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## SAFEGUARDING UNITED STATES ANIMAL INDUSTRIES AGAINST INCURSION OF FOREIGN ANIMAL DISEASES

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### **Abstract**

The Veterinary Services (VS) Strategic Plan of the United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA APHIS) lists "Safeguard the U.S. from the occurrence of adverse animal health events." as Goal 1.<sup>3</sup> "Exclusion," or prevention of foreign animal disease entry into the country through ports and across borders, is a front-line effort in the overall safeguarding plan. A survey of stakeholders representing commodity, trade and other industry groups along with responsible state agencies revealed that human travel, animal product imports and the importation of exotic birds represented the highest risks of animal disease introduction in their minds. U.S. Customs Service figures for FY 2000 list 489,000,000 passengers and pedestrians crossing U.S. Borders, and almost 140,000,000 conveyances including trucks, buses, ships, aircraft and cars. The volume is expected to double by 2009.<sup>2</sup>

Keeping the animal industries of our country free of foreign pathogens is a multi-agency federal and state government effort that depends upon the cooperation of the various industries themselves. The effort attempts to balance plant and animal issues, and encompasses much more than just border and port inspections. The Animal Health Safeguarding Review, Results and Recommendations (2001) states "Effective exclusion activities are a continuum from the gathering of international animal health information and trade negotiations through the promulgation of import regulations, review of import requests, and the physical inspection activities at ports of entry; to domestic surveillance and monitoring systems which include field and laboratory infrastructure designed to detect the incursion of foreign animal disease."<sup>2</sup>

It is no longer just an agricultural issue. Threats of bio- or agri-terrorism have made U.S. biosecurity a national, military, and food security issue. Beyond the well-documented economic devastation caused to food animal producing industries in major disease outbreaks, free-ranging and captive wildlife populations, commercial animal populations, and companion animal populations are all at risk. In 2001 the U.S. livestock industry was estimated to be worth about \$100 billion. The poultry industry alone has suffered losses estimated at as much as \$12,000,000 per week in international trade interruptions resulting from recent avian influenza (AI) outbreaks in the Northeast and other parts of the country, most of which were low pathogenic AI strains. Some diseases harbor zoonotic potential, making their introduction by whatever means a public health concern as well.

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Prior to the creation of the Department of Homeland Security (DHS) in 2002, many of the port inspection activities of passengers and animal product shipments were conducted by the Plant Protection and Quarantine (PPQ) division of USDA APHIS, in collaboration with U.S. Customs Service officers. These were the people you saw at airports or seaports when entering the country. In March 2003, DHS established the Customs and Border Protection (CBP) division, absorbing the majority of USDA's PPQ employees nationwide, along with their responsibilities and functions.

Veterinary Services maintains a presence at major border crossings and air and sea ports through which live animals enter the country. Livestock species and most types of birds are subject to import quarantine. There are three major Animal Import Centers in the United States. The New York Animal Import Center (NYAIC, Newburgh NY) and the Miami Animal Import Center (MAIC, Miami FL) are both fully staffed and operated by USDA employees. The Animal Import Center in Los Angeles, CA is privately owned and operated, but overseen by Veterinary Services personnel. In addition, USDA operates a bird quarantine station in San Ysidro, CA. Ruminants and swine are held in quarantine for a minimum of 30 days, horses for 3, 7, or 60 days (depending on country of origin and its endemic diseases). Birds remain in quarantine for 30 days, except for smuggled birds, which stay a minimum of 45 days. During their quarantine, animals "...shall be subject to such inspections, disinfection, blood tests, or other tests as may be determined by the Administrator, to determine their freedom from disease."<sup>1</sup>

In addition to USDA-operated facilities, approved, privately owned and operated quarantines for "commercial" birds (intended for zoos, conservation, or pet trade) are located in port cities such as Los Angeles, New York and Miami. These facilities are carefully inspected and monitored on a daily basis by USDA Veterinary Medical Officers or Animal Health Technicians. In the summer and fall of 2003, Exotic Newcastle Disease (END, formerly known as VVND) was identified by virus isolation from cloacal swabs in three different private commercial bird quarantines, prompting depopulation of nearly 6,000 birds, some of which were headed to zoos around the country.

USDA APHIS VS recognizes its responsibility to global conservation efforts of endangered and threatened species. Every effort is made to return such species to their country of origin in cases where they are cohorts in shipments of disease-positive birds but are themselves negative. In rare instances, such species have been allowed to enter the United States after extended quarantine and repeated negative testing. A recent example (summer 2003) involved four juvenile Jabiru storks (*Jabiru mycteria*) that had the misfortune of sharing a private commercial bird quarantine with African hornbills that were END-positive.

Since publication of the Safeguarding Review in October 2001, there have been major outbreaks of AI in the Northeast and Texas, END in California and parts of the Southwest, and a single case of Bovine Spongiform Encephalopathy (BSE) in Washington State whose political and financial ramifications are still being felt. These outbreaks have strained the Agency, requiring

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diversion of personnel and resources away from safeguarding and other duties. Despite this, USDA is dedicated to implementing fully the recommendations of the Safeguarding Review, including major improvements in funding, staffing, training, information technology and physical facilities.

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## RENAL MYXOZOANOSIS IN WEEDY SEA DRAGONS (*Phyllopteryx taeniolatus*)

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### Abstract

Weedy sea dragons (*Phyllopteryx taeniolatus*) are related to seahorses and pipefish, and are found in reefs and sandy underwater regions of Western Australia, South Australia and further east along the coastline of Victoria, Australia. These fish are protected under Australian law, and have become popular recently for aquarium exhibits in the United States. With the exception of a few parasites,<sup>1-3</sup> little is known of the pathogens of sea dragons. Myxozoanosis has not previously been reported in sea dragons. This report describes the occurrence of renal myxozoanosis in 11 weedy sea dragons.

Results of this study are summarized in Table 1. The sea dragons with renal myxozoanosis were from three separate aquaria in the United States, and all cases were diagnosed during May-June of 2003, except one case that was diagnosed in March 2002. All were wild caught, and had been in captivity at the National Aquarium, Baltimore, MD (Case 1), Point Defiance Zoo, WA (Cases 2-7), and Aquarium of the Pacific, CA (Cases 8-11). All were adults of unknown age. Six were female, four were male, and one was undetermined. Inappetence was the most common clinical sign. Skin erosions and snout abrasions or deformities were the most common gross lesions. Important concurrent disease processes included cutaneous and/or systemic ciliated protozoan infection (seven cases), hepatic lipidosis (six cases), steatitis (four cases), mycobacteriosis (three cases), and biliary myxozoanosis (two cases).

Histologically, all animals had large numbers of developing myxosporeans in the lumina of renal tubules and collecting ducts. In all cases, this finding was associated with a mild degree of renal tubular dilation, hypertrophy of tubular epithelial cells, and accumulations of cellular debris and some proteinaceous fluid in the tubules.

Myxozoan spores in wet mounts and histologic sections had morphology consistent with that of the genus *Sinuolinea*.<sup>4</sup> Members of this genus have been described from the urinary bladder of a

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variety of marine fishes. Spores were characterized from one specimen as spherical or sub spherical (16.7  $\mu\text{m}$  length  $\times$  16.5  $\mu\text{m}$  width) with two spore valves joined by a sutural line that curved around the spore, forming a well marked sutural ridge. The two polar capsules (5.4  $\times$  5.2  $\mu\text{m}$ ) located anteriorly were set widely apart and contained polar filaments with approximately six turns. Analysis of the 18S rDNA gene will help clarify the taxonomic position of this isolate.

The significance of renal myxozoanosis to the health status of captive weedy sea dragons is not known. All fish had one or more concurrent disease processes that could account for morbidity and/or mortality. Morphologic alterations associated with the presence of these parasites in the renal tubules were generally mild, although the impact of this infection on renal function was not determined. Antemortem detection of this parasite may be possible, as spores are shed into the urine. Because all of the fish were recent captives, it is possible that this infection occurs naturally in wild weedy sea dragons. Further characterization of the parasite may provide additional information regarding its life cycle, and the potential for spread to other fish on exhibit.

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**Table 1.** Signalment, history and clinical signs, gross and microscopic pathology of weedy sea dragons (*Phyllopteryx taeniolatus*) with renal myxozoanosis.

Case	Origin <sup>a</sup>	Age <sup>b</sup>	Sex <sup>c</sup>	Clinical Signs	Gross Pathology	Additional Disease Processes <sup>d</sup>
1	naq	A	m	6 wk rostral ulcer, weight loss, died	missing appendage, tan kidneys	sepsis, + hepatic lipidosis
2	pdz	A	m	none	pale snout, skin erosions	ciliate bronchitis, intestinal cestodiasis, steatitis
3	pdz	A	m	several days of inappetance	pale snout, skin erosions	systemic ciliated protozoan infection, intestinal nematodiasis and cestodiasis, steatitis
4	pdz	A	m	sick for 2 wk, abnormal swimming pattern, died	ventral abdominal purple discoloration, sloughing of skin and scales	biliary myxozoanosis, systemic ciliated protozoan infection, steatitis, mineralized swim bladder
5	pdz	A	f	wild caught 2 wk earlier, 2 wk inappetance, abnormal swim pattern, died	None	ciliates in brain, cestodiasis, ulcerative cellulitis, ++ hepatic lipidosis
6	pdz	A	f	wild caught 2 wk earlier, 2 wk inappetance, died	erosion on snout	ulcerative cellulitis with ciliates, steatitis, ++ hepatic lipidosis
7	pdz	A	f	wild caught 3 wk earlier, 2 days of misshapen snout, died	nodular snout, skin erosions, facial laceration	ulcerative cellulitis with ciliates, unidentified intestinal parasite, ++ hepatic lipidosis
8	ap	A	F	wild caught 2 mo earlier, 2dy inappetance, died	white patches on skin, flagellates seen in skin scraping	emaciation, unidentified intestinal parasite, biliary myxozoanosis
9	ap	A	f	wild caught 2 mo earlier, found dead, no clinical signs	None	mycobacteriosis, biliary nematodiasis, ++ hepatic lipidosis
10	ap	A	f	wild caught 3 mo earlier, flagellates on skin, inappetance, morbid, euthanized	rostral abrasion	mycobacteriosis, unidentified intestinal parasite, emaciation
11	ap	A	-	wild caught 3 m earlier, found dead	white patches on skin, flagellates seen in skin scrapings	mycobacteriosis, ulcerative cellulitis with ciliates, + hepatic lipidosis

<sup>a</sup>naq = National Aquarium, Baltimore; pdz = Point Defiance Zoo; ap = Aquarium of the Pacific.

<sup>b</sup>A = adult.

<sup>c</sup>m = male, f = female.

<sup>d</sup>+ = mild, ++ = moderate, +++ = severe.

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## INVESTIGATION OF A FATAL MYCOPLASMA INFECTION IN VAAL RHEBOK (*Pelea capreolus*)

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### Abstract

The on-site Molecular Diagnostics Laboratory of the Zoological Society of San Diego investigated an outbreak suspected to be a foreign animal disease. Within a 4 wk period in February 2003, three male Vaal rhebok (*Pelea capreolus*), endangered South African antelopes, were presented to the veterinary hospital for evaluation of subdued behavior and decreased appetite of 24 hr duration. Additional clinical signs were variable and included ataxia, increased bronchovesicular lung sounds, excessive salivation, and head tremor. Radiographic changes supported a respiratory disease component in each case. Despite supportive treatment, all three were either euthanatized or died within 2 days of presentation.

Post-mortem examination showed that the animals had one or more lesions consistent with a systemic infection (pneumonia, cellulitis, and lymphadenitis). However, the etiology could not be determined histologically. The affected animals also had USDA permanent post entry quarantine (PQ) status that severely hindered the amount of diagnostic testing that could be done outside the grounds of the San Diego Zoo. Consequently, the zoo relied on its on-site Molecular Diagnostics laboratory to investigate possible viral or bacterial etiologies.

Using polymerase chain reaction (PCR) methods, the laboratory found that the animals were infected with mycoplasma of the mycoides cluster. These mycoplasmas are of particular concern, in that all species of this cluster are pathogenic in ruminants, and thus have a tremendous effect on livestock industries by causing death and disease in cattle, goats and sheep.<sup>3</sup> The mycoides cluster of mycoplasmas consists of six species that are closely related genetically and phenotypically.<sup>4</sup> Among these, *Mycoplasma mycoides* subsp. *mycoides* small-colony (MmmSC) and *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp), which are the causes of contagious bovine pleuropneumonia (CBPP) and contagious caprine pleuropneumonia

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(CCPP) respectively, are of particular concern. These mycoplasmas are classified by the Office International des Epizooties as A and B list pathogens<sup>1,2</sup> and are considered to be foreign animal diseases in the United States because of the threat they pose to domestic animals.

Due to the possibility of the agent being a foreign animal pathogen, the USDA was notified and archived samples from affected animals were submitted for evaluation. The USDA excluded the presence of MmmSC and Mccp, and confirmed our results that the disease-causing agent is a mycoplasma of the mycoides cluster. However the zoo was still faced with the dilemma of an infectious mycoplasma that had caused acute disease in the rhebok, and could possibly affect other animals. Further molecular analysis allowed the identification of each species of infecting mycoplasma, and the development of diagnostic tests to track possible contact animals. The results of this study illustrate that although mycoplasmal infections are ubiquitous and usually non-pathogenic, some species of animals can be particularly susceptible to them. The ability to use on-site molecular diagnostics in the disease investigation of PQ status animals can be an invaluable tool in determining the possible cause of the disease, and in future prevention of outbreaks through surveillance.

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## MORTALITY OF WINTERING MONARCH BUTTERFLIES: ARE EMERGING PATHOGENS THE CAUSE OF DECLINING POPULATIONS?

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### Abstract

The health of ecosystems is directly related to the health of species, in which the presence of disease in individuals and populations can reflect ecosystem health. Habitat fragmentation and destruction have led to ecosystem disruptions, including altered patterns of disease transmission and emerging infectious diseases (EID), the accumulation of toxic pollutants, and the invasion of alien species and pathogens. Using the criteria that define EIDs of humans, EIDs have been identified affecting domestic animals, crops, insects, wildlife and wild plants.<sup>1</sup> Knowledge of butterfly diseases is rudimentary; however, there are many agents, including baculoviruses and nuclear polyhedrosis viruses, bacteria, and fungi that may cause disease in butterflies.

The monarch butterfly (*Danaus plexippus*) is one of the best known North American butterflies, because of its annual migration. Unable to withstand freezing weather in the northern range, in autumn tens of millions fly south, roosting in huge numbers in oyamel fir (*Abies religiosa*) forest fragments in Mexico. In winter 2003-2004 approximately 10 million butterflies per ha were concentrated on a hibernation range of 11 ha. Monarch butterflies use the same trees year after year, and their journey can cover thousands of miles. Since the larval food plants do not grow in their overwintering sites, the spring generation flies back north to regions where milkweeds of the family Asclepiadaceae are plentiful. Monarch butterflies spend the summer in either the New England-Great Lakes area or the canyons of the eastern Rocky Mountains. It was not until 1976 that the overwintering grounds were discovered. The Great Lakes population spends the months of November-March in the Sierra Madre mountains of central Mexico.<sup>3,5</sup>

Mortality of monarch butterflies on the wintering grounds may be due to effects of global climate change, deforestation, starvation, desiccation, freezing, and predation by wild birds, the scansorial black eared mouse (*Peromyscus melanotis*), ladybugs, and ants. Mortality due to predation by wild birds may reach 9-15%. Overwintering monarch butterflies tolerate only a narrow range of temperature and wetness; a combination of freezing temperatures and rain can be lethal. Following a snowstorm in winter 1995-1996, mortality reached 7%, while in January

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2002 over 80% of the monarch population was killed.<sup>3</sup> Recent studies indicate that infection with the protozoan parasite *Ophryocystis elektroscirrha* may be related to low survival of infected larval and adult butterflies, induced by the sporozoite, which potentially causes severe damage to the gut, and mortality.<sup>2,4</sup>

During 2004, we sampled four sites in the Monarch Butterfly Biosphere Reserve, including Las Palomas, El Rosario, Llano de Toro (Sierra Chincua) and Coala (Sierra Chincua). We collected 10 live and 10 moribund or recently dead butterflies from each site for bacteriology, mycology, virology and toxicology. In addition, using pieces of Scotch tape, we collected samples of abdominal scales from 500 live butterflies per site, which then were released unharmed. Bacteriologic analysis focused on anaerobic, aerobic and environmental microorganisms. Several culture media were inoculated for fungal growth and characterization, and three cell lines (Vero, HeLa and HEp-2) were inoculated for virus isolation and identification.

Oocysts of *O. elektroscirrha* were documented at all sites during two sampling episodes. In January/February 2004, oocyst prevalence was: Palomas - 2.2/1.8%, El Rosario - 7.0/5.0%, Llano de Toro - 3.0/1.4%, Coala - 3.4/2.0%. Bacterial and fungal isolations were similar at all sites, *Corynebacterium* spp., *Bacillus* spp., zygomycetes and slow-growth fungi predominating. We currently are attempting to characterize a fungus similar to that found in the abdominal scales of live butterflies. A virus that causes cytopathic effects in Vero cells and plaques on the chorioallantoic membranes of chicken embryos also was isolated. Strong synergism between infection with *O. elektroscirrha* and a fungus that penetrates wing scales may contribute to severe mortality, perhaps abetted by other potential pathogens, including the virus isolated.

The Mexican government is reviewing the decree that originally protected the overwintering sanctuaries in 1986, and is getting input from many people with scientific knowledge of the locations of overwintering sites and the needs of monarch butterflies. The primary conservation issue is the high rate of oyamel fir deforestation. Supporting programs providing alternative sources of income for landowners are required to secure the long-term survival of this charismatic butterfly species.

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## IRIDOVIRUS INFECTIONS OF TURTLES AND TORTOISES

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### Abstract

Iridoviruses are large, variably host-specific, dsDNA viruses that infect invertebrates and poikilothermic vertebrates. Five genera are recognized, of which members of the genus *Ranavirus* have been shown to infect fish, amphibians and reptiles.<sup>3-5</sup> Several accounts of iridovirus infection have been documented in chelonians.<sup>1,2,5-8</sup> In the United States, only three cases have been reported; a Russian tortoise (*Testudo horsfieldii*), and a box turtle (*Terrapene carolina*) in which no pathology was described<sup>5</sup> and a wild gopher tortoise (*Gopher polyphemus*) that had signs of respiratory disease.<sup>8</sup> Between July and October 2003, a captive Burmese star tortoise (*Geochelone platynota*) from Georgia, a wild gopher tortoise (*Gopherus polyphemus*) from Florida and five Eastern box turtles (*Terrapene carolina carolina*) from Pennsylvania were found to be infected with *Ranavirus*. Clinical signs were similar to those seen with herpesvirus infection and included palpebral edema, ocular and nasal discharge, and oral plaques. The most consistent histologic lesions were necrotizing and ulcerative stomatitis and/or esophagitis, and fibrinous and necrotizing splenitis. In addition, several animals had varying degrees of multicentric vasculitis or thrombosis, necrosis of hematopoietic tissues, and multifocal necrotizing tracheitis, conjunctivitis or gastritis. In some cases, basophilic intracytoplasmic inclusion bodies were observed within epithelial cells of the oral mucosa, esophagus, stomach and trachea, or within endothelial cells, macrophages and hematopoietic cells. In each case, a virus compatible with iridovirus was isolated in *Terrapene* heart cells. PCR was used to amplify a segment of the gene encoding the iridovirus major capsid protein.<sup>5,6</sup> Approximately 400 base pair amplicons were sequenced and BLAST analysis indicated the most closely related virus to be Frog Virus 3, a *Ranavirus*. An iridovirus was isolated from an ill leopard frog (*Rana utricularia*) near the Burmese star tortoise enclosure with an identical sequence as the star tortoise iridovirus, suggesting that amphibians may serve as a reservoir host for a ranavirus

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transmissible to chelonians. The identification of a *Ranavirus* in box turtles and tortoises at widely separated sites over a 3-mo period either suggests an emerging disease or one that has been previously unrecognized.

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**TRYPANOSOMIASIS (SURRA) IN THE CAPTIVE SUMATRAN RHINOCEROS  
(*Dicerorhinus sumatrensis sumatrensis*) IN PENINSULAR MALAYSIA**

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**Abstract**

Five captive Sumatran rhinoceros (*Dicerorhinus sumatrensis sumatrensis*) housed in a facility in Selangor Malaysia died in a biphasic epidemic that spanned 18 days. Four of the five rhinos had been wild-caught in peninsular Malaysia and translocated into captivity; one was the only offspring of a female that had been pregnant at the time of capture. Clinical signs included initial depression and anorexia followed by rapidly progressing incoordination, muscle tremors, nasal hemorrhage, recumbency and labored breathing, followed by death. Despite broad-spectrum antibiotic and supportive therapy, all five rhinos succumbed. Trypanosomes identified as *Trypanosoma evansi* were detected in blood smears taken just prior to death from the last two animals. Gross pathology was nonspecific; however, histopathologic examination revealed multi-systemic disease compatible with historic reports of surra in other animals. Three animals had intralesional trypanosomes and extravascular hemolysis; three of four animals for which spleen was available had unique and characteristic splenic lesions consisting of marked enlargement of periarteriolar sheaths with lymphoid depletion. Trypanosomes were identified in the brain of one animal in association with endothelial hypertrophy. Immunohistochemistry was performed on tissue samples to further characterize the disease. A herd of buffalo located adjacent to the Sumatran Rhino Conservation Center shared a common fence with the 10-acre reserve where the first animal had been housed for the 2 wk immediately prior to its death. This outbreak represents the first report of surra in the Sumatran rhinoceros.

