics behind each capture is crucial in ensuring both the safety of the animals and every team member. Between 2008 to 2012 the authors' NGO group has sent teams of volunteers to assist with the capture and handling of over 100 wild manatee.

During times of a disaster, collaborative efforts between an NGO and a zoo or aquarium would have far reaching benefits towards the safety and care of the facility animals. It is obvious that an NGO that has been assisting a zoo or aquarium with its research program would offer a huge source of trained and experienced volunteers. During a disaster, these volunteers could assist with many of the daily chores and husbandry duties (e.g. preparing food, feeding, or cleaning of pools). These volunteers would also insure that essential equipment such as pumps and generators are filled with fuel or maintenance of that equipment is kept going day and night to make sure that no animals suffer or die when electrical power is not available. This would free up more experienced staff or management to do other critical responsibilities. An NGO may have volunteer veterinarians and veterinary technicians who are trained with many hours of handling wildlife and could now become part of the disaster planning efforts and response. Training could be shared between both the wildlife facility and the NGO during research efforts thereby making it easier to have volunteers who are prepared to respond. As well, having an NGO who is already part of the facility's research programs and is included within its disaster planning and response efforts would ensure a compatibility of ethics towards the efforts of conservation of captive and wildlife species.

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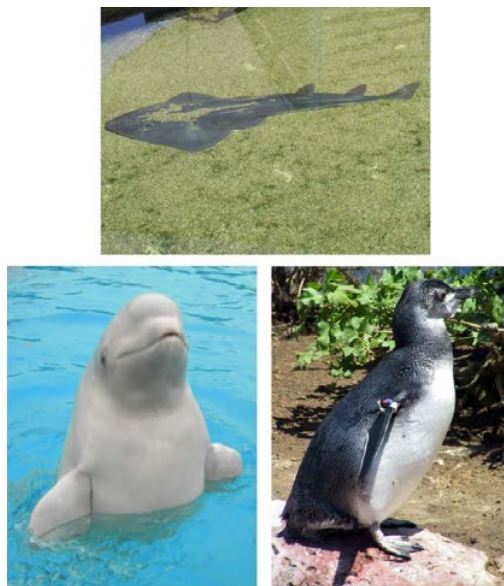
**Figure 1.** House destroyed by storm surge.



**Figure 2.** Tree stripped bare by high winds.



**Figure 3.** A facility destroyed by storm surge.



**Figure 4.** A wide variety of species are located in zoos or aquariums that require care after a disaster.



**Figure 5.** Volunteers from the Oceanographic Environmental Research Society, a Non Governmental Organization, assisting with the capture of a wild manatee.



**Figure 6.** Veterinarians and veterinary technicians working alongside Oceanographic Environmental Research Society volunteers.