**Introduction**

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The Sumatran rhinoceros (*Dicerorhinus sumatrensis*) is considered the most endangered rhinoceros species, even though the estimated 300 animals outnumber the rarer Javan species (*Rhinoceros sondaicus*). The Sumatran rhino remains the most threatened of the five extant species largely due to poaching for the rhino's horn, compounded by forest degradation. Following the tragic loss of five animals in Peninsular Malaysia, just eight Sumatran rhinos survive in captivity worldwide, four in zoological facilities in the USA and four in sanctuaries in Southeast Asia.

Wild populations increasingly face risk of emerging diseases, especially where domestic animal reservoirs exist near populations of captive or wild species that may not share similar disease ecology. Recent examples of diseases that are infecting new species or crossing environmental barriers include BSE, CWD, West Nile virus, highly pathogenic avian influenza, monkey pox and ebola virus, and the list continues to grow. Zoonoses are also becoming more prevalent as more people and their livestock move into new environments.<sup>7,16,17</sup> Many of these diseases, including surra, are emerging directly or indirectly because of mankind's exploitation of the earth's resources for food, fuel, medicines, and agriculture - problems compounded by the modern trend towards globalization.<sup>16,17</sup>

## **Methods**

The epidemiology and pathology of an acute epidemic mortality event involving the last captive Sumatran rhinoceros in Peninsular Malaysia was investigated. In addition to extensive necropsy and laboratory testing conducted in Malaysia, partial pathologic tissue sets collected from each animal by the attending veterinarian or the Universiti Putra Malaysia were imported into the USA for further examination. Epidemiologic data were reviewed. This included assessment of chronologic and demographic information; local staff interviews; evaluation of scientific reports and laboratory analysis of samples taken during the outbreak and of testing of domestic animals surrounding the reserve; and review of the literature.

## **Results and Discussion**

Five Sumatran rhinoceros (four females and one male) housed in a species conservation facility in Selangor Malaysia died in an epidemic that was a significant setback to an already struggling captive propagation program. Four of the five rhinos had been wild-caught in peninsular Malaysia and translocated into captivity; one was the only offspring of a female that had been pregnant at the time of capture. The biphasic epidemic spanned just 18 days in October and November of 2003, during the peak of the rainy season.

The first animal died on October 30, within 24 hr of being moved from the adjacent forested reserve back to the barn. This death was followed ten days later by the first clinical signs in the male. Despite broad-spectrum treatment that included antibiotics, fluid therapy, anthelmintics, anti-inflammatory medications and other supportive measures, the animal died. Clinical signs

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included lethargy, depression, anorexia, and weakness that progressed to posterior paresis, labored respirations, recumbency and death, which are compatible with those seen in surra in other species.<sup>1,6,20</sup> The next day another female was affected with similar signs, and soon succumbed. The remaining two animals, which became ill about the same time, appeared to be recovering with treatment. However, 6 days later severe clinical signs recurred and death followed. Blood samples collected during the event showed mild anemia and monocytosis, but an otherwise unremarkable leukocyte count.

An initial diagnosis of trypanosomiasis was made by identification of trypanosomes on thick and thin blood smears at the Universiti of Kebangsaan in Kuala Lumpur, Malaysia.<sup>4</sup> The organisms were classified as *Trypanosoma evansi* based on size and morphology. Unfortunately, this diagnosis was not made until after all of the animals had succumbed to the disease. Additional diagnostic tests, including enzyme-linked immunosorbent assays (ELISA), card agglutination test for trypanosomiasis (CATT), mouse inoculation test (MIT), immunohistochemistry (IHC), and polymerase chain reaction (PCR), have been developed to help improve detection of *T. evansi*, surveillance for infection, and diagnosis of surra.<sup>11,12,14,15,18,23,24</sup> These methodologies often are used to monitor for recurrent parasitemia post-treatment.<sup>24</sup>

A unique lesion was observed in three of the four spleens examined microscopically. The splenic white pulp was greatly expanded by histiocytes, with central lymphoid depletion, a pathologic lesion observed in both natural and experimental surra infections and classified as depletion of periarteriolar lymphoid sheaths.<sup>3</sup> Anemia, circulating hemosiderophages and marked splenic hemosiderosis were documented, suggesting extravascular hemolytic disease. One animal had evidence of disseminated intravascular coagulation, a condition attributed to trypanosomiasis in humans.<sup>2</sup> Histopathology revealed presumptive trypanosomes in the brain of one animal, in association with endothelial hypertrophy, and in multiple other organs. Further testing of frozen and formalin-fixed tissues, including reproductive organs, for trypanosomes was facilitated by immunohistochemistry. Infertility has been associated with trypanosome infections, which disrupt spermatogenesis in domestic boars and rams.<sup>13,19</sup>

The salivarian trypanosomes, such as *T. evansi*, are readily transmitted by tabanid flies and other diptera.<sup>6,8,22</sup> Tabanids were abundant at the rhino center, with increased numbers prevalent at the time of this epidemic, likely because of the rainy season conditions.<sup>9</sup> Transmission would likely be efficient in the captive environment because of the concentration of susceptible animals exposed to infected vectors, and the unique nature of trypanosome biology.<sup>22</sup> A herd of buffalo located adjacent to the Sumatran Rhino Conservation Center shared a common fence with the 10-acre reserve where the first animal had been housed for the 2 wk immediately prior to its death, and may have been a reservoir of infection for local tabanids.

Perissodactylids as a group appear to be highly sensitive to trypanosomiasis, with high mortality reported in domestic horses.<sup>6,20,21</sup> Trypanosomes have been associated with disease in African black rhinoceros (*Diceros bicornis*), although that species appears relatively resistant to disease, unless stressed by translocation, presumably because of innate resistance.<sup>5,10</sup> Such tolerance of

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trypanosome infection would provide an adaptive advantage for a species like the black rhinoceros that has co-evolved with these blood parasites. Since *T. evansi* is a relative newcomer to Southeast Asia,<sup>6</sup> Sumatran rhinos probably have had little opportunity to adapt to infection with this agent. Hence, surra might be expected to appear as an epidemic in a group of susceptible animals exposed to infection at a common site over a short period.

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## GENOMIC ANALYSIS OF THE SONGBIRD STRAIN OF *Mycoplasma gallisepticum*

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### Abstract

Mycoplasmal conjunctivitis was first observed in house finches (*Carpodacus mexicanus*) at backyard birdfeeders in the Mid-Atlantic States in 1994.<sup>3</sup> The epidemic spread rapidly throughout the population of house finches in the eastern U.S. and southeastern Canada.<sup>1</sup> The causative agent of the epidemic in songbirds, *Mycoplasma gallisepticum*, has long been an economically important pathogen of domestic poultry, both in the U.S. and worldwide.<sup>4</sup> Together with other investigators, we have previously speculated that infection with *M. gallisepticum* may have been recently acquired by the house finch as a result of transmission from domestic poultry. Alternatively, this epidemic may have resulted from the emergence of a virulent form of a *M. gallisepticum* strain associated with songbirds but not previously recognized in these hosts.

To address this question we have initiated a molecular approach to determine whether the songbird strain of *M. gallisepticum* is a newly acquired pathogen of songbirds or whether it has a long, well-established relationship with this host. This study begins to address this question by determining the genome size of songbird isolates of *M. gallisepticum* and comparing this with the published genome sizes of a number of *M. gallisepticum* strains isolated from domestic poultry.<sup>2,5-7</sup> Genomic DNA was purified from three *M. gallisepticum* isolates obtained from house finches in distinct geographic locations in 1994, 1995 and 2001, digested with the restriction enzymes, *SmaI*, *EagI* and *NaeI* and mobilized by pulsed field gel electrophoresis. The genome size was estimated by comparison of the DNA fragments with lambda DNA markers. All three house finch isolates produced similar banding patterns with each of the restriction enzymes tested. The estimated molecular weight of 975 kilobases is smaller than the 1030-1070 kilobase genomes previously reported for *M. gallisepticum* isolates from poultry.

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## THE PATHOLOGY OF CALIFORNIA MARINE LIFE POLICY

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### **Abstract**

In 1998-99, California overhauled marine life policy with the enactment of the Marine Life Management Act (MLMA) and the Marine Life Protection Act (MLPA). The successful campaign to pass these two acts was a turning point in marine management politics. Their enactment provided hope that marine fisheries and ecosystems would be managed sustainably and depleted fisheries would be restored. The Department of Fish and Game has had to shift its entire approach to managing marine fisheries. However, new conflicts have arisen as stakeholders and scientists have wrangled over the implementation of these new laws. Commercial fishermen have fought with sport fishermen over allocation of specific fisheries pursuant to the MLMA. The sport fishermen have waged a political battle against establishing marine reserves under the MLPA. What is the future of marine life policy in California?

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## **WILDLIFE DISEASE MANAGEMENT IN THE UNITED STATES NATIONAL PARKS: WHY DON'T PARKS DO SOMETHING?**

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### **Abstract**

The United States National Park Service (NPS) manages over 385 units comprising nearly 34 million hectare. About 270 of NPS units have significant natural resource components. Wildlife management actions in these units are based on the NPS mission, legal mandates, management policies, and public expectations. Among the most important NPS policies is the goal to maintain naturally functioning systems. Therefore, diseases are not inherently bad or in need of management if they are a native component of the system. Although policy guidance to all NPS units is consistent, planning outcomes and management actions vary among units due to differences in purpose and enabling legislation that originally designated the site. For example, this difference is evidenced in disease management approaches to address bovine brucellosis in free-ranging bison in Yellowstone National Park versus those that resulted in elimination of the disease in the fenced bison population in Wind Cave National Park. Adherence to the National Environmental Policy Act (NEPA) also plays a significant role in planning management actions by all federal agencies but the NPS is held to a particularly high standard for compliance by its constituency. In the realm of wildlife diseases, this is exemplified in the approach that NPS has taken for decision-making on the use of oral rabies vaccination and management of elk populations affected by chronic wasting disease. Although the processes used and management alternatives implemented by the NPS may vary from state wildlife departments, or even other federal agencies, the NPS is an active partner in addressing wildlife disease issues that threaten natural processes.

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## SOCIAL BIOSECURITY: COMMUNITY RESPONSE TO AN ANIMAL DISEASE OUTBREAK

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### **Abstract**

When animal disease occurs within human settlements, coordinated community response is an important element in any official control or eradication strategy. Yet communities' capacity for cooperative response is strongly influenced by pre existing social, economic and political factors. This paper presents some lessons from an animal disease control effort on the manner in which community organization can be incorporated into effective animal disease response.

Exotic Newcastle Disease (END), a virulent poultry viral disease, broke out in southern California in 2002-2003. While the outbreak included a number of commercial poultry facilities, it was initially found in backyard birds in urban and suburban communities. Within these communities, commercial, backyard, pet, and wild bird populations exist close proximity to one another. As a function of the region's diverse cultural landscape, bird owners, ethnic groups, and enterprises have quite different social and economic agendas for their animals.

This study looks at the way in which four communities in southern California responded to the outbreak of END and a government effort for its eradication. It examines critical variables in each community for developing a capacity for social biosecurity for animal disease. These are the variables that largely determine the degree of community cooperation with animal disease control strategies.

Crucial variables include basic demographics of the community, pre existing relations with authorities, attitudes and practices regarding birds and animals, and settlement patterns. It describes how some official actions and politics of outside authorities tended to foster cooperation and how others tended to exacerbate conflict. Finally it makes recommendations on the way in which official disease control strategies can seek to incorporate community dynamics into a design for social biosecurity related to animal diseases.

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**VETERINARY MEDICAL RECORDS: WHAT ARE OUR RESPONSIBILITIES AND WHAT ARE THE LEGAL ISSUES?**

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**Abstract**

Certainly, the Animal Welfare Act (AWA) requires records of animals. Adequate veterinary care implies that veterinary medical records should be maintained, but what details are required. April 2003, the USDA proposed to amend the Animal Welfare Act medical records regulations to require that research facilities, dealers and exhibitors maintain medical records as part of their program of adequate veterinary care. The proposed regulations would specifically require a detailed accounting of certain aspects of medical histories.

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## CLINICAL WILDLIFE MEDICINE: A NEW PARADIGM FOR A NEW CENTURY

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### Abstract

Clinical wildlife medicine has mostly been applied to wildlife rehabilitation which is defined as the temporary care of injured, diseased and displaced indigenous animals and the subsequent release of healthy animals to appropriate habitat in the wild.<sup>5</sup> Few have doubted the important role these activities have played in improving the welfare of many wild animals that are often injured as a result of human activities. However, it is unlikely that the rehabilitation of injured individuals of a common species has any significant effect at the population level.<sup>7</sup> Therefore, legitimate questions have been raised regarding the justification of such activities and whether they could lead to interference in natural selection, increase disease transmission among and between species, and result in the inappropriate translocation of animals. The Wildlife Center of Virginia (WCV) has developed policies and procedures, health screening protocols,<sup>8</sup> and a preventive medicine program that are designed to eliminate or minimize the potential harm that could result from wildlife rehabilitation. In addition, the WCV has expanded upon the traditional role of the treatment and release of wildlife to include many other veterinary scientific, conservation, public health, and public policy activities. Veterinary training is one of the most important justifications for our activities, and the WCV has training programs for veterinary students, veterinary interns and veterinarians training to be specialists in wildlife medicine through an American College of Zoological Medicine approved residency program. These training programs emphasize clinical medicine, wildlife population health management, and conservation medicine, or the ecological context of health. Conservation medicine is a new discipline and has developed in response to the emergence of new diseases and threats to human and animal health from anthropogenic ecological changes.<sup>1</sup> The WCV has been documenting anthropogenic effects on wildlife health for 20 yr and the animals presented to the WCV are used as biomonitors for ecosystem health.<sup>2</sup> The WCV has also adapted the Epi Info software package as its disease-monitoring database.<sup>4</sup> This database has allowed us to identify significant temporal changes in animal admissions, and spatial clustering of clinical cases.<sup>2,3</sup> Wildlife can also serve as early warning indicators or sentinels of disease outbreaks in humans and domestic animals and a syndromic surveillance system is currently being developed. Finally, the WCV uses the clinical cases and research projects to educate the public, modify human behaviors that are detrimental to wildlife, and influence public policy decisions.<sup>6</sup> The program outlined above is a potential model program for other wildlife centers and universities that receive wildlife in a clinical setting.

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## THE ROLE OF THE VETERINARIAN IN WILDLIFE REHABILITATION: MORE THAN THE ANIMALS

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### Abstract

West Nile virus (WNV) was first detected in North America in 1999 and has rapidly spread across the continent. An unusual characteristic of the North American epidemic is high avian mortality. The effect of WNV on wild bird populations was highlighted during the 2002 WNV season. Wildlife rehabilitation centers in 14 states reported a sudden increase in admissions of sick and dying raptors, particularly great horned owls and red-tailed hawks, at the height of the epidemic. Soon after, several anecdotal reports were received from rehabilitators who developed symptoms they believed to be due to WNV infection. Although mosquitoes are known to transmit the virus, many of these rehabilitators believed they acquired WNV while treating the sick birds (i.e., non-vector transmission). Some reported confirmation of WNV infection by their physician. Using the 2002 National Wildlife Rehabilitation Association (NWRA) directory, a telephone survey of randomly selected wildlife rehabilitators in the 14 states was conducted to determine the proportion of rehabilitators that had acquired WNV infection and to assess the possibility of direct bird-to-human transmission of the virus. Forty-two raptor rehabilitators participated in the survey. 34 (81.0%) reported admitting WNV-suspect or WNV-confirmed raptor cases. Common personal protective measures taken included wearing gloves (leather and/or latex) and hand washing. Masks, face shields and/or goggles to protect against aerosol or body fluid exposure were used on an “as needed” basis, if at all. Half of the respondents reported using no protection against mosquito exposure, despite some reporting uncountable number of mosquito bites that season. Nonspecific symptoms that could have been due to WNV infection were reported in nine respondents, but only one (11.1%) sought medical care, after which the diagnosis was unknown. Based on this study, it is impossible to determine the likelihood illness occurred as a result of direct contact with sick birds. The states in which these rehabilitation facilities were located included those that experienced high WNV activity during the 2002 WNV season. It was impossible to determine whether illness was due to WNV and whether exposure occurred by route other than via mosquito. Further studies evaluating risk of WNV infection in personnel caring for wildlife are needed. The study does demonstrate the need for guidance to rehabilitators regarding protective practices to prevent injury and diseases they may acquire while treating sick animals. Veterinarians who provide services to wildlife rehabilitation facilities are in a unique position to provide guidance regarding protective measures needed while working with sick wildlife, as well as to emphasize the importance of personal health and the need to seek medical attention when symptoms arise.

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## INTEGRATING MULTIDISCIPLINARY INFORMATION AND AGENCIES

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### **Abstract**

Once one has developed proficiency in a professional field, it is challenging, but often necessary, to cross over to the language and information of another to better understand the significant links. The recent appearance of two emerging diseases in North America, West Nile virus and Chronic Wasting Disease, has made discipline overlaps quite apparent, and highlighted the need for information resources to facilitate interdisciplinary exchange. New volumes of the WILDPro Electronic Encyclopedia on these diseases will be demonstrated, showing how this may be accomplished.

There is increasing interest in the integration of disease databases that exist in multiple agencies and institutions. One of the most significant incentives for this is as a mechanism for biosurveillance for potential bioterrorists attacks. However, even without this current heightened awareness, there are significant compelling reasons for all agencies to understand how information contained within their systems could be useful when combined with others. One of the most important factors in facilitating database integration is the use of a standardized vocabulary. Current integrated information, surveillance, and records projects such as WILDPro, ZIMS, NAHLN, BioPortal, and BioWatch will be discussed, and the features of each explained.

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## UNDERSTANDING THE ECOLOGY OF NIPAH VIRUS: AN EMERGING ZOOTIC PARAMYXOVIRUS

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### Abstract

During the 1990s two novel, closely-related, paramyxoviruses emerged in Australia and Malaysia which resulted in the significant loss of human life.<sup>1,2</sup> Hendra virus (HeV) and Nipah virus (NiV) each moved from natural fruit bat reservoirs into intermediate domestic animal hosts (horses and pigs respectively) and then into humans, with fatal consequences. These two pathogens have been described as members of a new genus of paramyxovirus: *Henipavirus*. While the number of human cases of HeV was limited, (3 cases, 2 were fatal) in Malaysia there were 265 cases of NiV with a near 40% case fatality rate. Recurrent neurologic infection has affected approximately 7.5% (n = 160) of those who survived NiV infection.<sup>3</sup> Outbreaks of novel Nipah-like viruses have occurred within the past 4 yr in South Asia resulting in the loss of human lives, with the most recent outbreak occurring in Bangladesh in January, 2004.<sup>4</sup> The Henipavirus Collaborative Research Group, funded through the NIH Fogarty International Center, is working to understand the ecological and anthropogenic factors that drive the emergence of henipaviruses, as well as the mechanism for transmission between their wildlife hosts and humans. We are testing three main hypotheses in this study:

Did anthropogenic pressure on fruit bat habitat and populations via deforestation and hunting alter the distribution and movement patterns of fruit bats, bringing a higher than usual concentration of infected bats to the index farm prior to the 1998-9 outbreak?

Did climatic factors including the 1997 El Nino Southern Oscillation and land-use change, including the expansion of fruit orchards, alter the distribution of food availability for flying foxes, causing them to aggregate near the site of Nipah virus emergence and allow for an emergence in pigs to occur?

Did an expansion or intensification of pig farming in Malaysia provide the correct conditions for a change in host-pathogen dynamics that allowed a repeatedly introduced virus to become enzootic, then epidemic in pigs?

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*Pteropus vampyrus* and *Pteropus hypomelanus* have been found to carry NiV-neutralizing antibodies at a significantly high prevalence, with virus having been isolated from *P. hypomelanus*.<sup>5,6</sup> These pteropid bats are considered the probable reservoirs for Nipah virus in Malaysia. We are using satellite telemetry (Microwave Technologies, Maryland) combined with ground-truthing to describe the distribution and long-range movement patterns of *P. vampyrus*, which has been located at the point of emergence of Nipah virus. We are also conducting disease distribution surveillance in *P. vampyrus* and both distributional and longitudinal disease surveillance in the Island flying fox, *P. hypomelanus*. To date, approximately 26.3% of *P. vampyrus* (n = 38) and 20% of *P. hypomelanus* (n = 157) have carried serum neutralizing antibodies to Nipah virus.<sup>5,7</sup> Computer models are being used to analyze the dynamics of NiV emergence in domestic swine and its spread between pig farms, with the goal being to identify a threshold density at which the infection can sustain itself long enough for an outbreak to occur. Laboratory studies are also underway to determine the mechanisms of transmission of henipaviruses between pteropid bats and between bats and other species.

Ultimately, our goal is to be able to prevent future outbreaks of known pathogens such as Hendra, Nipah, and other Nipah-like viruses, and by improving our understanding of the factors that drove their emergence, we also hope to prevent the emergence of new, potentially more lethal, paramyxoviruses.

#### ACKNOWLEDGMENTS

This study is funded by an NIH/NSF "Ecology of Infectious Diseases" award via the John E. Fogarty International Center of NIH, TW05869 and by core funding to the Consortium for Conservation Medicine from the V. Kann Rasmussen Foundation. We would like to thank the consultants to our group, Tom Ksiazek (CDC, Atlanta) and Sai Kit Lam (Univ. Malaya).

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## PRESENT SITUATION OF WEST NILE VIRUS IN MEXICO

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### Abstract

The initial outbreak of West Nile virus (WNV) in North America was recognized in New York City in August 1999, with deaths reported in humans, horses, and numerous species of birds.<sup>8</sup> Since then, the geographic distribution of WNV in North America has greatly increased, reaching Mexico in 2002, where a vast number of new potential hosts (avian, mammalian, reptilian) have been exposed to the disease. In Mexico, mosquito vectors are available throughout most of the year creating serious, long-term threats to human health, horses and vulnerable avian populations in the region.<sup>9</sup>

During the summer of 2002, the Agricultural Ministry of Mexico (SAGARPA) began to receive reports of encephalitis-like illness in horses from several different areas of Mexico, concurrent with reports of WNV encephalitis outbreaks in horses along the Texas border in the states of Coahuila, Tamaulipas and Chihuahua.<sup>1,2</sup> Other suspected cases were reported from several southern Mexican states.<sup>2</sup> In July 2002 antibodies to WNV were detected in horses in the state of Yucatan.<sup>8</sup> The mode of entry of the virus into the Yucatan peninsula is unknown; however, the virus may have been brought in by migration because this area is a principal landfall of many species that migrate from the north-eastern and midwestern United States. Antibodies to WNV reported in certain species of migratory birds (gray catbird, rose-breasted grosbeak, and indigo bunting) supports this hypothesis.<sup>3,8</sup> There is even a report in mid 2001 that neutralizing antibodies to WNV were detected in a bovine in the southern state of Chiapas.<sup>1,8</sup>

The first evidence of WNV transmission among birds in northern Mexico, was in March 2003, 796 birds representing 70 species were captured and assayed for antibodies to WNV. Nine birds had flavivirus-specific antibodies by epitope-blocking enzyme-linked immunosorbent assay; four were confirmed to have antibody to WNV by plaque reduction neutralization test.<sup>4</sup> During 2003, several epizootics characterized by neurologic disease occurred on farms housing *Crocodylus moreletii* and *C. acutus*. Crocodylians may serve as an amplification host for this virus.<sup>5</sup>

In early 2003, a nation-wide surveillance network for the detection and prevention of WNV in zoological institutions was formed in conjunction with the Wild Life National Agency (Dirección General de Vida Silvestre) incorporating 33 Institutions in the surveillance network and influencing a total of 85 zoos, aquariums and breeding facilities, from the Mexican Association of Zoological Parks and Aquariums (AZCARM). A specific protocol was put

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together and made available for these institutions in order to have a uniform approach to surveillance, diagnostics, case reporting and prevention of disease.

On May 5, 2003 a dead captive raven (*Corvus corax*) from a zoological park in the city of Villahermosa, Tabasco State, was analysed, and virus isolation of WNV was done on tissue samples at the CPA-SAGARPA biosafety level 3 facility in Mexico City.<sup>2</sup> Phylogenetic studies indicate that this isolate, the first from Mexico, is related to strains from the central United States but has a relatively high degree of sequence divergence.<sup>2</sup> Out of this isolate, a vaccine is being developed by the National Producer of Veterinary Biologics, mainly for the vaccination of horses in the army and for a cost-accessible product for mass vaccination (Hector Castell-Blanch personal communication).

Although WNV has already been detected in 13 states of Mexico, there is some difference in the impact of WNV in comparison with the USA experience, with a low rate of morbidity and mortality in both animals and humans. It has been hypothesized that extensive prior exposure to another *flavivirus* such as dengue (DEN), St. Louis encephalitis (SLEV), Venezuelan equine encephalitis (VEEV) or yellow fever virus, could attenuate the effects of WNV due to antigenic cross-reactivity of *Flavivirus* antibodies, especially after a second or sequential *Flavivirus* infection in the same host.<sup>11</sup>

For example, dengue and dengue hemorrhagic fever (DHF) have been present since 1982, when Mexico reported serotypes 1 and 2 and in 1995 serotypes 3 and 4 (hyperendemicity),<sup>7</sup> an outstanding increase of DEN-3 circulation was identified.<sup>10</sup> Risk factors include the numbers of infected and susceptible human hosts, size of mosquito population, (*Aedes aegypti*) feeding habits, and temperature (which affects vector distribution, size, feeding habits, and extrinsic incubation period).<sup>6</sup> There is also a Mexican isolate (200787/1983) which is antigenically unique by signature analysis with respect to all other dengue-2 topotype viruses. This strain is also unique in biologic behaviour (neurotropism) and is of epidemiologic significance in Mexico.<sup>10</sup>

The biologic and epidemiologic consequences of these mosquito-borne viruses co-circulating in the same ecosystem could either attenuate disease due to cross-protective antibodies or enhance disease due to immune enhancement. In the case of dengue, enhancement of virus replication by heterologous *Flavivirus* antibodies and T-cell activation are thought to occur in some patients during a second or sequential dengue infection, resulting in hemorrhagic fever or shock. In contrast, animal data indicate that prior infection with a heterologous *Flavivirus* reduces the severity of subsequent challenge with WNV. Results of experimental studies with rodents, monkeys, and pigs, suggest that heterologous *Flavivirus* antibodies protect against or modify subsequent infection with WNV.<sup>11</sup>

In the north-eastern region of the United States, the diagnosis of WNV infection has been relatively easy, since most people and animals were experiencing their first *Flavivirus* infection.<sup>11</sup> However, as WNV spreads into geographic regions where people and animals have

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other pre-existing *Flavivirus* antibodies, the interpretation of diagnostic tests becomes more difficult, and the prediction of the consequences are more challenging.

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## COMPARING IMPLEMENTATION OF A LIVE TEST AND CULL PROGRAM FOR CHRONIC WASTING DISEASE IN WILDLAND AND URBAN SETTINGS

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### Abstract

The mule deer (*Odocoileus hemionus*) population that winters in Estes Park, Colorado and the eastside of Rocky Mountain National Park, Colorado (RMNP) is currently sampled for chronic wasting disease (CWD) using a live tonsillar biopsy test. Utilizing the live test as a population level experimental management technique involves cooperation between federal and state field teams working in their respective jurisdictions. The Colorado Division of Wildlife (CDOW) works primarily in the town of Estes Park, Colorado and recently published a paper evaluating the feasibility of the live tonsillar biopsy strategy in this urban environment.<sup>1</sup> The costs and logistics associated with implementing this program in RMNP are considerably different. We compare and contrast the feasibility of implementing a live testing program for CWD in mule deer populations in urban versus wildland environments.

Costs associated with mule deer live testing are estimated on a per deer basis. The two major categories of expenditures are supplies/equipment and personnel services. Supplies and equipment are fixed costs and include wildlife pharmaceuticals, darts, ear tags, telemetry transmitters, vehicle, and lab fees. Supplies and equipment costs are similar between urban and wildland environments. Colorado Division of Wildlife reported a supplies/equipment cost range of \$297 - \$341 per animal dependent upon the drug combination used. Rocky Mountain National Park costs per deer are comparable. The real difference in feasibility between wildland and urban settings lies in personnel service costs. These costs are substantially higher per deer in a wildland setting. The time required to locate deer in wildland settings greatly increases personnel costs. In 28 field days, CDOW sampled 181 mule deer, averaging 6.5 deer per day. Rocky Mountain National Park sampled only 41 deer in 28 days, averaging 1.5 deer per day. These differences in efficiency between wildland and urban environments are important considerations for wildlife managers who may be considering using this technique.

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## EPIDEMIOLOGIC ANALYSIS OF RISK FACTORS FOR MYOCARDITIS AND DILATED CARDIOMYOPATHY IN SOUTHERN SEA OTTERS (*Enhydra lutris nereis*)

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### Abstract

Cardiac disease is an important cause of mortality for southern sea otters in California. This condition was newly described and had no known etiology in beachcast sea otters necropsied from 1998 and 2001 at the California Department of Fish and Game's Marine Wildlife Veterinary Care and Research Center. The objectives of this study were to characterize cardiac lesions observed in southern sea otters and evaluate common sea otter pathogens and potential infectious, toxic and nutritional etiologies for their relationship with cardiac disease. Characterization of cardiac lesions by gross and microscopic necropsy findings has allowed the definition of two overlapping cardiac disease syndromes in otters: (1) myocarditis, characterized by lymphocytic inflammation of myocardium and (2) dilated cardiomyopathy (DCM), characterized by grossly enlarged atria and ventricles with concurrent myocarditis. Major risk factors associated with myocarditis included adult age, good body condition (likely as a result of an acute death), exposure to *Sarcocystis neurona*, and suspected exposure to domoic acid. Domoic acid, a marine toxin produced by *Pseudo-nitzschia australis*, is a common cause of mortality in sea lions and causes characteristic clinical signs involving the central nervous system. While there may be other factors associated with myocarditis that were not evaluated here, these findings suggest that *S. neurona* and domoic acid may both be important causes of myocarditis in sea otters. Myocarditis associated with exposure to *S. neurona* occurred predominantly in the northern part of the sea otter range, while domoic acid-related myocarditis occurred largely in the south, where domoic acid blooms were more frequent. A spatio-temporal cluster of DCM was identified in the southern aspect of the sea otter range in California from May to November 2000. Adult age and suspected previous exposure to domoic acid were associated with an increase risk of DCM. Also, otters with DCM had significantly lower concentrations of myocardial L-carnitine than controls and otters with myocarditis. Dilated cardiomyopathy may be an advanced stage of domoic acid-induced myocarditis in sea otters, possibly following chronic and repeated exposures to domoic acid blooms. While the relationship between domoic acid and myocardial L-carnitine concentrations requires further research, myocardial L-carnitine might play a key role in the progression of myocarditis to DCM in sea otters.

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## WILDLIFE AND THE ECOLOGY OF ANTIMICROBIAL RESISTANCE

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### **Abstract**

Antimicrobial agents are essential elements in the prevention and treatment of bacterial infections in humans, animals, and, to a lesser extent, in plant agriculture and aquaculture. In food animals, these agents are also used to promote growth and enhance feed efficiency. The increasing emergence of bacterial resistance to antimicrobials is of global public health concern, most notably due to the increasing incidence of multiple-drug resistant bacterial infections and higher rates in morbidity and mortality due to treatment failure. However, the impact of antimicrobial resistance may not be limited to human health. Because the emergence and spread of resistance is the result of complex interactions between bacterial communities, antimicrobial agents, the host species, and the environment, animal health and environmental health effects are also likely. This paper explores the evidence for an impact on wildlife species and their potential role in the dissemination of antimicrobial resistance throughout ecosystems.

Potential routes of transmission of antimicrobial resistance within ecosystems include contacts between animals, animal products, humans, manure, soil, and surface water.<sup>13,19</sup> Bacteria may acquire resistance to an antimicrobial agent as the result of a mutation or incorporation of transferable genetic resistance determinants via conjugation, transformation or transduction.<sup>2,15</sup> Exposure of bacterial populations to antimicrobials may alter the bacterial populations through the elimination of susceptible bacteria and enables the survival and amplification of resistant bacteria, thus creating a selective pressure for resistance. Factors such as the method of administration, dosage, and frequency and duration of use are likely to impact the magnitude of this selective pressure.

Although they receive the most attention, it is important to note that pathogenic bacteria are not the only populations of concern with respect to antimicrobial resistance. Commensal bacteria comprise a large potential reservoir of resistance genes for bacterial pathogens. As the number of resistant commensal bacteria increases, the pool of genetic resistance determinants also increases, facilitating more frequent transfer of resistance to pathogenic bacteria. The indirect transmission of resistance via commensal bacteria and the environment may be as significant as direct transmission through the food chain or direct contact between humans and animals.<sup>9</sup> The high prevalence of antimicrobial resistance in commensal bacteria of humans probably reflects both the selective pressure exerted by antimicrobial usage in an environment as well as the potential for resistance in future infections in both humans and animals.<sup>7,8,12,20</sup>

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Although the high global prevalence of antimicrobial-resistant bacteria is widely attributed to use of antimicrobials in humans and domesticated animals, it is important to bear in mind that some bacterial populations are intrinsically resistant to certain antimicrobials, and others probably evolve resistance to certain of these drugs due to exposure to naturally produced antimicrobials in the environment. The extent to which the prevalence of resistance in bacteria from wildlife reflects such “natural” sources of resistance has not been well-characterized. For example, in a retrospective study of *Enterobacteriaceae* isolated from wild mammals in Australia, Sherley, et al. found that the rates of resistance were equal to rates seen in the natural, “antibiotic-free,” environment.<sup>17</sup> In contrast, in a Finnish study of wild moose, deer and vole, there was an almost complete absence of resistance in *Enterobacteriaceae*, from which the authors concluded that resistance is not a universal characteristic of bacterial populations and most likely results from use of these drug classes in humans and animals.<sup>14</sup>

While studies exploring antimicrobial resistance in wildlife are limited in number, resistance has been demonstrated in multiple species of pathogenic and nonpathogenic bacteria within free-ranging and captive wild mammals,<sup>5,10,17</sup> birds,<sup>4,18,21</sup> reptiles,<sup>1,6</sup> and aquatic species.<sup>11</sup> In particular, the spread of antimicrobial resistant bacteria and their persistence in the environment may be enhanced by wild birds populations due to their mobility and distances traveled during migration. Wild birds have been implicated as a possible source of *Salmonella* infections in humans and farm animals.<sup>3,16</sup> Antimicrobial-resistant *Salmonella* spp. have been observed in double-crested cormorants and common loons in Florida,<sup>21</sup> and *Salmonella typhimurium* were identified in black-headed gulls in the Czech Republic.<sup>18</sup> In a study in the United Kingdom, the range and serotypes of *Salmonella* carried by gulls was similar to the bacterial flora of human the population, which led the authors to suggest sewage as a possible source of infection.<sup>4</sup> Evidence for cycles of anthrozoönotic and zoonotic transfer of *Salmonella* infection demonstrates the potential for transfer of resistance determinants between animal, human and environmental sources.

*Escherichia coli* is a common component of the commensal fecal flora in humans and most animals. In one study of *E.coli* strains from captive mammals, birds, and reptiles in Trinidad, approximately 97% of the isolates tested demonstrated resistance to one or more of eight antimicrobials tested.<sup>6</sup> In a related study of *E.coli* isolates from both wild and captive mammals in Trinidad and Tobago, close to 96% of the isolates were resistant to one or more antimicrobial agents. In the second study, prevalence of resistance among the isolates from captive mammals was significantly higher for three of the antimicrobial agents tested. However, for ampicillin and cephalothin, the prevalence of resistance among the free-ranging animals was significantly higher than those from captive animals. The authors concluded that the high prevalence of resistance to antimicrobials among *E.coli* isolates within free-ranging and captive populations of wild animals may adversely affect treatment options available to veterinarians. In addition, the presence of resistant enteropathogenic serotypes among the isolates could pose a health hazard to consumers of wildlife meat.<sup>1</sup>

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In an ongoing study in the United Kingdom of *E.coli* isolates in wood mice and bank voles, wood mice are significantly more likely to carry antimicrobial resistance than bank voles, even though they occupy the same habitat (N. Williams, personal communication). In an earlier related study in the U.K., vancomycin-resistant enterococci (VRE) were discovered to be part of the normal flora in wood mice, bank voles, and other species of wild mammals. However, while both wood mice and bank voles are reservoirs for VRE, the bank voles did not excrete VRE.<sup>10</sup> Wood mice tend to be omnivores and travel long distances in search of food and territory, whereas bank voles are herbivores and have limited territories. It was unclear how the animals acquired VRE and whether they were long-term carriers. Exposure to avoparcin (an antimicrobial related to vancomycin) was an initial consideration, but avoparcin had not been used in food animals in proximity to the study site and samples were collected after avoparcin was banned as a growth promoter.<sup>10</sup> In any case, it seems possible that host mammal species, ecological niche, and geographic location may influence the antimicrobial resistance profile of isolates and the potential for transfer and spread of resistance within the environment.

These and other studies suggest that free-ranging and captive wildlife are involved in the complex ecology of antimicrobial resistance. Both pathogenic and commensal bacteria in wildlife may serve as reservoirs of antimicrobial resistance that may be selected for, amplified, and spread through the environment via various pathways. While the presence of antimicrobial resistance in wild mammals, birds, reptiles and aquatic species does not necessarily pose a risk to these populations (assuming they are not treated with antimicrobials), they may play an important role as reservoirs for the potential transfer of resistant bacteria and genetic determinants, potentially impacting the health of humans, companion animals, food animals and captive wildlife. Enhanced surveillance of pathogenic and commensal bacteria in wildlife is therefore warranted. Both the extent and significance of the risk to humans and nonhumans from antimicrobial resistance in wildlife should be evaluated, and, if deemed prudent, steps should be taken to mitigate the impact of resistance in wildlife populations.

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## WILD BIRD SPECIES AND THE ECOLOGY OF VIRULENT AVIAN INFLUENZA

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### Abstract

Avian influenza (AI) viruses have evolved little in 60 yr in their natural hosts (wild waterfowl, shorebirds and gulls) that nearly always remain asymptomatic. They evolve much faster in aberrant hosts (pigs, chickens) in which they can become lethal. Although often blamed, wild birds are not significant in spreading virulent AI. Responses to AI outbreaks must be based on facts and not media hype. Poultry present the real danger.

### Introduction

The two surface glycoproteins of influenza A viruses, haemagglutinin (H) and neuraminidase (N), are the most important for inducing immunity and therefore vary the most.<sup>2</sup> Few H and N subtypes have been isolated from mammals, but all 15 H and all 9 N subtypes have been isolated from migrating water-fowl and shorebirds worldwide. Natural influenza A infections have been reported in humans, pigs, horses, marine mammals, mustelids and birds.<sup>23,3</sup>

Although influenza viruses infect a wide variety of birds and mammals, the natural hosts are wild ducks, geese, swans, gulls and terns that intermittently transmit AI to other avian and even mammalian species (chickens, turkeys, pigs, horses, seals, whales, humans).<sup>18,5</sup> Avian influenza viruses evolve slowly in their natural hosts because of the brief avian lifespan and replication in their intestines and may be in evolutionary stasis.<sup>8</sup> The evolutionary rate accelerates rapidly in new (aberrant) host species due to selective pressures to adapt.<sup>19</sup>

Avian influenza is mostly asymptomatic in aquatic birds.<sup>18,21</sup> Viral replication in aberrant hosts is usually limited and overt disease is rare.<sup>5</sup> AI viruses bind preferentially to SAa2,3-galactose. Human strains preferentially bind to SAa2,6-galactose.<sup>24</sup> Thus, AI viruses do not replicate well in humans, and must reassort or adapt in an intermediate aberrant host before emerging in human populations. Pigs have receptors for both avian and human influenza viruses and are a likely intermediate host. The recent transmissions of avian H5N1 and H9N2 viruses directly to humans showed poultry can also be intermediate hosts.<sup>21</sup>

The first known direct transmission of virulent avian influenza (Hong Kong H5N1) from poultry to people killed 6 of 18 infected people in 1997 (thousands more people were exposed to infected chickens).<sup>24</sup> Human-to-human transmission was rare,<sup>7</sup> and no more human cases occurred after

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all poultry in Hong Kong were culled. H5N1 was evolving rapidly in the new chicken host and had acquired a number of amino acids that correlate with replication in humans. Eradicating 1.6 million chickens eliminated the immediate opportunity for H5N1 viruses to infect humans.<sup>24</sup>

Before depopulation, H5N1 virus was isolated from 20% of chickens and 5% of waterfowl in Hong Kong markets, but was not isolated from other birds, including other gallinaceous species, pigeons, and caged passerine and psittacine birds, or from wild birds. Chickens were the only clinically affected species in the live markets.<sup>12</sup> It is likely that H5N1 viruses are now widespread around Hong Kong. The multiplicity of H5N1 genotypes circulating in poultry in the wider region increases the opportunity for the emergence of pandemic strains by developing efficient human-to-human transmission through further reassortment.<sup>5</sup> Prior to 2003, wild ducks were not found to maintain virulent H5 influenza viruses.<sup>14</sup> H5 viruses can become highly pathogenic in domestic poultry but usually remain non-pathogenic in ducks.<sup>1</sup>

Avian influenza viruses are rarely isolated from passerines or psittacines. However, passerines are common near intensive poultry production worldwide and limited evidence supports the potential perpetuation and transmission of AI by passerines near intensive poultry production.<sup>12</sup> Most AI viruses from psittacines have been isolated during quarantine. The H9N2 viruses isolated from two ring-necked parakeets imported from Pakistan into Japan shared high sequence similarities with the 1997 H5N1 and 1999 H9N2 viruses transmitted directly from birds to humans.<sup>12</sup> The two H9N2 isolates identified 1 yr apart were closely related, indicating they belong to the same lineage that must have been established in Pakistan for at least 1 yr. These isolates were non-pathogenic in chickens and mice.<sup>10</sup> Although psittacines are not significant in the epidemiology of influenza A viruses, they can harbour and possibly transmit AI. This risk is greatest in countries with local, regional and international trading of wild birds.<sup>12</sup>

When tested with the 1997 H5N1 virus, seven gallinaceous spp. (chicken, turkey, Japanese quail, bobwhite quail, pearl guinea fowl, ringneck pheasant and chukar partridge) and zebra finches were the most susceptible (high morbidity, mortality >75%) with high viral re-isolation. Geese, emus, house finches and budgerigars were less susceptible and virus re-isolation was low.<sup>12</sup> Ducks, house sparrows and gulls showed mild or no disease, and viral re-isolation was low to moderate. Pigeons, starlings, rats and rabbits resisted infection. This contrasts with previous experiments in which H7N7 virus killed all starlings and spread to contact starlings, but killed only 30% of sparrows and failed to spread to contact sparrows.<sup>11</sup> Thus, although the virulence of a single AI virus can vary substantially between avian species, including species within the same order, passerines and psittacines appear to play very minor roles in the natural epidemiology of AI.<sup>12</sup>

The pattern of spread of the virulent 2003/2004 H5N1 outbreak strongly suggests the virus was carried by smuggled poultry, a practice widespread in Southeast Asia. The genetic sequence of the virus isolated from a Vietnamese victim matched most closely one from Chinese poultry. Five of the eight genetic strands were almost identical to an H5N1 from duck meat smuggled from eastern China to Taiwan in 2003.<sup>13</sup> Some experts blamed migratory birds, but there is no

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direct evidence of wild birds spreading virulent AI. Wild birds were affected near big poultry outbreaks but regular monitoring of migratory birds in Thailand and elsewhere did not reveal the virus.<sup>13</sup>

The genetic diversity of AI viruses circulating in poultry in southeastern China has increased sharply since 2001. This shows H5 is circulating widely somewhere, under unusual selective pressures.<sup>22</sup> Asia's growing prosperity has caused a boom in intensive poultry production. Since 1997, many Chinese producers have vaccinated with inactivated H5N1. If a vaccine is a poor match, as is the case with the H5N1 strain that swept Asia, AI can still replicate in animals that show no disease. Intensive vaccination in south China ( $>11 \times 10^6$ ) may have allowed the virus to spread widely unseen.<sup>13</sup> Vaccines that provide partial immunity and mask disease but allow hosts to continue to shed may speed viral evolution.<sup>9</sup> Vaccination may have led to the evolution of more virulent H5N1 strains that evaded vaccine protection.

After AI hit Bangkok, a special hotline received nearly 1,200 reports of "mysterious" bird deaths. However, the birds, mostly budgerigars and parrots, were dying from shock and starvation after being released by their fearful owners. Most callers were so panicky they demanded an immediate diagnosis over the telephone. Up to 128,091 caged birds were waiting to be tested for AI. Bangkok crows were sampled after the deaths of two crows at a zoo were linked to an H5 AI. In Thailand 500 migratory open-billed storks and another 300 birds died in wetlands. Only 30-40% of the dead storks were infected with AI. There were no reports of AI in Bangladesh from where Asian open-bill storks migrate. Thus, the storks most likely were infected in Thailand.<sup>13</sup>

In late 2002, H5N1 killed non-domestic birds in parks and a zoo in Hong Kong, including waterfowl, greater flamingos, gray herons and egrets. In February 2003, avian H5N1 was isolated from two humans, one of whom died. Despite high genetic homology ( $>99.0\%$  in all genes), the human isolates showed a very different reactivity pattern compared with the H5N1 viruses isolated from the wild waterfowl. All H5N1 isolates from 1997 to 2001 were non-pathogenic in ducks but the H5N1 isolates from late 2002 were highly pathogenic in ducks. This is the first time since 1961 that influenza viruses are known to have killed waterfowl.<sup>19</sup> Despite the 2002 outbreak, there is little evidence the 2003/2004 H5N1 strain significantly affected wild bird populations or that wild birds spread it. Of 6000 wild birds tested in Hong Kong, one peregrine falcon was positive for the H5N1 strain. However, as the 2003/2004 H5N1 strain killed migratory wild birds, serologic studies in wild birds across Asia are needed to determine whether it became established in wild populations.<sup>4</sup>

Despite a lack of evidence, governments in China, Thailand, Cambodia, Japan and Hong Kong were quick to implicate wild birds in the spread of the virulent 2003/2004 H5N1.<sup>13</sup> Responses ranged from the logical and effective to the fanciful and irresponsible. In China, authorities were required to monitor and disinfect the habitats of migratory birds, collect their excrement and sanitize it. Hong Kong closed parks and zoo exhibits but not poultry or wild bird markets. Despite the revelation that a chicken farm in Kyoto failed to report mass deaths due to H5N1 and

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continued to ship live chickens, eggs and meat while experiencing massive mortality, Japanese authorities supposed the virus was carried by migrating birds from Korea because there was no variation in the virus' sequences in Japan.<sup>13</sup>

The Thai Agriculture Ministry wanted to cull migratory birds because killing almost 30 million chickens on 40,000 farms had not controlled the epizootic. In Thai provinces where AI re-emerged, the infection was found mostly in fighting roosters. Authorities suspected infected fighting roosters smuggled out of red zones during the first outbreak then returned to the areas had probably re-kindled the infection. Some owners refused to slaughter their prized fighting roosters. Yet a spokesperson for the University's faculty of veterinary science said AI virus in yellow zones was due to the failure to eradicate all fowl and that birds in natural habitats should also be culled, not just chickens.<sup>13</sup>

### **Vaccination Trial**

Virulent AI and SARS outbreaks in Hong Kong caused widespread fear and greatly reduced visitation to Ocean Park. Although H5N1 viruses are highly variable, there is much cross-protective immunity from H5N1 vaccines and non-pathogenic AI viruses such as H5N3.<sup>6</sup> To allay public fear and to protect collection birds, we tested a killed H5N3 vaccine (HK/goose/1999 H5 and A/duck/Germany/1215/73 N3). This allows differentiation between infected and vaccinated birds by testing for different neuraminidase antibodies. The number of birds with protective titers (>1:16) 28 days after a single vaccination are shown in Table 1.

Vaccinations were repeated 28 days later (titers not yet available). Titers will also be determined 6 mo after the second vaccination to see whether protective levels last for the whole influenza season (6 mo). The titer levels indicate very high protective levels in most species. Three of 28 ducks and 3/7 swans maintained protective levels for over 12 mo following single prototype vaccinations in 2003. Results for psittacines are pending also but after the single 2003 prototype vaccination, 2/5 parrots developed protective levels.

### **Discussion and Recommendations**

Live-bird markets provide outstanding conditions for genetic mixing and spreading of AI viruses<sup>22</sup> and are critically important in the perpetuation and transmission of AI viruses to other avian species and to mammals, including humans.<sup>15</sup> In contrast, free-ranging wild birds appear to play a much lesser if any significant role in the ecology and epidemiology of virulent AI. Intensively reared poultry provide excellent opportunities for AI viruses to increase in virulence due to:

- Genetic uniformity in novel hosts providing intense advantage to mutant forms
- High density, large populations
- Confinement so that uninfected birds cannot avoid diseased birds or shed virus
- Food and water readily available to prolong the lives of severely ill birds

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- Assisted transportation in vehicles to markets, farms and other businesses

Wild bird populations do not provide these opportunities due to:

- High genetic diversity avoiding intense selection for a single new mutant strain<sup>16</sup>
- Mostly low density, dispersed metapopulations
- Freely able to avoid ill birds and low risk of contacting contaminated sources
- Must work extremely hard for food under intense competition such that often even mildly affected individuals succumb rapidly, minimising pathogen spread
- No assistance for dispersal. Ill migratory birds unlikely to travel far

Because all known influenza A subtypes exist in wild aquatic birds, avian influenza is not eradicable. Prevention and control are the only realistic goals.<sup>22</sup> Governments should strive to:

- Monitor birds in live markets and exports/imports closely
- Close live bird markets while virulent AI is circulating in the region
- Improve biosecurity measures (eg prevent contact with wild aquatic spp.)
- Separate land-based poultry, pigs and aquatic avian species in farms and markets
- Monitor the movement of poultry between farms and markets closely
- Only allow controlled effective vaccination in response to virulent outbreaks
- Conduct serologic and other epidemiologic studies in wild birds across Asia to determine whether virulent AI became established in wild populations

Zoos and other organisations dealing with wild birds should:

- Avoid birds from commercial sources such as markets and dealers, especially those trading internationally
- Vaccinate at-risk or high profile birds with an effective vaccine prior to the risk period
- Minimise the exposure of collection animals to wild birds, especially aquatic species.
- Separate aquatic species from passerines, psittacines and other species.

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**Table 1.** No. birds with protective titers (>1:16) 28 days after vaccination with experimental H5N3 vaccine.

Titer	Ducks	Swans	Flamingos	Goose	Ibis	Heron	Crane
No. >1:16	28	7	23	1	0	2	1
No. <1:16	4	0	0	0	1	0	0
Mean titers	521	370	355	256	0	256	128

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## EPIDEMIOLOGIC INVESTIGATION OF A *Mycobacterium tuberculosis* INFECTION OF MULTIPLE ANIMAL SPECIES IN A METROPOLITAN ZOO

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### Abstract

From 1997 to 2000, six cases of *Mycobacterium tuberculosis* (TB) infection were diagnosed in three species of animals at, or recently originating from, the Los Angeles Zoo. Restriction fragment length polymorphism (RFLP) analysis showed that five of six animal isolates shared an identical IS6110 pattern, with the sixth differing only by one additional band. A multi-institutional epidemiologic investigation was conducted to identify and interrupt possible transmission among the animal cases, and to screen personnel for active TB infection and TB skin-test conversion.

### Animal Cases

In April and October of 1994, Asian elephant (*Elephas maximus*) #1 and Asian elephant #2 arrived at the Los Angeles Zoo from a private elephant facility where they had lived together. They were housed together at the zoo until November of 1996 when elephant #2 was returned to the facility for several months before transfer to another zoo. In the spring of 1997, Elephant #1 (30 yr old) died of salmonellosis, with *M. tuberculosis* found in granulomatous lymph node lesions from the thoracic and abdominal cavities, and Elephant #2 (30 yr old) was found to have a positive trunk wash culture for *M. tuberculosis*. In July of 1998, one of a closed herd of three Rocky Mountain goats (*Oreamnos americanus*) consisting of a sire and two offspring, died of pulmonary *M. tuberculosis* at 6 yr of age. The goat's asymptomatic herdmates were screened and had negative chest radiographs and tracheal wash cultures, but one of the two goats was positive on tuberculin skin-test. In October of 1998, a clinically normal Black rhinoceros (*Diceros bicornis*) was diagnosed with *Mycobacterium tuberculosis* after a positive skin test and nasal wash culture. In the winter of 1998, the two remaining goats were evaluated again with negative

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chest radiographs and tracheal wash cultures. However, 1 yr later, both were humanely euthanatized at 8 and 12 yr of age due to clinical evidence of tuberculosis on chest radiographs (both animals), and active clinical signs in one (neither were able to be orally treated). In January of 2001, a rhino was humanely euthanatized after a protracted illness that was non-responsive to aggressive treatment. The rhino was found to have severe multifocal hemosiderosis and atypical mycobacterial infection in her lungs, with no *M. tuberculosis* cultured. This animal had been treated with oral Isoniazid and Rifampin for 1 yr, cultured routinely, and was never culture positive again.

### **Epidemiologic Investigation**

Investigators examined medical and location histories of the affected animals, animal handling practices, health-care procedures, and performed an infection control assessment of the animal compounds and health-care facilities (including measuring air flow in the compounds by smoke testing). We conducted a review of zoo employee medical records for evidence of TB symptoms, tuberculin skin-test results, and chest radiograph information. A list of current and former employees was cross-matched with reported TB cases in the California state registry from 1985 to 2000. As part of the annual occupational health screening in June of 2000, zoo employees underwent questioning regarding TB symptoms, received tuberculin skin tests, and completed a questionnaire on medical history, job type, and history of contact with the infected animals.

### **Epidemiologic Findings**

No common cross-species contact outside the animal compounds and no contact with an infectious human were found. The distance at which the public was kept from the animals and the distance of the compounds from each other (the elephant compound was 27 meters from the rhino compound and the goat compound was 90 m from both) suggests that direct transmission was unlikely. No active TB cases in humans were found, and no matches were found in the database of reported cases. The RFLP analysis of this strain of *M. tuberculosis* matched that of three elephants with which #1 and #2 were housed at a private elephant facility from September of 1993-February of 1994.<sup>1</sup> We hypothesize that elephants #1 and #2 were infected at the private facility and were shipped with latent *M. tuberculosis* infection in 1994, subsequently infecting the black rhino and Mountain goats at the Los Angeles Zoo.

Of interest, animal caretaking and animal contact were not associated with a positive tuberculin skin-test, while groundskeepers were found to have an increased risk of tuberculin skin-test conversion compared with other job categories. Employees attending the elephant necropsy and employees who trained elephants were more likely to have tuberculin skin-test conversion than those who did not.

### **Conclusion**

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This is the first documented human and veterinary epidemiologic investigation of *Mycobacterium tuberculosis* affecting multiple species in a zoo.<sup>2</sup> No evidence of transmission from humans to animals or active infections in humans were found. Genotyping evidence strongly suggests transmission from one species to another, although no evidence of transmission was discovered. Human tuberculin skin-test conversions associated with the elephants were most likely due to lack of respiratory protection for these employees when the risk of TB infection was not known. The finding that groundskeepers and not animal handlers were associated with a higher risk of tuberculin skin-test conversion was surprising, and we hypothesized that this may have to do with groundskeepers as a group being more likely to have been born outside of the United States.

Control measures to eliminate the spread of disease to people and animals were undertaken immediately and throughout this outbreak, and no further cases of *M. tuberculosis* have been diagnosed at the zoo in the past 3 yr despite ongoing surveillance. Four elephants and three rhinos that had direct contact with the infected animals remain TB negative by trunk and nasal wash culture methods as outlined by the USDA for elephant TB surveillance. Methods of indirect transmission in mammalian zoo species and causes of variability in infection and morbidity within and among species warrant further investigation. Ongoing vigilance, occupational health programs and infection control measures in potentially exposed animals are recommended to prevent ongoing transmission of *M. tuberculosis* in zoo settings.

### **Acknowledgments**

The authors thank the Animal Care and Animal Health staff of the Los Angeles Zoo who cared so well for these animals, and the veterinarians (including consulting pathologists), technicians, and medical records staff who collected, analyzed, and organized the clinical data. We could not have performed this evaluation without Sue Thisdell, Safety Officer at the Los Angeles Zoo; Jothan Staley and Donna Workman-Malcom of the City of Los Angeles Occupational Health Services Division; Lee Borenstein, Elenor Lehnkering, Patrick Ryan, Jeanne Soukup, and Annette Nita of the Los Angeles County Department of Health Services; and Diana Whipple for her RFLP expertise.

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### **ZOONOTIC PATHOGENS RECENTLY FOUND IN WILD GOLDEN LION TAMARIN (*Leontopithecus rosalia*) AND COMMON MARMOSET (*Callithrix jacchus*) IN THE**

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## STATE OF RIO DE JANEIRO, BRAZIL, AND THEIR POTENTIAL FOR TRANSMISSION TO HUMANS

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### Abstract

A health evaluation study was conducted in eight wild groups of golden lion tamarins (GLT) and nine groups of common marmosets (CM) in a 1000 ha fragment of lowland Atlantic Rainforest in the State of Rio de Janeiro, Brazil. We analyzed 75 fecal samples from 35 individual GLT and 13 individual CM using the spontaneous sedimentation method. A total of 1676 parasite eggs were collected from the primates, representing four different parasitic helminthes. All four species of parasites were found in both GLT and CM. A comparison of parasites according to primate species showed neither morphometric nor statistical differences. The helminth eggs were classified as *Prosthenorchis elegans* ( $68,47 \pm 4,36 \mu\text{m} \times 46,10 \pm 15,89 \mu\text{m}$ ; n = 693); Ancylostomatidae ( $50.14 \pm 0.804\mu\text{m} \times 29.57 \pm 9.34 \mu\text{m}$ ; n = 876); *Ascaris* sp. ( $64.33 \pm 3.68 \mu\text{m} \times 52.46 \pm 5.46 \mu\text{m}$ ; n = 68); and Oxiuridae ( $60.71 \pm 4.76 \mu\text{m} \times 27.86 \pm 2.11 \mu\text{m}$ ; n = 34).

Blood samples from 13 GLT and 55 CM were tested for the human serotype of *Trypanosoma cruzi*. Nine CM from four groups were positive by the indirect immunofluorescence assay (IFAT) with titers ranging from 1:20 to 1:320. Questions relating to the epidemiologic position of these primates in the Chagas disease cycle and their potential susceptibility to disease caused by *Trypanosoma cruzi* are still unresolved. Studies are underway to identify the vector in the forest ecosystem and to evaluate the common marmoset as a possible reservoir.

### ACKNOWLEDGMENTS

This research was made possible by the financial and logistical support of the Golden Lion Tamarin Association, LCA/UENF, FENORTE, Ecology Lab/ENSP/FIOCRUZ, PROBIO and Trypanosomatids Biology Lab/IOC/FIOCRUZ.

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## ROLE OF BULLFROGS (*Rana catesbeiana*) IN THE SPREAD OF AMPHIBIAN CHYTRIDIOMYCOSIS

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### Abstract

Chytridiomycosis is a recently-discovered fungal disease of amphibians responsible for mass mortality and population declines in a number of regions globally.<sup>1</sup> It appears to have recently expanded in geographic range and has therefore been classed as an emerging infectious disease (EID).<sup>2</sup> Since its discovery in 1998, a series of experimental and field studies have led to a hypothesis that anthropogenic introduction of chytridiomycosis is largely responsible for its recent emergence.<sup>3</sup> First, the pattern of amphibian declines in Central America and Australia, and the biologic traits of the causative agent (*Batrachochytrium dendrobatidis*) are typical of an invasive pathogen moving through naïve hosts.<sup>3</sup> Second, molecular studies of global isolates have shown that there is little variation between isolates from widely separated regions, suggesting a recent emergence event.<sup>4</sup> Finally, this pathogen has been reported from amphibians traded nationally and internationally for food, as pets, for ornamental backyard release, for zoo exhibits and captive breeding programs, and in amphibians introduced for biocontrol.<sup>4</sup>

In this presentation, we present the following evidence from field and experimental data that implicate bullfrogs (*Rana catesbeiana*) in the introduction of chytridiomycosis in some regions:

- Experimental inoculation of bullfrogs led to infection in some cases, but no infected animals showed clinical signs of chytridiomycosis. The lesions were focal and not typical of animals which had died of chytridiomycosis.
- Bullfrogs farmed for food in Uruguay showed high prevalence of infection by *B. dendrobatidis*, but no clinical signs of chytridiomycosis.
- The causative agent of chytridiomycosis has been identified historically in bullfrogs from the Savannah River Site, where 25 yr of amphibian population data demonstrate the absence of long-term declines.
- In a population of recently-introduced bullfrogs in Venezuela, *B. dendrobatidis* was found in 48/48 individuals, none of which showed clinical signs of chytridiomycosis.

These studies, and recent work by other groups in the USA suggest that bullfrogs are efficient carriers and are involved to some extent in the introduction of chytridiomycosis into regions in the USA and abroad. We propose that the trade in bullfrogs for food, and their introduction into

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new regions or countries should be more closely monitored, and surveillance measures introduced for this disease.

#### **ACKNOWLEDGMENTS**

This study is funded by NSF IRCEB award IBN-9977063 and NSF IRCEB award DEB-02133851 and by core funding to the Consortium for Conservation Medicine from the V. Kann Rasmussen Foundation.

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## EPIDEMIOLOGY OF EMERGING TICK AND RODENT-BORNE DISEASES IN TRENTINO, NORTHERN ITALY

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### Abstract

Lyme disease, human granulocytic ehrlichiosis, tick-borne encephalitis (TBE) and hantavirus infections are among the most predominant zoonotic diseases emerging in Europe. The rodent species (yellow-necked mouse, *Apodemus flavicollis* and bank vole, *Clethrionomys glareolus*), both widely occurring in the forest ecosystems of Trentino, play a central role in the ecology of these infections. We assessed the spatial distribution of *Borrelia burgdorferi* sl., *Anaplasma* sp., TBE virus, Cowpox virus, Murid Herpesvirus and Hantavirus by serologic and molecular analysis of blood and tissue samples from 367 rodent individuals trapped during 2002. The rodent species, *A. flavicollis*, (n = 238) was found to be infected with most of the pathogens investigated, with infection prevalence ranging from 3.3% for TBE virus to 24.5 % for Murid Herpesvirus. The other rodent species, *C. glareolus*, (n = 108) had high infection prevalence for Cowpox virus (40%), but no individuals were infected with TBE virus. Using advanced GIS-based mapping procedures, these findings were combined with other data previously collected and recent serologic analyses of wild and domesticated ungulates and humans to develop risk maps for Trentino.

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## AN ALTERNATIVE TO EUTHANASIA FOR POPULATION CONTROL OF NUISANCE WILDLIFE

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### **Abstract**

Resident wildlife populations present a threat to captive animal collections through predation, competition for food, and serving as a source of infectious disease. Many facilities employ a protocol for trapping and euthanasia to reduce these populations; however, this process often creates a vacuum allowing new animals to move into the territory at a faster rate. The Columbus Zoo and Aquarium employed a trap-and-euthanate protocol for several years, and animals that were euthanated were submitted for necropsy to the Ohio Department of Agriculture. A review of the pathology reports over the past 4 yr revealed an average of 115 animals submitted per year. None of the animals submitted during this time period were diagnosed with rabies or distemper.

A preventive health program was implemented at the Columbus Zoo and Aquarium for resident wildlife frequently trapped. The raccoon, (*Procyon lotor*), is a common inhabitant of the zoo grounds and was selected as a trial species for this program. This carnivore often enters animal housing areas and raids food supplies or predate the captive collection, while also contaminating the area with urine, feces, and saliva. In order to reduce the risk to both the animal collection and the human workers that potentially encounter contaminated material and/or raccoons, a protocol was developed to address viral and parasitologic risks.

Live traps were set strategically around zoo grounds and keepers checked traps every morning. Once a raccoon was captured, it was transported to the hospital for processing. Each animal was immobilized, weighed, and a physical exam was performed. Blood was collected for serology and serum banking, and a fecal sample was evaluated for parasites. Surgical sterilization was performed, (males were vasectomized, females had tubal ligations performed), and each animal was permanently identified with a microchip and metal eartag. Vaccinations included rabies, canine distemper combination including leptospirosis, and feline parvovirus were administered. A long-acting anthelmintic, (moxidectin, ProHeart 6, Fort Dodge Animal Health, Overland Park, KS 66225 USA), was given, and topical flea and tick preventive, (fipronil, Frontline, Merial United States, Athens, GA 30601 USA), applied. Raccoons were then recovered in crates and released at the trap site in the evening. In the event a raccoon was re-trapped within 1 mo, it was released immediately. Any animal trapped 30 days beyond initial examination was re-immobilized for venipuncture, fecal collection, and repeated vaccinations/de-wormer if necessary.

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This program has been in place for almost 2 yr, and data collected indicates that there is a relatively static population of raccoons on and around the zoo grounds. Thirty one animals were trapped and processed in 20 mo. Twenty-two of those animals were re-trapped, and most were repeatedly re-trapped. Trapping procedures and frequency have not been altered in 6 yr. These results indicate populations of resident raccoons have established home ranges that include zoo property. The significant decrease in number of raccoons trapped from prior to initiation of program to present may be due to territorial defense, which would slow the vacuum effect of trap-and-removal protocols. Establishing a 'protected' population of raccoons on grounds also potentially reduces the risk of exposure to infectious diseases to both collection animals and human workers.

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## GENETIC SUSCEPTIBILITY TO BOVINE TUBERCULOSIS IN WILD BOAR

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### Abstract

Bovine tuberculosis (bTB) is an important re-emerging zoonotic disease, causing major economic losses and constraining international trade of animals and their products. Despite evidence that genetic factors may influence resistance to bTB in cattle, this aspect remains unstudied in natural populations. In order to investigate the relative importance of genetic heterozygosity in determining susceptibility to bTB infection in natural populations of wild boar, we studied 177 wild boars from eight estates in Southern Spain with different wild boar density and bTB prevalence. Each individual was assessed for age class, sex, and bTB infection. Disease dissemination (spread of lesions within an animal) was determined following anatomopathologic examination. We used a panel of 20 microsatellite markers previously cloned from domestic pigs to calculate each individual's 'internal relatedness' (IR), a derivative of heterozygosity which estimates parental similarity. An initial analysis revealed a significant association between bTB infection and IR, relatively less heterozygous (= high IR) wild boars being more likely to be infected (GLM,  $F_{1, 170} = 8.96$ ,  $P < 0.003$ ).

A more complicated model incorporating age, wild boar density, and estate management practices showed that bTB infection was strongly associated with IR and age (both  $P < 0.0005$ ), and less strongly with abundance ( $P < 0.005$ ). Testing each marker separately revealed evidence of a disproportionate effect on bTB susceptibility associated with chromosomes 7q and 13q, regions with plausible connections to immune function. Our results show that regardless of differences in management practices, genetic heterozygosity is a key predictor of both infection and disease progression. This study provides the first convincing evidence of genetic factors influencing the maintenance and severity of bTB in natural populations.

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## EXPERIMENTAL INFECTION OF DOMESTIC PIGS WITH PSEUDORABIES VIRUS ISOLATED FROM FERAL PIGS

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### **Abstract**

Many feral swine populations are infected with pseudorabies virus. Infection was confirmed through virus isolations from the genital tract and transmission is believed to be venereal in feral swine herds. The objectives of this project were to determine if domestic pigs could be infected with feral swine strains of pseudorabies virus via the genital and respiratory routes and to determine the extent and sites of virus shedding and latency. Domestic gilts and boars were inoculated, either intranasally or via the genital tract, with a strain of pseudorabies virus that was isolated from the prepuce of a feral pig. We found that this virus could infect both gilts and boars by either route of inoculation and that virus shedding was primarily from the area of inoculation. Although virus was difficult to reactivate via steroid treatment, it did appear to colonize the central nervous system with the sites of latency dependent on route of inoculation. Thus, strains of pseudorabies isolated from the genital tract of feral swine have the potential to spread to domestic swine by either venereal or oronasal transmission and once introduced latent infection could develop.

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## EXOTIC NEWCASTLE'S DISEASE AND THE JABIRU STORK (*Jabiru mycteria*): WHEN EXPOSURE DOESN'T HAVE TO MEAN THE END

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### Abstract

The Jabiru stork (*Jabiru mycteria*) is a very rare bird in captivity. Many years ago, there was a small but stable population in a few zoological institutions, but none had success reproducing them in captivity. The Dallas World Aquarium has spearheaded an effort to bring in young birds in order to infuse new founder stock into an aging and faltering population. In their range in Venezuela, Jabiru chicks are often illegally collected by the native people and literally “fattened up” for later consumption. The wildlife department of the Venezuelan government (FUNZPA) confiscates these birds when they are discovered and place them in local zoos if space permits. The Dallas World Aquarium has many *in situ* projects within the country of Venezuela and has an excellent working relationship with the government, thus we are often considered for placement of confiscated animals as well.

On 16 May 2003, four juvenile (estimate 1-1.5 yr of age) Jabiru storks entered a U.S. Department of Agriculture (USDA) approved quarantine station in Miami, Florida. It was an unremarkable shipment and the birds settled into the routine of the station easily especially since they had been in some form of captivity for most of their lives. They were to be cleared after 30 days and shipped but were delayed due to an unexpected heat wave and subsequent restrictions of the airlines in June. When contacted to begin the crating process once the weather had improved, we were notified that they were awaiting some test results so we wouldn't be able to ship that week either. It was the morning of 11 June 2003 that I got the call from Dr. Cambre, the director of the Miami USDA quarantine station that the private station we were using, as well as other stations in the area had tested positive for Exotic Newcastle Disease (END). All imports originating in Tanzania were testing positive for the virus. It was at that time we learned the private station we were currently using did not have any solid dividers separating the birds in quarantine. Our storks were not in direct contact with the positive birds but they had consumed some Cordon Blues who had escaped their cages and were basically feral within the station itself. Their swabs had tested negative for Exotic Newcastle's disease but per USDA regulations would have to be destroyed with all the other birds within this quarantine station.

The Jabiru stork is a Appendix I Endangered Species regulated by the Convention on International Trade in Endangered Species (CITES) which gave these birds protection from the mass disposal, however, this station would have to be completely fumigated and left empty for 2-3 mo. Where would these birds go? On 16 July 2003, all the birds in the quarantine were destroyed leaving only the Jabirus in the quarantine station. The original plan from USDA was

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to issue a refusal of entry order and return the birds to their county of origin. There they would have to be housed in a biosecure location for 120 days, after which we could re-import them providing that they had continued to test negative. With a refusal of entry order we would be able to reuse our CITES import permit. There was some debate as to the testing location of the cloacal swabs, and would we need a CITES permit to send these to NVSL in Ames, IA? This plan seemed like our only option and the birds were scheduled to be shipped on 22 July 2003.

However, Venezuela was not willing to accept potentially END positive birds into the country and refused import. Now we had a problem. We were told that we would have to find another country that had endemic Newcastle's and see if they would be willing to take the birds. Dr. Amand graciously offered any support or assistance the AAZV could provide in locating a biosecure location. Venezuela then told us they were going to meet to discuss the situation and for us to wait and see what they could work out. We waited 1 wk while the veterinarians, government officials, and our representatives discussed options in Caracas. During this time, USDA both in Riverdale and in Miami was looking at back-up plans in the event Venezuela continued to refuse entry. On 1 August 2003, Venezuela gave a final "No" for re-entry. On 4 August 2003, I received a conference call from Riverdale and Miami informing us that the USDA had thought of a plan to allow these birds to stay in this county.

The four birds were to be relocated to the USDA quarantine station in New York. This station was selected because the Miami station did not have room in its facility for these birds. They were to be shipped basically in sealed crates and shipped directly to New York where they would be transported by a USDA vehicle to the station. There they were housed in a barn with a flock of sentinel chickens. The birds would be tested monthly and after a 120-day quarantine would be released to our possession. The day they were to be shipped, Dr. Cambre drew a blood sample for virology. The birds did show an antibody titer to END, so they had been exposed. Luckily they continued to test negative the entire time they were in quarantine and on 11 November 2003, the four Jabirus arrived at our facility. They went through a 90-day quarantine from the main collection here at The Dallas World Aquarium. Currently, the birds are thriving and we are grateful to the USDA Veterinary Services for seeing the importance of a species and not being unwilling to set a precedent when the opportunity presents itself.

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## CLINICAL MANAGEMENT IN CAPTIVITY AND FIELD APPLIED TO REINTRODUCTION OF ENDANGERED PARROTS IN SOUTHEASTERN BRAZIL

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### Abstract

The Fundação Florestal/CEMAS Conservation Program aims to reintroduce endangered parrot species to an area where they once existed, and its status is irrelevant within this context. The purpose of the program is the conservation of critical species threatened with extinction due to intense illegal trade and habitat destruction. This objective will be met in Southeastern Brazil, through rehabilitation and clinical management in captivity and establishment of wildlife reserves in order to recover natural populations and their habitats. Focusing on endangered parrot species, the objective is to evaluate pathogenicity and virulence of agents that threaten parrots in captivity and in the wild in order to establish guidelines for reintroduction programs.

We have improved the captive population for the reintroduction of red-tailed Amazon *Amazona brasiliensis*, blue-cheeked Amazon *Amazona rhodocorytha*, Illiger's macaw *Ara maracana*, green-winged macaw *Ara chloroptera* and golden-capped conure *Aratinga auricapilla* recovered from confiscations. For instance, samples were collected of 14 wild individuals of *A. brasiliensis* from South coast of São Paulo state aiming to diagnose pathogens related to clinical management. As a result, we had 3 positive samples to *Chlamydia psittacii*, 1 positive sample to *Mycoplasma* sp. (PCR), and 3 positive samples to *Proteus* sp., here indicating that this group had presented clinical symptomatology and also *Enterobacter* sp., *Streptococcus* sp., *Salmonella* sp. and *Staphylococcus* sp. had been isolated from cloacal swab cultures. Concerning to the management of the nestlings from the wild, a comparative study among *Candida* spp. was developed isolating them from the crop contents. We found *C. guilliermondii*, *C. famata* was recovered from those individuals and, using the same techniques as the captivity nestlings we found only one strain of *C. albicans*. That program experimented with various reintroduction and monitoring techniques, and the results could be instructive for other reintroduction projects in Brazil.

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## CONSERVATION AND REINTRODUCTION OF ENDANGERED PRIMATES IN SOUTHEASTERN BRAZIL: CLINICAL ASPECTS

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### Abstract

The objective of the reintroduction program ongoing at The Fundação Florestal-CEMAS is to reintroduce primate species to the area where they previously existed. The restoration of these species to the São Paulo forests from which they have been extirpated has broad public interest and support. It symbolizes the importance of protecting and managing whole ecosystems. The Fundação Florestal-CEMAS reintroduction program has experimented with various techniques for reintroduction and monitoring which can be carried over to other projects. An added benefit of the program has been the opportunity to study clinical aspects of primates in captivity and wilderness. The purposes of the Fundação Florestal-CEMAS include the conservation of critical species threatened with extinction in southeastern Brazil through rehabilitation and clinical management in captivity and establishment of wildlife reserves to recover natural populations and their habitats. With primate species the objective is to evaluate pathogenicity and virulence of agents which affect primates in captivity and in the wild in order to establish guidelines for reintroduction programs.

The captive population of nonhuman primates consists of the following: brown howler monkey *Alouatta fusca*, buffy-tufted-ear marmoset *Callithrix aurita*, black-tufted-ear marmoset *C. penicillata*, masked titi monkey *Callicebus personatus* and woolly spider monkey *Brachyteles arachnoides*. These animals originate from traffic and rescues from the wilderness or where animals in urban forest parks were found suffering traumatism and sent to CEMAS veterinary hospital. Samples collected from 98 brown howler monkeys from the Cantareira State Park, located in southeast São Paulo state showed the following results: 9 samples positive with rabies (Rhabdovirus), 5 samples positive with *Leptospira interrogans* and none positive for *Leishmania* sp and *Herpes simae*. In the area of coproparasitology, all animals studied showed positive for *Enterobius* sp, and after a short time in captivity 70% were positive for *Giardia* sp. Bacteriology of feces in immune depressed animals with clinical symptoms showed a proliferation of *Pseudomonas aeruginosa* in all individuals and *Proteus* sp, *Streptococcus* sp and *Corynebacterium* sp, in lesser proportions. Necropsy examinations in 2 brown howler monkeys showed the presence of *Dipetalonema reconditum* in the abdominal cavity. These findings together with data from other primate species have resulted in the development of a specific standard for the reintroduction programs for endangered primates in Brazil.

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## REINTRODUCTION OF OSPREYS TO *THE WILDS*, IN OHIO: I. ENVIRONMENTAL RISK ASSESSMENT THROUGH TOXICOLOGIC ANALYSIS OF FISH

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### Abstract

Eight ospreys (*Pandion halietus*) were hacked and released at The Wilds (Cumberland, Ohio) in June 2003 as part of a statewide reintroduction program initiated by the Ohio Department of Natural Resources. Through the Department of Wildlife and Conservation Medicine at The Wilds, a toxicologic risk assessment was performed to evaluate the potential for exposure of the released birds to contaminants and to provide a predictor of success for the reintroduction plan. Two species of fish, bluegill (*Lepomis macrochirus*) and large mouth bass (*Micropterus salmoides*), were taken from local ponds and lakes and tested for organochlorine pesticides, polychlorinated biphenyls (PCBs) and heavy metals. No pesticide contaminants or PCBs were detected and heavy metals were found to occur at sub-threshold levels. Mercury and other mineral contaminants were still present, however, and remain a cause for concern. Continued monitoring is warranted.

### Introduction

In conjunction with an ongoing Ohio Department of Natural Resources (ODNR) Division of Wildlife reintroduction project, eight nestling ospreys (*Pandion halietus*) were transported to The Wilds for hacking and release in June 2003. Similar releases are planned to continue annually through 2006. The suitability of The Wilds as osprey habitat has been debated. The Wilds property comprises a vast expanse of reclaimed land and many of its ecological components are yet to be thoroughly investigated. Following decades of surface mining for coal, the reclamation process resulted in a large system of ponds and lakes extending throughout the property and surrounding area. There is no federally mandated bio-monitoring of soil, water or fish in the region due to local restrictions on public access and little is known regarding local ecosystem health. Through ongoing ecological and conservation medicine studies at The Wilds, efforts are being made toward developing a greater understanding of this unique habitat.

Prior to the initiation of the current reintroduction plan, the last known osprey nest in Ohio was abandoned in 1941. Ospreys were listed as endangered in this state in 1977 and were granted protected status in 31 states by the year 2000. Like many birds of prey, ospreys suffered major declines following exposure to environmental contaminants such as DDT, PCBs and mercury, which negatively impacted reproduction.<sup>15</sup> Given their piscivorous diet, ospreys are particularly

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susceptible to contaminants that biomagnify through the food chain.<sup>7</sup> Although the reintroduction project has been met with initial success (40 birds hatched from 22 nest this year), the distribution of the returning birds has been limited to the northern half of the state for reasons yet to be determined. The Wilds was therefore requested to support a release site in 2003 based on (1) the long-standing partnership between ODNR and The Wilds, (2) the southeastern location and excellent potential osprey habitat in The Wilds environs, and (3) the local expertise.

The Wilds staff recently participated in an investigation of African fish eagles (*Haliaeetus vocifer*) as biomonitors of environmental contamination in Uganda.<sup>12</sup> This, and similar research on bald eagles (*Haliaeetus leucocephalus*) in the United States,<sup>2</sup> prompted a more comprehensive approach to the health aspects of osprey reintroduction at The Wilds. Based on the uneven distribution of osprey return within the state of Ohio, the known ongoing effects of contaminants on osprey reproduction in some areas of the United States<sup>5,11</sup> and the unknown status of contaminants at The Wilds, a specific toxicologic investigation was indicated. Whereas exposure to contaminants that ospreys encounter during their southern migrations cannot be controlled, environmental investigations allow the suitability of the release site to be maximized.

## Materials and Methods

### Environmental Suitability Assessment

When addressing potential risk factors in the reintroduction of ospreys to The Wilds, persistent organic pollutants (POPs) were considered a significant non-infectious disease threat based on preliminary information including the following:

- Ospreys disappeared from Ohio due to heavy exposure to POPs such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs) and heavy metals. Mercury, PCBs, and DDT have proven to be among the most toxic contaminants to raptors due to bioconcentration, bioaccumulation and biomagnification processes.<sup>7</sup>
- The process of surface mining for coal and the resulting acid mine runoff can facilitate accumulation of disproportionately high levels of several heavy metals in the environment.<sup>1</sup>
- Ohio is a largely agricultural state and agricultural runoff has been associated with increased environmental burdens of chlorinated pesticides such as DDT.
- Coal plants such as those upriver of the watersheds of The Wilds are a potential source of contamination with PCBs, arsenic, cadmium, lead, mercury, and selenium.<sup>4</sup>
- POPs bioaccumulate in fish. While many POPs are being detected in decreasing levels in fish in Ohio and surrounding regions, in some areas contaminants such as mercury and lead have been found in slowly increasing levels.<sup>3,13</sup>
- Ohio has widespread POP-based fish consumption advisories.
- Dietary contaminantation has proven to be the primary source of exposure to POPs in piscivorous raptors such as ospreys.<sup>7</sup>

Fish Collection

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Blue gill and large mouth bass were selected for study because they are the prevalent fish species found at The Wilds and consequently will comprise the main prey base of resident ospreys. Fish samples were collected by hook and line and consisted of two same-species fish of similar size (< 10% length disparity) per sample. Collection parameters were based on the upper end of the length range for typical osprey acquisition (bass 30-35 cm; bluegill 17.5 - 22.5 cm). A total of 18 samples were collected from 9 different lakes. Length and weight measurements were recorded and whole body composites were frozen within 4 hr of collection.

### Sample Analysis

Samples were analyzed for contaminants at Michigan State University Animal Health Diagnostic Laboratory. Tissues for analysis of chlorinated pesticides (DDT and metabolites, aldrin,  $\alpha$ -BHC,  $\beta$ -BHC, hexachlorocyclohexane, dieldrin, endrin, heptachlor epoxide, lindane, nonachlor) and PCBs (total aerochlors) were extracted and purified using procedures previously described.<sup>10</sup> Linear standard curves at 0.002-0.5 ppm range for pesticides and 0.05 - 2.0 ppm range for PCBs were used for quantification. Concentrations were determined by gas chromatography with electron capture detector (Varian 3400 gas chromatograph, Varian Instruments, Walnut Creek, CA). The quantification limits were 0.001 ppm for pesticides and 0.01 for PCBs. The recovery rates were 70 – 100 %. Samples were analyzed for mercury by cold vapor atomic absorption spectrophotometry at 253.7 nm with a quantification limit of 0.005 ppm. Accuracy was monitored by concurrent analysis of procedural blanks. Mineral panel analysis was performed with inductively coupled plasma-atomic emission spectrometry (ICP-AES) for the following elements: barium, iron, sulfur, copper, manganese, antimony, sodium, cobalt, molybdenum, arsenic, lead and potassium.

### Results

Neither chlorinated pesticides nor PCBs were detected in any samples. Of 18 OSHA-regulated toxic metals measured, 11 were detected and are presented in Table 1. The following minerals were not detected at the corresponding quantification limits (ppm): selenium, 2.0; boron, 1.0; thallium, 2.5; antimony, 1.0; molybdenum, 0.2; arsenic, 0.5; and lead, 0.5. A literature review revealed proposed dietary risk thresholds for birds for eight minerals. Of these, cadmium, copper, manganese, arsenic and zinc were not detected at quantities above threshold. Levels of mercury and lead were found in sub-threshold levels for most references,<sup>6,14</sup> but were above some US EPA references for low toxicity values for dietary exposure in birds. The quantification limit for selenium was below the generally accepted threshold for dietary exposure in birds (3.0 ppm), but above a proposed threshold 0.23 ppm found in one reference.

### Discussion

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Quantification limits for PCBs and chlorinated pesticides are well below both the critical toxic dietary thresholds proposed for reproductive failure in ospreys (0.5 ppm<sup>9</sup> and 6.0-9.0 ppm ww,<sup>15</sup> respectively) and the threshold for effects on shell thickness in birds (0.1 ppm ww<sup>8</sup>).

Heavy metal concentrations quantified in this study were similar to national averages reported in the early 1990's for freshwater fish from comparable areas. Little data is available regarding risk thresholds for many of these contaminants in birds. Of the minerals detected, mercury is of most concern. Mercury levels fell into the high-end range of those levels reported for southeast Ohio by the EPA in 1997, however, no sample exceeded the proposed risk thresholds for reproductive failure in osprey (dietary 0.5 ppm ww).<sup>14</sup> Mercury levels have not appreciably declined across the country since 1974<sup>13</sup> and have in fact increased in some osprey populations.<sup>3</sup> Mercury is of particular concern because it readily bioaccumulates, bioconcentrates and biomagnifies as methylmercury<sup>7</sup> which can reduce reproduction in birds with low level chronic exposure.<sup>6</sup> Furthermore, the majority of mercury that accumulates in eggs and offspring, and therefore presents the greatest risk to successful reproduction in adults, is acquired from the breeding area,<sup>6</sup> which is typically within twenty miles of the male's fledging site. Although mercury is ubiquitous, liberation of ecologically toxic methylmercury from the inert form may be exacerbated in this study area by mine run-off, coal-fired electric plants, seed treatment, and man-made lakes.<sup>13</sup>

Pre-introduction and translocation assessments are not new concepts. The variables and considerations, however, are ever expanding and in the past decade the complexities associated with translocation have been increasingly revealed. While issues of disease spread and susceptibility were well discussed in the early 1990's (J Zoo Wildlife Med: Vol. 24:3), issues such as endocrine disruptors and unknown environmental toxicants as obstacles to successful animal movements have been introduced more recently.<sup>2,7</sup> The success of wildlife translocation is increasingly contingent upon interdisciplinary efforts and planning and the scientific community is responding with a broadened approach. In regards to piscivorous raptors in particular, much has been learned from ongoing contaminant monitoring and individual species are becoming increasingly validated as contaminant biomonitors.<sup>2,6,12</sup> It is therefore prudent that the information be used as part of a preventive medicine regime for these birds as well.

## **Conclusions**

The toxicologic analysis of local fish is considered a valuable determinant of risk associated with osprey reintroduction at The Wilds. In terms of known risk factors in food chain contamination, the results of this study indicate that The Wilds offers suitable habitat for an osprey reintroduction program.

The true extent of toxic risk from lead, cadmium and newer pesticide chemicals remains largely unknown and further study is necessary. Newer, non-accumulating pesticide chemicals are showing increasing evidence of adverse health effects on wildlife and expanded testing protocols are indicated. Mercury, PCBs and chlorinated pesticides represent a particular health hazard to

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osprey and some of these chemical are found in excessive levels in the Great Lakes region to the north of Ohio. Periodic monitoring of fish and possibly returning osprey or their offspring would be prudent.

Additional aspects of the merit of this reintroduction project warrant emphasis. The benefits go beyond those of a toxicologic risk assessment. This project offered a unique opportunity for public education and awareness. Through hands-on involvement with monitoring, feeding, banding and veterinary examination of the ospreys at The Wilds, dozens of Ohio children were directly involved with the reintroduction of an endangered species in Ohio. Furthermore, the hawk tower could be seen from public tour routes, furthering public interest and education. Finally, the reintroduction strengthened ties between The Wilds and the ODNR as well as the Columbus Zoo, Muskingum College and the OHEPA

#### ACKNOWLEDGMENTS

This project was funded by the Columbus Zoo and Aquarium Conservation Fund. We gratefully acknowledge the Columbus Zoo for their support, Wilson Rumbelha (MSU ADDL) for assistance with fish tissue analysis and interpretation, Dennis Mishne (Ohio EPA) for access to fish tissue contaminant data, and John Estinek (Ohio EPA) for toxicologic advice.

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**Table 1.** Metals detected in largemouth bass and blue gill at The Wilds, 2003.

Mineral	Mean (ppm)	Range (ppm)	n
Mercury	0.153	0.01-0.39	18
Barium	0.41 <sup>a</sup>	<0.05-1.51	16
Iron	23.77	4.53-121	18
Chromium	0.43 <sup>a</sup>	<0.20-0.72	14
Calcium	13,269	3780-26,000	18
Magnesium	479	310-697	18
Zinc	16.3	9.8-23.7	18
Cadmium	0.1 <sup>a</sup>	>0.1-.10	1
Copper	0.45	0.23-1.01	18
Manganese	5.84	0.38-31.9	18
Cobalt	0.225 <sup>a</sup>	<0.10-0.225	2

<sup>a</sup>Samples below detection limit not included in mean.

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**SEROSURVEY OF INFECTIOUS DISEASES IN *EX SITU* GIANT PANDAS (*Ailuropoda melanoleuca*) AND RED PANDAS (*Ailurus fulgens*) IN CHINA**

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**Abstract**

Nearly 60% of China's *ex situ* giant panda population is currently located in two facilities. The potential vulnerability of these concentrated groups to infectious diseases is of concern. As a first step to evaluate disease risks, a serologic survey of giant pandas was conducted, with a comparative assessment made for a red panda population maintained in the same facility. All serum samples for the survey came from the Chengdu Research Base for Giant Panda Breeding, which currently maintains 26 giant pandas and 35 red pandas. Serum samples from 19 giant pandas (total 44 samples; ages 3-18 yr) were banked from 1998, 1999, 2000, 2001 and 2003, mostly during the breeding season (March-May). The eight red pandas (1-5 yr of age) were sampled in 2003. At this facility, adult giant and red pandas were generally vaccinated with a product from China developed for domestic dogs that included attenuated distemper, parvo, rabies, herpes and parainfluenza viruses; since 2002, the vaccine also has contained coronavirus. Vaccination of cubs generally began after 1 yr of age.

Serum samples were tested for antibody titers to canine distemper (CDV), adeno (CAV) and corona (CCV) viruses by serum neutralization assay; parvovirus (CPV-2) by hemagglutination inhibition assay; *Toxoplasma gondii* by indirect hemagglutination assay; *Neospora caninum*, *Leptospira interrogans* (serovars pomona, hardjo, icterohemorrhagiae/copenhageni, grippotyphosa, canicola), and canine herpes (CHV) and canine parainfluenza (CPIV) viruses by indirect fluorescent antibody assay. All samples were negative for *Leptospira*, CPIV, CCV, CAV, CHV and *Neospora*. Positive titers to *T. gondii* ranged from 1:40 to 1:160; 57% of giant panda and all red panda samples were negative. All but 6 (13%) giant panda samples had significant titers to CDV, ranging from 1:16 to 1:2,560, with 50% in the range of 1:24 to 1:160. Six giant pandas with low titers are known to have been vaccinated within 2 mo of at least two of the sampling dates. All red panda samples had titers (1:24 to 1:10,240) with the highest titers occurring in unvaccinated yearlings. Titers to CPV-2 were  $\geq$  1:20 in all but one sample (range 1:20 to 1:10,240), with 80% of giant panda and 75% of red panda samples  $\geq$  1:80. The high degree of variation in titers to CDV and CPV and the lack of vaccine response to CCV, CPIV

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and CHV suggest that alternative measures must be considered to more effectively protect giant and red pandas in China's *ex situ* population against these agents of infectious disease.

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## LEARNING FROM ROADKILLS: IMPLICATIONS FOR CONSERVATION OF NATIVE SNAKES

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### Abstract

Published studies of free-ranging snake populations in North America have not included health surveys to date. Some information is available for other regions (e.g., *in situ* green anaconda health assessments) but is typically restricted to a species-specific investigation rather than including a more comprehensive sampling of other taxa of snakes. This pilot study investigates the feasibility of using dead-on-the-road snakes as one component of assessing the health status of free-ranging snake populations. Formalin-preserved organ samples were obtained from fresh dead-on-the-road (DOR) specimens of snakes collected in Arizona and H&E stained sections were observed by light microscopy. Nine DOR snakes collected in 2002 were analyzed. 88.9% (8/9) of the DOR snakes were adult males. One DOR snake was a juvenile crotalid and the gender was not determined. Seven of nine (77.8%) of the DOR snakes were crotalids and 22.2% (2/9) of the DOR snakes were colubrids. Three of nine (33.3%) of the DOR snakes had histologic lesions suggesting an inflammatory or infectious process. Two of nine (22.2%) of the D.O.R snakes showed signs of inflammatory processes. One of nine (11.1%) had no etiologic agent identified with the inflammatory process. This was a Western longnose snake (*Rhinocheilus lecontei lecontei*) with multifocal very mild lymphoplasmacytic hepatitis. One of nine (11.1%) showed inflammatory processes and a parasite. This was a western diamondback rattlesnake (*Crotalus atrox*) with lymphoplasmacytic gastritis, and hypertrophic enteritis with coccidian-like intraepithelial protozoa. One of nine (11.1%) showed a parasite (microfilaria) without obvious signs of inflammation. This was a Sonoran gopher snake (*Pituophis catenifer affinis*) that had massive numbers of microfilaria in the liver vessels and sinusoids. No inclusions similar to inclusion body disease of ophidian paramyxovirus were detected in any of the nine DOR snakes. Additional surveys are recommended to help inform reintroduction and recovery programs.

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## SEROLOGIC EVIDENCE OF INFECTIOUS BURSAL DISEASE VIRUS EXPOSURE IN CAPTIVE WHOOPING CRANES

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### Abstract

Between September 2001 and March 2002, unusually high morbidity and mortality was observed during releases of endangered, captive-reared whooping cranes (*Grus americana*) in central Florida. An ongoing epidemiologic investigation has implicated infectious bursal disease virus (IBDV) as the likely etiologic agent. The source of this virus remains unknown. A serologic survey of the two primary US captive populations was undertaken to ascertain the historic exposure of the cranes to IBDV. Archived serum samples from the International Crane Foundation, Baraboo, WI and the USGS Patuxent Wildlife Research Center, Laurel, MD were screened for IBDV serotype 2 neutralizing antibodies. At ICF, samples were tested from healthy resident adults (n = 32, collected annually 1998 - 2002) and 3-5 mo-old juveniles (n = 54, samples collected at pre-release quarantine exams 1997 - 2003). Twelve percent of adults and 7% of juveniles were seropositive in this survey (titer  $\geq$  1:32). Seropositive adults first appeared at ICF in 1998, and seroprevalence peaked at 15 % in 2002. Seropositive status varied across years within three individuals with a complete serologic history. The geometric mean titer of adults increased during the study (range 1:4 in 1998, 1:11 in 2002). Seropositive juveniles first appeared at ICF in 2003, when 4 of 9 (44 %) cranes had elevated titers. At Patuxent, preliminary results suggest that 4 of 32 (12 %) juveniles in recent years were seropositive for IBDV. In December 2003, IBDV infection was confirmed at necropsy by PCR assay of spleen and bursa in a juvenile whooping crane that demonstrated a neutralizing antibody titer of 1:256. In addition, several Patuxent sandhill cranes (*Grus canadensis*) used in a West Nile virus vaccination study during 2003 developed IBDV antibody titers  $\geq$  1:64. Interpretation of these data suggests that exposure of captive whooping cranes to IBDV serotype 2 has occurred historically as early as 1998, and that exposure is more common compared to several years ago. The low titer levels observed in the vast majority of the cranes are suggestive of exposure to the virus and not believed to be associated with active infection nor the presence of a disease state, though at least one exception has been found. The risk for whooping crane recovery may lie in pathogen transfer to environments that promote disease expression such as areas used for reintroduction.

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## IMMUNIZATION OF BLACK-FOOTED FERRETS AGAINST PLAGUE (*Yersinia pestis*)

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### Abstract

Sylvatic plague, caused by the bacterium *Yersinia pestis*, is primarily a disease of wild rodents that is transmitted between mammals via flea-bite, direct contact, or inhalation. Since its introduction into the United States in the early 1900's, plague has become firmly established in native rodent populations throughout the west, causing frequent epizootics in prairie dogs (*Cynomys* spp.) and other wild rodents. The disease has had devastating effects on many prairie dog populations, often killing 90-100% of individuals in affected colonies. Black-footed ferrets (*Mustela nigripes*) depend primarily on prairie dogs for both food and shelter and thus may be exposed to the bacteria either by consumption of plague-infected prey or by flea-bite. Once thought to be extinct, a captive breeding and recovery program was established for black-footed ferrets in 1987 after an outbreak of canine distemper nearly decimated the last known wild colony that was discovered 6 yr earlier. The occurrence of plague in prairie dog populations and its potentially devastating effect on black-footed ferret re-establishment is a major impediment to the captive breeding and recovery program.

We conducted an experiment to assess the feasibility of vaccinating black-footed ferrets (BFF) against plague using a recombinant fusion protein consisting of F1 and V antigens from *Y. pestis*. On days 0 and 28, post-reproductive BFFs were immunized with the fusion protein by subcutaneous (s.c.) injection. Control animals received a placebo by the same route. Two weeks after the second immunization, mean antibody titers to *Y. pestis* F1 antigen were measured and found to be significantly higher in vaccinates than their pre-immunization value ( $P < 0.001$ ) and significantly higher than the control value ( $P < 0.001$ ). Six months post-immunization, 16 vaccinates and eight controls were challenged with approximately 8,000 colony forming units of virulent *Y. pestis* by s.c. injection. Eleven of 16 vaccinates survived challenge with no ill effects; their survival rate was significantly different ( $P = 0.02$ ) from the eight control animals, all of which died within 3-6 days. Two months later, the 11 surviving vaccinates were challenged

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again by ingestion of a plague-infected mouse. None of the animals showed any ill effects and all survived. In contrast, seven control animals fed infected mice died of plague within 2-4 days, including one animal that did not actually ingest the mouse, but likely sniffed or licked it. This study demonstrates that immunization of black-footed ferrets with the recombinant F1-V fusion protein can induce significant antibody responses and reduce their susceptibility to plague infection. Until other methods of plague control are developed, the F1-V vaccine might be useful in protecting black-footed ferrets in captive-breeding facilities and animals intended for reintroduction programs.

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## INFECTIOUS BURSAL DISEASE VIRUS ASSOCIATED WITH A WASTING SYNDROME IN RELEASED WHOOPING CRANES IN FLORIDA

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### Abstract

Whooping cranes (*Grus americana*) have been reintroduced into central Florida beginning in 1993 until the present. Bobcat predation of otherwise healthy cranes in good nutritional condition was the most common cause of mortality. However, released cohorts in the years 1997-8 (14/22 died) and 2001-2 (14/27 died, 5/27 clinical illness) experienced unusually high morbidity and mortality. Positive serum neutralizing titers for infectious bursal disease virus (IBDV) were identified following the 2001 event, and an epidemiologic study of released birds and the captive source flocks was initiated. Serotype 1 (Lukert and Variant A) tests were mostly negative. Serotype 2 testing resulted in many more positives. Polymerase chain reaction (PCR) positive results for IBDV were obtained from two whooping cranes; one that died during the first epizootic in Florida in 1998, and one captive bird that died in 2003 at Patuxent Wildlife Research Center. The serotype remains unconfirmed at this time. Virus isolation has not been accomplished.

The disease was characterized by chronic weight loss in young of the year birds that were actively foraging. Other observations included granulomatous oral lesions, bill bruising and fractures, anemia, abnormal submissive behavior, and infection with *Megabacteria* and *Eimeria*. Eight of 10 released cranes with titers  $\geq 1:128$  had detectable evidence of illness. Older birds sharing the same habitat and food remained unaffected. Some sick birds were able to recover and become members of bonded pairs. The first seropositive case occurred in 1993. Seropositive birds are not randomly distributed by year indicating differential exposure or susceptibility. The prevalence of seropositive birds (titer  $\geq 1:32$ , n = 256, 1993 to 2003) increased from 7% of birds leaving captive centers to 33% of birds that had spent 2 wk in Florida. Significantly more of the older birds captured, either because they were clinically ill or to change transmitters, were seropositive (75%). Within the wild flock, seroprevalence increased with age. In wild birds monitored frequently, fluctuation in titers indicated multiple re-exposures. The source of exposure, whether within the whooping crane flock, sandhill cranes, or another species, remains unknown.

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## REPRODUCTIVE EVALUATION IN WILD AFRICAN ELEPHANTS PRIOR TO TRANSLOCATION

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### Abstract

Translocations of wild African (*Loxodonta africana*) elephants have increased significantly since 1993 after Clem Coetsee developed a new method to move adult elephants in Zimbabwe. Since then the technique have been optimized mainly by the staff of the Kruger National Park (KNP) and over 750 elephants in family units and almost 100 mature bulls have been translocated by the KNP capture team.<sup>1</sup> The translocations were mainly performed for reducing the number of elephants in KNP and for stocking other reserves. Few elephants were also moved for overseas export to international zoological institutions. However, each elephant translocation is always a logistic challenge and is extremely costly. Therefore, it is very important to select the right elephants or elephant groups for the future translocation. If the main goal of a translocation is the establishment of a new breeding group, it is especially important to select infertile individuals and highly pregnant females which could have a miscarriage due to the transport stress. The IZW team developed a field applicable portable ultrasound technique which allows the reproductive evaluation of an immobilized wild elephant in about 15 min.<sup>2</sup> So far, ultrasonographic assessments have been used for the selection process of the immobilized elephants during two translocations projects in KNP (1997) and the Royal National Parks Mkaya and Hlane in Swaziland (2003). A total of 8.13 elephants were sonographically examined. Due to underdeveloped genital tract, reproductive disorder, late pregnancy or perinatal stage 2.3 elephants (approx. 24%) from the originally selected 21 individuals were reversed and released to the wild. The transrectal ultrasound evaluation offered a new opportunity for the accurate selection of reproductive healthy individuals for translocation and it helped to avoid the transport of late pregnant cows with the high risk of miscarriage.

### ACKNOWLEDGMENTS

The authors thank the South African capture crew, Mickey Reilly (Kingdom of Swaziland), Mike Bester (Bester Birds and Animals South Africa), Eric Zeehandelaar (FJ Zeehandelaar, Inc), Dr. Larry Killmar (ZSSD), and Randy Rieches (ZSSD).

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## THE SALTON SEA: PESTILENCE, POLITICS, AND POSSIBILITIES

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### Abstract

California's largest lake, the Salton Sea, located 30 miles north of the U.S.-Mexico border, has become a critical stopover site for millions of migrating birds, in part due to the loss of over 90% of the state's wetlands. More than 400 species of birds have been reported in the Salton Basin – two-thirds of all the species in the continental United States. Banding returns illustrate that birds from all over western North America utilize the Salton Sea ecosystem, with several of these bird species depending heavily on the Sea. During recent years, over 45% of the entire U.S. population of the threatened Yuma clapper rail (*Rallus longirostris yumanensis*), 80% of the American white pelican (*Pelecanus erythrorhynchos*) population, and 90% of the continental population of Eared grebes (*Podiceps nigricollis*) have been supported by the Salton Sea.<sup>4</sup> As recently as 1999, over 200 million fish were estimated to reside in the Salton Sea, attracting numerous fish-eating birds and supporting a large sport fishery.<sup>2</sup>

The capacity of the Salton Sea to sustain such a rich avifauna community and fish populations that provide the food base for many of these birds is being threatened by numerous factors, including rising salinity, excessive nutrient run-off from agriculture, and proposed water transfers that would decrease water inflow to the Sea. In addition, unprecedented and massive mortality of fish and birds from a variety of causes signify that this ecosystem is severely stressed. Bird mortality and the number and frequency of disease outbreaks have increased substantially since the 1980s. In 1992, an estimated 150,000 Eared grebes died at the Salton Sea from avian cholera and an undetermined cause.<sup>1</sup> This was the largest documented epizootic event in Eared grebes in United States history, killing approximately 10% of the North American population of this species. Avian cholera continues to kill grebes annually and is taking a heavy toll on Ruddy ducks (*Oxyura jamaicensis*), becoming perhaps the most important cause of winter mortality for this species. During 1996, nearly 20,000 pelicans and other fish-eating birds at the Salton Sea died from an atypical outbreak of avian botulism, including an estimated 15% of the western subpopulation of American white pelicans and over 1,400 Brown pelicans (*Pelecanus occidentalis*), an endangered species.<sup>3</sup> Newcastle disease and salmonellosis are also recurring causes of avian mortality at the Salton Sea.

As significant as these mortality events are, of even greater importance is the continued degradation of the Salton Sea that has severely impacted the fishery and its dependent bird populations. Gill-net surveys, creel results and bird surveys from 2002 to the present time

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indicate a substantial and dramatic decline in all sport fish populations with concomitant declines in the abundance of fish-eating birds. Attempts to address these critical ecosystem health problems at the Salton Sea are mired in controversy over water rights. It is urgent that stakeholders resolve these issues before an ecological collapse of the Salton Sea becomes inevitable.

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## APPLIED CONSERVATION AND WILDLIFE HEALTH IN SOUTHERN CALIFORNIA

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### **Abstract**

San Diego County and the surrounding areas in southern California harbor a wealth of wildlife and people. However, maintaining healthy wildlife populations into the future will require a concerted effort between scientists, managers, and the public. Wildlife health issues often cross disciplinary boundaries, and solutions require that you follow the problems wherever they lead. Ecosystem health is more than just a buzzword – in fact it requires a broad ecosystem-based approach to identify and ultimately solve problems.

The Wildlife Health Center's Southern California Program began in the early 1990s as a research study into the cause of bighorn sheep population decline in the Peninsular Mountain Ranges of San Diego and Riverside Counties. Although infectious disease appeared to be the primary cause of mortality, field investigations revealed that mountain lion predation was the major factor limiting bighorn sheep population growth. Lions normally utilize deer as their primary prey, but here we had lions literally driving a bighorn sheep population to near extinction. To further complicate the picture, the conflict between lions and bighorn sheep occurred simultaneously with a sharp increase in lion attacks on people nearby.

Clearly there was more to this wildlife health issue than just the two species (lions and bighorn sheep, or lions and people). To identify, understand, and address the issues in this region, the WHC's Southern California Program has developed an ecosystem-based approach that encompasses mountain lions, bighorn sheep, deer, people and habitat. The picture that is emerging suggests that the pervasive influence of humans in this region has dramatically altered the system. "Problems", whether they involve bighorn sheep or lions, have largely been created by humans and human activities. For example, habitat loss (urban development) and modification (fire suppression and catastrophic wildfires) has altered the distribution and abundance of lions, bighorn sheep, and deer. In addition, it has altered the interactions between people and wildlife in ways that pose new problems for people (pets and livestock killed by lions) and wildlife (lions killed for killing domestic animals).

It takes a multidisciplinary, team approach to solve wildlife or ecosystem health problems. The Southern California Program has formed partnerships with state and federal wildlife and wildland agencies, as well as with land trusts and private stakeholders, to develop, fund, and implement solutions. More information on the program is available at <http://www.vetmed.ucdavis.edu/whc/scp/default.htm>.

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## HIGHLY MIGRATORY SPECIES IN THE CALIFORNIA BIGHT: IMPLICATIONS FOR WILDLIFE DISEASE

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### Abstract

The California Bight supports a diverse community of marine vertebrates including four robust pinniped populations and at least a dozen resident or transient cetacean species. These species vary in their geographic and vertical use of the coastal marine environment. Marine mammals undergo seasonal migrations that carry them across U.S. political boundaries into Mexico or Canada and into habitats as diverse as the relatively remote offshore islands, to beaches and embayments near high-density urban areas, to regions impacted by agricultural or industrial run-off and sewage.<sup>1, 2, 3</sup> They are therefore exposed to and regularly move among areas of varying water quality<sup>4</sup>. “Pathogen pollution” from anthropogenic sources has been implicated as a contributory factor in wildlife diseases, including those affecting marine species.<sup>5, 6</sup> Epidemics of disease may result when infected animals move into an area occupied by naïve populations; conversely, healthy animals moving into new areas (e.g., in response to environmental perturbations such as El Nino<sup>7</sup>) may experience morbidity and mortality associated with endemic pathogens in that area.<sup>8</sup> Infectious diseases in marine mammals vary in their etiology, pathogenesis, zoonotic potential and ecological impact. Pinnipeds may serve as reservoirs for pathogens that can have substantial impacts on terrestrial populations, including humans (e.g., influenza B virus<sup>9</sup>), domesticated food animals and terrestrial wildlife (e.g., brucellosis<sup>10</sup>). An understanding of marine mammal movements and disease exposure - not just in U.S. waters but in all the regions they inhabit - is critical to understanding marine ecosystem health in the California ecoregion.

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## A SOUTHERN SEA OTTER (*Enhydra lutris nereis*) UNUSUAL MORTALITY EVENT: FINDINGS FROM ANALYSIS OF FRESH DEAD ANIMALS

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### Abstract

The southern sea otter (*Enhydra lutra nereis*) population, located on the coast of central California, is listed as “threatened” under the Endangered Species Act. Recent surveys estimate that the entire population numbers approximately 2500 animals and that the population has failed to increase significantly since 1995.<sup>4</sup> An abundance of evidence suggests that high mortality is a key factor in the slow recovery of this population.<sup>4,8</sup> From February through April 2003, southern sea otter carcass recovery rates exceeded 10-yr averages and an “unusual mortality event” (UME) was jointly declared by the US Fish and Wildlife Service (FWS) and the National Oceanic and Atmospheric Administration (NOAA).<sup>3</sup> Because of ongoing sea otter mortality and documented domoic acid (DA) intoxication in other marine mammals and birds in the Central California region during this same time period, the FWS and NOAA extended the UME investigation to include otters dying between January 1, 2003 and October 1, 2003.

The total number of fresh and non-fresh southern sea otters stranding in 2003 (n = 262) was 23% greater than any previous year and was 58% above the 10-yr running average of 166 animals per year.<sup>3</sup> For fresh dead sea otters, the most common significant and grossly discernable lesions included: acanthocephalan peritonitis (18%); shark predation (15%); cardiomyopathy syndrome (12%); boat strike (6%); mating trauma (6%); and geriatric (e.g., dental) disease (6%). On histopathology, suspected or confirmed protozoal (*Toxoplasma gondii* and/or *Sarcocystis neurona*) meningoencephalitis or systemic disease<sup>4</sup> was observed in 12% of otters. Moderate to severe brain lesions consistent with domoic acid (DA) intoxication<sup>2,3,7</sup> were observed in at least 11.6% of otters, with further review in progress. In addition, gross or histopathologic lesions consistent with cardiomyopathy syndrome (gross myocardial mottling, fibrosis, necrosis, vacuolization, nonsuppurative inflammation and ganglioneuritis or cardiac dilation)<sup>5</sup> were observed for at least 27.5% of freshly dead otters stranding during the UME. The proportion of southern sea otters reported with gross or histopathologic lesions suggestive of cardiomyopathy syndrome has increased in recent years, and this syndrome has been recognized as a significant

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source of southern sea otter mortality.<sup>5</sup> Recent epidemiologic data supports an association between cardiomyopathy syndrome in sea otters and previous or current DA exposure,<sup>5</sup> and cardiac abnormalities have been described in other marine mammals with confirmed or suspected DA intoxication.<sup>2,7</sup> In the present study, over 70% of fresh dead sea otters had DA present in urine (as detected by receptor binding assay<sup>6</sup>), with DA levels ranging from 29 to >42,000 ng/ml. Domoic acid was present in sea otter urine in all months tested (January through September, 2003), suggesting that environmental DA exposure may be chronic for otters living along the central California coast. However, a higher proportion of otters with high urine DA concentrations (>200ng/ml) was detected in late spring 2003, corresponding with a peak in otter mortality and a recognized DA event in other marine wildlife from the same area. Therefore, DA intoxication may have contributed to the high level of southern sea otter mortality observed in 2003.

#### ACKNOWLEDGMENTS

Special thanks to Brian Hatfield, Jim Estes and Tim Tinker for compiling data on overall mortality and data on non-fresh otters, and to Greg Sanders, USFWS, Trevor Spradlin, NOAA and the UME Working Group for facilitating this work. Thanks to Jack Ames, Mike Harris, Brian Hatfield, Jim Estes, Mike Kenner, Greg Sanders, Erin Dodd, Heather Harris, Sharon Toy-Choutka, Eva Berberich, Kat Starzel, Sandra Wong, Dan Rejmanek, Spencer Jang, Barry Puget, Ann Melli, Andrea Packham, Woutrina Smith, Mike Murray, Michelle Staedler, Andy Johnson and Marty Haulena for facilitating carcass collection, necropsy, sample collection and diagnostics. Thanks also to Kathy Burek and Jim Hill for assisting with sea otter histopathology efforts, and to the staff, students and volunteers of the Monterey Bay Aquarium, the Marine Mammal Center, USGS/BRD, University of California, Moss Landing Marine Laboratory and CDFG Marine Wildlife Veterinary Care and Research Center for facilitating carcass recovery and sampling. Miles Reed, Clare Stavely and others assisted with the development and maintenance of the MWVCR sea otter necropsy database.

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## CAUSES OF DEATH IN RELEASED CALIFORNIA CONDORS (*Gymnogyps californianus*) FROM 1992-2002

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### Abstract

The reintroduction of California condors (*Gymnogyps californianus*) to the wild began in 1992 after the last wild bird was brought into captivity in 1987. A successful captive breeding program produced a first chick in 1988, and has increased the size of the California condor population from a low of only 22 birds in 1982, to 63 birds in 1992, and 197 birds in 2002. Currently, there are 90 birds in the wild: 44 in California, 41 in Arizona (this includes the first successfully wild-hatched and fledged chick), and five in Baja California, Mexico.

To date, 174 birds have been released, with an overall mortality rate of 33%. Carcasses of most dead condors (85%) have been recovered and necropsied by the Department of Pathology at the San Diego Zoo (the exception being carcasses serving as evidence in legal proceedings, which go to the US Fish and Wildlife Service for necropsy). Causes of mortality of condors that have died during the first 10 yr of the program from 1992-2002 have been analyzed: 17% of all mortalities have been due to power line collisions; 13% due to lead toxicity or suspected lead toxicity; 11% due to predation; and 6% have been due to emaciation. Causes of mortality have not been determined in 21% of the mortalities because of advanced autolysis or scavenging of the carcass. Other causes of mortality have included wildfires, shooting, propylene glycol poisoning, drowning, and neoplasia.

In the first 2 yr after releases began in 1992, five of eight released birds were killed in powerline collisions, so in 1994 we began a powerline aversion program for all pre-release birds, which appears to have successfully reduced powerline collisions as a significant cause of mortality, with only four powerline deaths documented in the last 10 yr. Currently, lead toxicity ranks as the largest threat to California condors. Birds appear to be at increased risk for lead poisoning once they reach sexual maturity at approximately 6 yr of age and begin foraging for food on their own. From 1999-2002, lead toxicity was the cause of death in 21% of recovered carcasses, and we suspect that the cause of mortality in a significant percentage of the unrecovered carcasses was likely due to lead toxicity, given the social feeding habits of this species, as well as the

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numbers of birds known to be mature and foraging for food on their own at the time they died. Lead toxicity is not a new threat: it is thought to have been a significant contributing factor to the demise of the California condor in the wild, with three cases reported in the early 1980s.<sup>1</sup> A comprehensive monitoring, screening and treatment program for lead toxicity was instituted for wild condors, and a multiagency task force and the U.S. Fish and Wildlife Service (USFWS) are proceeding with hunter awareness programs to educate the public about ways to eliminate this threat by retrieving or burying carcasses or gut piles and using lead-free ammunition.

#### **ACKNOWLEDGMENTS**

The authors wish to recognize the field biologists of the USFWS, Peregrine Fund and Ventana Wilderness Society who work hard in difficult conditions to monitor and care for these birds in the wild, and who perform the often difficult task of immediately recovering dead birds for pathology. We thank the pathologists at the San Diego Zoo for their contributions to this data, Ron Jurek at the California Department of Fish and Game for keeping meticulous track of population size and distribution. We wish to thank the veterinary and animal care staff of the breeding facilities at the Los Angeles Zoo, San Diego Wild Animal Park and World Center for Birds of Prey for their efforts in providing healthy and behaviorally well-prepared birds for reintroduction to the wild. Special thanks to the members of the California Condor Recovery Team and its contributors who continue to actively and tenaciously manage this endangered species.

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**EVALUATION OF AN ORAL AND FOOD-BASED CANARYPOX-VECTORED CANINE DISTEMPER VACCINE IN ENDANGERED CHANNEL ISLAND FOXES (*Urocyon littoralis*)**

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**Abstract**

The Channel Island fox (*Urocyon littoralis*) was listed as endangered under the Federal Endangered Species Act on March 5, 2004 due to serious declines in populations of four subspecies over the last decade.<sup>1</sup> One of the four listed subspecies, *U. littoralis catalinae*, is present on Santa Catalina Island, where the population declined precipitously and dramatically by approximately 90% in 1999. This decline was believed to be the result of an outbreak of canine distemper.<sup>5</sup> Current population recovery efforts on Santa Catalina Island include a captive breeding program, and monitoring and vaccination of both captive and wild populations with a recombinant canarypox-vectored canine distemper vaccine (Purevax Ferret™, Merial, Inc., Athens, GA 30601). In 2001 and 2002, the vaccine was given intramuscularly to wild and captive foxes. Disadvantages of administering the vaccine by injection included: stress of capture and handling on the animals; potential for local or systemic reactions to the injected vaccine; costs for staff and staff safety concerns; and significant time delays between the initiation of the vaccination effort and achievement of vaccination of a significant portion of the population. Although antibody levels as measured by serum neutralization indicated that vaccine response occurred with intramuscular administration of the vaccine, the disadvantages of administering the vaccine by injection prompted us to investigate oral administration as an alternative.

Vaccine-laced bait has been used to effectively reduce labor costs, capture stress, and time required to accomplish vaccination in certain wildlife disease management efforts.<sup>2-4,9</sup> In 2003 we orally administered Purevax Ferret™ to 16 captive-raised juvenile island foxes. Two 1-ml vials of the vaccine were administered to each fox at approximately 16, 20, and 24 wk of age by one of two different oral routes: 1) direct administration of the rehydrated vaccine solution into the oral cavity, and 2) injection of the vaccine into a food item fed directly to the fox. Group selection was determined randomly within each of six pens housing foxes. Blood samples were collected from foxes to determine distemper antibody titers at 1, 3, and 5 mo post final vaccine administration (p.v.). Serum neutralization to measure antibody titers was conducted by the Cornell University Veterinary Diagnostic Laboratory. Foxes were sampled while still in captivity at 1 mo p.v., and the 3- and 5- mo p.v. samplings occurred after the juvenile foxes were released

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to the wild. One fox was subsequently removed from the study due to management considerations. All 15 foxes remaining in the study were captured for re-sampling at least once post-release. At 1 mo p.v., 13/16 foxes (81.3%) showed measurable antibody response to the vaccine (>1:6). Mean antibody titers were statistically significantly higher in the direct administration group ( $P = 0.003$  student t test mean  $\log_{10}$ ). Two foxes had interfering substances that rendered the first antibody measurement invalid. Initial antibody titers achieved in our study were consistent with levels demonstrated to be protective in some vaccine challenge studies in species with similar sensitivity to canine distemper virus.<sup>6-8</sup> Measurement of antibodies in samples obtained at 3 and 5 mo p.v. are underway, and antibody persistence in our two study groups is being evaluated and compared to previous years when the vaccine was given intramuscularly. Based on our initial findings, we believe direct oral administration of Purevax Ferret™ to be a viable option for vaccinating Channel Island foxes, but a better bait formulation may be necessary for food-based vaccination to be effective.

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## DEBILITATED LOGGERHEAD TURTLE (*Caretta caretta*) SYNDROME ALONG THE SOUTHEASTERN US COAST: INCIDENCE, PATHOGENESIS, AND MONITORING

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### Abstract

In 2003, there was a perceived increased occurrence of emaciated and barnacle-laden loggerhead turtles (*Caretta caretta*) found stranded (both dead and moribund) along the southeastern US Atlantic coast. To investigate this situation further, the Wildlife Conservation Society's St. Catherines Island (SCI) Wildlife Survival Center (WSC) and the Georgia Department of Natural Resources organized a workshop on SCI in November of 2003. Fifteen people attended including: turtle biologists from Florida, Georgia, South Carolina, and North Carolina, veterinarians, toxicologists, immunologists, and representatives from the National Oceanic and Atmospheric Administration (NOAA).

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The group determined that there was an increasing trend in strandings of debilitated sea turtles from 1992-2002 (approximately 11% annual increase). The number of debilitated turtles appeared to increase substantially in 2003 (NC 3%, SC 22%, GA 10%, and FL 20.2% of the total turtle strandings). The species composition of debilitated sea turtle strandings was primarily loggerheads, but a few green (*Chelonia mydas*), Kemp's ridley (*Lepidochelys kempii*) and possibly, in Florida, a hawksbill (*Eretmochelys imbricata*) turtles were affected. Temporally, the stranding of debilitated turtles occurred all year in Florida; however, strandings were found to be concentrated in the spring and summer (April through July) in the other states. Spatially, debilitated sea turtles were stranded across the southeastern US coastal region, there were areas of high stranding density in the southern part of North Carolina, the northern part of South Carolina (Georgetown and Horry Counties) and around Cape Canaveral in Florida (Brevard County). Many explanations for stranding patterns were discussed including ocean currents, winds, and cold-stunning events.

A debilitated turtle was defined as emaciated with small barnacles covering the skin. The flippers can also have lesions or be necrotic. While heavy epibiota can be a normal finding on the carapace and plastron of healthy loggerhead sea turtles, the skin is generally free of these commensals. Health assessment and necropsy data from these cases indicated the turtles were being affected by a wide range of secondary bacterial and parasitic infections with the primary cause still to be determined. Seven debilitated turtles showed significantly higher blood levels of polychlorinated biphenyls (PCBs) and organochlorine pesticides compared to apparently healthy turtles (Keller, et al., unpublished data). In a separate study, mercury concentrations in blood and scutes were 2 to 3 times higher in dead stranded turtles compared to live, apparently healthy turtles although the sample size was small (R. Day, unpublished data). It is still unclear at what levels these compounds become toxic to sea turtles. The high contaminant levels could be a secondary effect as debilitated turtles use up their fat reserves, causing organic contaminants to become concentrated in blood.

The group determined several areas that need to be addressed in 2004. First, a complete statistical analysis of debilitated sea turtle stranding trends (NMFS-Sea Turtle Stranding and Salvage Network Database) is needed to better define the extent of the problem. This analysis will assist in determining if there was a substantial and statistically significant increase of stranded debilitated turtles in 2003. Possibly the strandings correlate with overall increases in off shore populations. Ongoing studies at the St. Lucie Power Plant in Florida, indicate a significant increase in loggerhead sea turtle populations. The average annual captures of this species from 1992 to 2002 was 275, while in 2003, 538 turtles were captured. Stranding reporting protocols will be reviewed to ensure that debilitated turtle strandings can be accurately assessed. In the past, not all strandings were examined for signs of debilitation. Thus the percentage of debilitated turtles should be expressed as a proportion of turtles examined, not total strandings.

In order to provide consistent, standardized documentation on debilitated turtles that strand in 2004, protocols are being developed to include: visual assessment, physical examination,

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morphometrics, clinical pathology, contaminant analysis, immune function tests, gross necropsy and histopathology.

#### **ACKNOWLEDGMENTS**

We would like to thank the St. Catherines Island Foundation and the WSC for hosting the meeting on St. Catherines Island.

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## 101-YEAR REVIEW OF REPTILIAN NEOPLASIA AT THE PHILADELPHIA ZOOLOGICAL PARK

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### Abstract

A retrospective review of neoplasia in the reptile collection of the Philadelphia Zoological Park was conducted for the years 1901-2002. Necropsy reports were reviewed and all incidents of neoplasia were identified. Cases were histologically reviewed by one author (JGT) for confirmation of the original diagnosis. For a small number of cases, neither slide nor tissue blocks could be found. These cases were included in the study using the original diagnosis.

Incidence rates were calculated using the total number of reptile necropsies. The total number of necropsies before 1967 was obtained from a previous review, and manually counted for cases from 1968-2002.<sup>3</sup> To reduce the influence of sporadic losses of large clutches of neonates, cases determined to have died from “failure to thrive” (after 1967) were eliminated and the incidence re-calculated (Table 1).

The incidence of neoplasia in this collection varied considerably by taxa and time. No neoplasms were diagnosed in crocodylians. Chelonians had relatively low rates when compared to lizards and snakes. Though the overall incident rates are comparable to other reviews, the rates have gradually increased in both lizards and snakes over the 101-yr period.<sup>4-6</sup> The cause for this increase is unclear, though longer life spans, more thorough necropsies, and infectious agents are potential explanations.<sup>2</sup> A similar upward trend has been reported in snakes by another institution.<sup>7</sup>

A total of 94 neoplasms were found in six chelonians, 19 lizards, and 63 snakes. Two turtles, one lizard, and three snakes had multiple neoplasms. The locations of primary neoplasms are reported on Table 2. Overall, the liver was most often affected (22%), a finding common to previous studies, followed by the integument (15%) and endocrine systems (12%).<sup>4,7</sup> The relative frequency of hematopoietic neoplasia was low (6%) in comparison to reports in other collections.<sup>1,4</sup>

Continued surveillance and further investigations into the etiology of the various types of neoplasia found in this study is warranted.

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**Table 1.** Incidence of neoplasia in reptiles at the Philadelphia Zoo for 1901-2002. Results are reported as a percentage of neoplasms diagnosed/necropsies performed.

	Chelonians		Lizards		Snakes		All Reptiles	
	TIR <sup>a</sup>	AIR <sup>b</sup>	TIR	AIR	TIR	AIR	TIR	AIR
1901-1967	5.4	NC <sup>c</sup>	0.0	NC	1.2	NC	1.3	NC
1968-1979	0.0	0.0	0.6	0.7	2.7	3.4	1.8	2.2
1980-1991	2.4	2.4	3.0	4.1	4.0	5.5	3.4	4.5
1992-2002	0.0	0.0	4.7	5.4	6.5	10.2	4.5	5.7
All Years	1.6	1.6	2.3	2.7	3.0	3.6	2.6	3.0

<sup>a</sup>Total incidence rate.

<sup>b</sup>Adjusted incidence rates, reflecting the removal of failure-to-thrive cases (as defined as an animal less than 1 yr old with no post mortem diagnosis other than “inanition” or “starvation”, and with no recorded evidence of feeding).

<sup>c</sup>Not calculated.

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**Table 2.** Location of primary neoplasms (by body system) at the Philadelphia Zoo for 1901-2002.

Location	Chelonians	Lizards	Snakes	All Reptiles
Pulmonary	-	-	1	1
Circulatory	-	1	2	3
Endocrine	1	2	8	11
Liver	1	7	13	21
Gastrointestinal	1	2	7	10
Reproductive		1	6	7
Urinary	2	1	5	8
Integument	2	2	10	14
Musculoskeletal	-	-	1	1
Disseminated	-	1	5	6
Undetermined	1	3	8	12
Total	8	20	66	94

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**END TIDAL CARBON DIOXIDE VALUES FROM PASSIVE EXHALATIONS FROM PACIFIC WHITE-SIDED DOLPHINS (*Lagenorhynchus obliquidens*) AND BELUGA WHALES (*Delphinapterus leucas*) AT THE JOHN G. SHEDD AQUARIUM**

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**Abstract**

Marine mammal respiratory physiology differs from terrestrial mammals in that breath-holding results in oxygen storage challenges. These animals rely on hemoglobin and myoglobin oxygen storage while the alveoli collapse under pressure. As breath is held longer (sometimes for extended minutes) hypoxia increases with a concomitant increase in carbon dioxide.<sup>1</sup> Capnometers non invasively measure expired carbon dioxide (CO<sub>2</sub>), a by-product of metabolism excreted via the lungs, in millimeters of mercury. Although capnometers are usually used for anesthetic monitoring, another use is point of care testing to determine the ventilatory status of a non-anesthetized patient. A pilot study was developed to monitor baseline end tidal carbon dioxide (ETCO<sub>2</sub>) values for individual cetaceans at the Shedd Aquarium.

Pacific white-sided dolphins (*Lagenorhynchus obliquidens*, n = 5) and beluga whales (*Delphinapterus leucas*, n = 5) at the Aquarium were monitored via a Microcap<sup>®</sup> handheld capnometer (Oridion Capnography, Inc., Needham, Massachusetts 02494 USA). Many new capnometers can be used on non-intubated patients by placing the probe within the exhaled stream of gases. For this study, the probe was held above the blow hole to capture passive exhalations while the animal was at rest in a voluntary sternal “layout” position in the water. The dolphins participated in 15-18 sessions and the belugas in 10-11 sessions each. Each session consisted of capturing ETCO<sub>2</sub> from 2 consecutive passive exhalations per animal, yielding between 30-36 exhalations per dolphin and 20-22 exhalations per beluga. The mean ETCO<sub>2</sub> was generated for each individual animal for each session; all sessions were then combined for each animal and an overall mean ETCO<sub>2</sub>, standard deviation (SD) and standard error of the means (SEM) was derived per animal.

Since there are no published reference ranges for these species, the information in this study has provided the Aquarium with individual animal reference ranges of ETCO<sub>2</sub>. This information will be used to monitor the pulmonary health of each cetacean at the Aquarium, and as a baseline to compare to during times of illness.<sup>2</sup> In the future, cetacean population norms for ETCO<sub>2</sub> could be achieved if multiple institutions within the aquarium community participated in a larger study.

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## ***Salmonella arizonae* OSTEOMYELITIS IN A COLONY OF *Crotalus willardi*: AN ARGUMENT FOR VERTICAL TRANSMISSION**

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### **Abstract**

A long-term study of osteomyelitis in a colony of ridgenose rattlesnakes (*Crotalus willardi*) found a single *Salmonella arizonae* serotype, 56:Z4,Z23, cultured from affected bone in all but one animal.<sup>3</sup> The latter animal had *Providencia rettgeri* cultured from all tissues examined. In contrast this serotype was only grown once from the bowel (50 cloacal cultures and 3 intestinal cultures). All other *Salmonella* isolates belonged to subspecies *diarizonae* (14 serotypes) or *enterica* (two serotypes).

With serotype 56:Z4,Z23 being isolated so commonly from bone and not from the gastrointestinal tract, it seems likely that the organism is spread by a means other than the fecal-oral route. This idea is supported by the colony's husbandry, which generally keeps snakes isolated, except during the breeding season. Bacterial cultures of reproductive tracts at necropsy (n = 8) and non-fertilized yolk masses (n = 2), yielded nine serotype 56:Z4,Z23 isolations, in either pure culture or in mixed cultures. The testis of one snake grew *Salmonella diarizonae* 48:I-Z, but this organism was also isolated from the intestine of that snake. Similarly, five of six blood cultures grew *Salmonella arizonae* 56:Z4,Z23. Due to limited reproduction and the dispersal of the majority of progeny from this colony, determining if off-spring developed osteomyelitis cannot be made. Transmission of *Salmonella* spp. *in-utero* has been documented in chickens, snakes, and turtles.<sup>1,2,4</sup> Our preliminary data suggests the organism responsible for the bony lesions is transmitted to off-spring from the mother in-utero.

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## PHARMACOKINETICS OF FLORFENICOL AFTER A SINGLE INTRAMUSCULAR DOSE IN WHITE-SPOTTED BAMBOO SHARKS (*Chiloscyllium plagiosum*)

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### Abstract

Florfenicol is an often-used antibiotic in fish medicine, however little is known of its pharmacokinetics in these species. Pharmacokinetic studies in Atlantic salmon (*Salmo salar*) demonstrate a high bioavailability, a significant degree of tissue penetration, and high efficacy.<sup>1-3</sup> However, florfenicol pharmacokinetic data in red pacu (*Colossoma brachypomum*) indicated rapid elimination, necessitating high dosages (estimated at 20-30 mg/kg) administered every 24 hr.<sup>4</sup> The purpose of this study was to evaluate the pharmacokinetic properties of florfenicol in shark species, represented here by the white-spotted bamboo shark (*Chiloscyllium plagiosum*), and to quantify the concentration of florfenicol (bioavailability) in plasma and cerebrospinal fluid (CSF) after a single intramuscular injection in determination of its efficacy and longevity in treating a bacterial meningitis. This study is the only known report on florfenicol pharmacokinetics in a shark species and the only known report on florfenicol pharmacokinetics in CSF of a fish species.

A pilot study was performed with three sharks to determine an adequate therapeutic dose of florfenicol based on the approximate peak and duration of a single dose given intramuscularly. A therapeutic dose was defined in this study as one that provided plasma levels within the range of 4-8 µg/ml, based on the mean minimum MIC for the most common pathogenic organisms using NCCLS breakpoints, maintained throughout the dosing interval.<sup>5</sup> Plasma concentrations peaked at around 36 hr. This time was used to determine CSF sampling times for the study in order to minimize anesthesia time and CSF loss. From this data, the study dosage of 40 mg/kg was determined; a dose that maintained plasma levels above the MIC mark of 4-8 µg/ml and appeared "safe" enough to allow for accumulation when administering multiple doses. A 72-hr dosing interval was expected. Ten adult white-spotted bamboo sharks (five males and five females) were used for the pharmacokinetic study of florfenicol. On the first day of the study, each shark was anesthetized in 50 ppm tricaine methanesulfonate (MS-222<sup>®</sup>, Argent Chemical Laboratories, Redmond, Washington 98052 USA), examined, and weighed. "Pretreatment" (0

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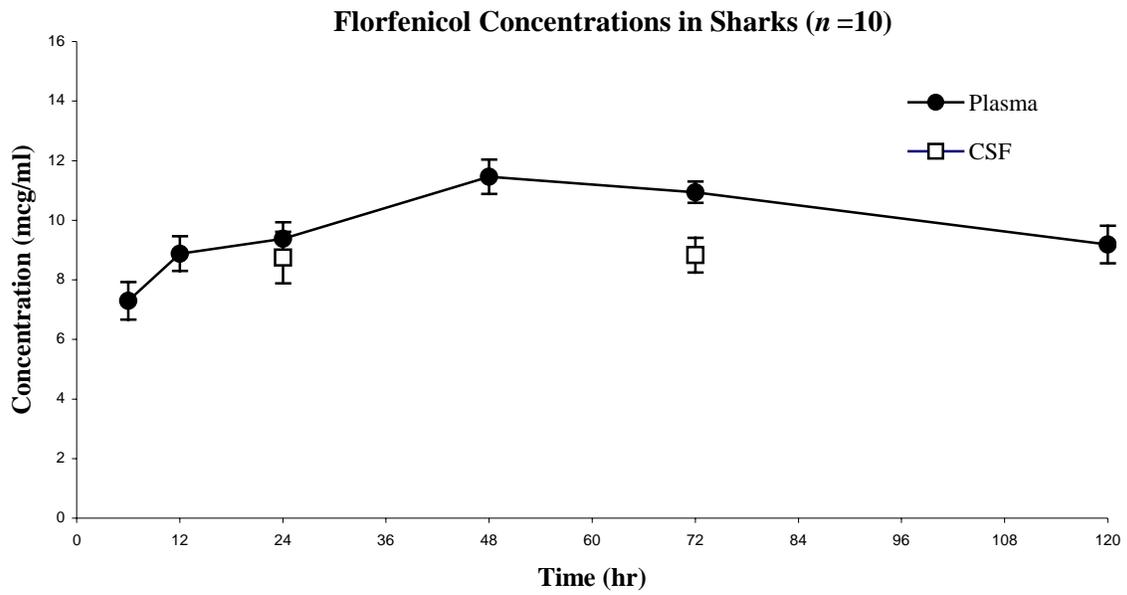
hr) plasma and CSF samples were collected, and each shark then received a single dose of florfenicol (Nuflor<sup>®</sup>, Schering-Plough, Union, New Jersey 07083 USA) at 40 mg/kg, which was administered deep intramuscularly (primarily delivered into white muscle) craniolateral to the dorsal fin. Blood samples were collected under manual restraint, except when CSF was collected in which case anesthesia was used. Blood samples were obtained from the caudal vein at 0, 6, 12, 24, 48, 72, and 120 hr post-injection via a 22-ga, 1-inch needle. CSF samples were collected under anesthesia at 0, 24, and 72 hr post-florfenicol administration via a 22-ga, 1-inch needle inserted at the soft depression on top of their head, medial and slightly caudal to the eyes. Quantitative analysis was performed on the samples through high performance liquid chromatography (HPLC) to determine florfenicol concentrations (Fig. 1). Florfenicol plasma levels appeared to peak at around 48 hr, and were maintained well above the target MIC of 6 µg/ml for 120 hr. CSF concentrations mirrored plasma concentrations, also surpassing the target MIC for a minimum of 72 hr. These results warrant the use of florfenicol as a primary choice in the treatment of systemic and CNS infections in sharks and possibly other fish species.

#### ACKNOWLEDGMENTS

The authors wish to thank the aquarists at the Henry Doorly Zoo including curator Sean Putney, the veterinary hospital staff including Gerald Huckmeier, Nicole Linafelter, Andrew DeJong, Jon Timmerman, and the staff at the North Carolina State University Clinical Pharmacology Laboratory for their assistance.

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**Figure 1.** Florfenicol concentrations versus time.

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## EFFECTS OF MALATHION ON INFECTIOUS DISEASE SUSCEPTIBILITY AND THE IMMUNE SYSTEM OF ENVIRONMENTAL INDICATOR SPECIES

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### Abstract

There is increased concern about the sublethal effects of organophosphorus (OP) pesticides on human and animal health.<sup>11,17</sup> Malathion, an OP compound, is one of the most widely used pesticides, applied to the environment at an annual rate of 4,486,000 ha in the United States alone.<sup>13</sup> It is used most commonly in the control of mosquitoes, flies, household insects, animal ectoparasites, and human lice. Malathion has been labeled with carbon, phosphorus, and sulfur and applied to fields to study its potential translocation and bioaccumulation.<sup>10</sup> Small rodents, insects and birds had detectable levels 1 yr after treatment.<sup>10</sup> While the most studied toxic effect of malathion is on cholinesterase in the nervous system, only a few studies have been conducted on its toxic effect on the immune system. Hermanowicz and Kossmam (1984) observed that humans occupationally exposed to OP compounds have marked impairment of neutrophil chemotaxis and had increased frequency of upper respiratory infections proportionate to the number of years of exposure to organophosphorus compounds.<sup>9</sup> Dulout, et al. (1983) demonstrated a dose-response relationship to malathion induced chromosomal aberrations in mouse bone-marrow cells.<sup>6</sup>

Taylor, et al. (1999a) published a model that demonstrated increased infectious disease susceptibility and mortality in Woodhouse's toads (*Bufo woodhousi*) externally exposed to field doses of an organophosphorus pesticide.<sup>14</sup> Amphibians were selected as the model species for investigation because they are considered highly sensitive, environmental health indicator species that inhabit the aquatic and terrestrial interfaces.<sup>14, 3, 2, 15</sup> Worldwide amphibian diversity and population numbers have been reported to be declining.<sup>14, 18, 16</sup> Pesticides are sometimes implicated yet few studies have been conducted to determine if and how pesticides actually present a hazard to them.<sup>7</sup> In addition, most published studies on the effects of pesticides on amphibians have been conducted on embryo and tadpole life stages.<sup>8, 4, 12, 5, 1</sup>

This current project further studies these initial findings on two environmental indicator species of large anurans, the bullfrog (*Rana catesbeiana*) and the marine toad (*Bufo marinus*), which inhabit widely different environments. The research showed an increased susceptibility to

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bacterial infection in these amphibian species following pesticide exposure, strongly suggesting an effect on immune suppression that could correlate with reported effects in humans. This work demonstrates the need to integrate human and wildlife health research and models to better assess potential ecological risks that could result in effects to both humans and wildlife.

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The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

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## ENVIRONMENTAL FACTORS INFLUENCING THE GROWTH OF *Uronema marinum* AND POTENTIAL TREATMENT MODALITIES FOR URONEMIASIS

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### Abstract

Uronemiasis is a disease of warm and cold water marine fishes caused by opportunistically pathogenic scuticociliates of *Uronema* sp. The disease can be devastating in aquaria and aquacultural environments where *Uronema marinum* is most often incriminated as the causative agent. Little is known about the prevention, diagnosis, and treatment of disease caused by this organism. Thus, the objectives of our studies were 1) to identify potential environmental factors associated with uronemiasis using an in vitro system and 2) to identify potential treatment modalities for uronemiasis using an in vitro system. For the first objective we looked at the survival, proliferation, and behavioral responses of *U. marinum* to temperature, salinity, and pH. *U. marinum* survived at a wide variety of specific gravities, pH's, and temperatures. However, low specific gravity and warm temperatures severely depressed growth and low pH caused rapid death. Thus, changes in water specific gravity, temperature, and pH potentially could be used to control the proliferation of *Uronema*. For the second objective *U. marinum* was cultured with various concentrations of formalin, methylene blue, malachite green, metronidazole, chloraquine, and H<sub>2</sub>O<sub>2</sub>. Formalin, malachite green, chloraquine, and H<sub>2</sub>O<sub>2</sub> all appeared useful for killing *Uronema* in water and on the surface of fish as long as the appropriate doses and treatment durations were used. H<sub>2</sub>O<sub>2</sub> was a very effective water treatment for killing *Uronema* at concentrations as low as 250 ppm. Chloraquine has potential as a systemic drug for the parenteral treatment of uronemiasis in individual fish.

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## TECHNIQUES USING VET BIOSIST™ IN AQUATIC ANIMALS

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### **Abstract**

Aquatic animal wounds are challenging to manage by the nature of the environment in which they live. Large open wounds are not conducive to bandaging and apposition of wound edges is often impossible. A xenograft, such as BioSisT™, provides an extracellular matrix in areas that are devoid of the natural scaffolding that is required for normal healing. This paper discusses how to use this product in a wet environment and sites examples of successful outcomes.

### **Introduction**

BioSIST™ is a non-reactive extracellular matrix produced from the swine small intestine submucosa (SIS) (Vet BioSIST™, Global Veterinary Products, Inc., New Buffalo, Michigan 49117 USA). The matrix contains essential collagens and growth factors, as well as the framework required for cell migration during wound healing. SIS has been used in small animal wound management for several years with numerous citations; its use in non-domestic species, however, is focused on laboratory animals used for assessment of human surgical procedures.<sup>4</sup> A few reports are available on its use in birds and, in one case, a reptile, but no reports of its use on aquatic animals were found through the authors' literature searches.<sup>1,2,4,5</sup>

### **Application/Technique**

#### **Fish**

Piscine skin has incredible and novel healing mechanisms. Recently, an excellent review on fish wound healing and management has become available and should be consulted.<sup>3</sup> Some large wounds are beyond the normal healing capacity of the animal. These lesions present a critical situation as fish rely heavily on intact skin to maintain osmotic balance. In these cases, SIS provides an excellent medium for providing a barrier against osmotic loss and for laying down tracks for cells to migrate.

SIS has a short half-life in water. In the author's experience, within 4 days most of the SIS has disintegrated except at suture sites. This may require re-application, the need to use smaller pieces tacked down over the entirety of the wound, or the placement of secondary barrier material over the SIS. Barriers can include Tegaderm™ (3M Center, St. Paul, Minnesota 55144 USA) or BioDres® (DVM Pharmaceuticals, Miami, Florida 33169 USA). All of these methods

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have been used successfully, but due to the variable nature of each wound, it is difficult to recommend a single way to use SIS.

### *General Recommendations*

Maintain the animal out of water while flowing water over the gills (for general anesthesia principles, see other texts). Debride wounds well. Teleost epidermis is not vascularized, therefore bleeding is not a good indicator of exposing healthy tissue, rather, tissue texture must be used to assess viability.<sup>3</sup> Cut and place the dry sheet of BioSIST™ over the wound. Use simple interrupted sutures to tack the edges of the material around the wound, then randomly place stay sutures in the center of the cut piece of SIS.

Note: When opting to use secondary barrier material as mentioned above, it should be placed and tacked down simultaneously with the SIS. If BioDres® is chosen as a secondary barrier, sutures should be tied loosely since the BioDres® material imbibes water and expands several millimeters. The authors have tied the tacking sutures over a hemostat in order to allow room for the expansion. Tight sutures will constrict (it imbibes with water) the BioDres® material and it will fall off.

### *Select Cases*

1) An adult, 383 g blackspot puffer (*Arothron nigropunctatus*) with a 5 cm white, raised wound on the peduncle. Examination of the wound revealed extensive necrosis over 30 % of the lateral body wall and exposure of muscle. Mucous smears were negative for infectious organisms and previous aggression in the enclosure suggested bite-related trauma. SIS was tacked in place over the entirety of the wound with numerous stay sutures in the center. The animal was treated with antibiotic and fluid-injected foods for several days. Handling was minimized to avoid disruption of the product. Within days the SIS sheet was disrupted but numerous fragments were attached to each suture site. Early epithelialization was noticeable. By the second week, normal pigmentation, including markings became obvious; examination after immobilization revealed apparently normal tissue.

2) An adult, 1.6 kg laced moray eel (*Gymnothorax favagineus*) with conspecific trauma to the mandible.<sup>5</sup> Examination of the wound revealed a symphyseal fracture and 2 cm of tissue loss on the mandible resulting in exposure of the bone. SIS with an overlay of tegaderm was sutured into available tissue; the fracture was stabilized with suture material. At week 1 there was a thin layer of granulation tissue over the bone. Closer examination revealed that the tegaderm was in place but only remnants of SIS were still present. At week 3 there was a thick granulation bed present. At 2 mo, the animal had one section of “scar” tissue, while the surrounding tissue was pigmented with normal markings.

3) A juvenile, 1.3 kg forktail Lates (*Lates microlepis*) with a descemetocoele. SIS was sutured over the descemetocoele with fine suture material. Gentamycin and flurbiprofen drops were

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placed on the eye once daily. The SIS material was unfortunately dislodged during capture at day 2, but there was sufficient healing for the corneal epithelium to have sealed the perforation. Keratotomy was performed 1 wk later because there significant defect present. Currently, the animal exhibits minor corneal scarring. This outcome was extremely successful because without intervention corneal lesions can become advanced and can result in complete perforation and loss of the eye. In other fish, minor corneal perforations and descemetocoeles have also been successfully treated using SIS provided that the lesions were not progressive or extensive.

4) A 17 yr 55 kg Queensland grouper (*Epinephalus lanceolatus*) with a surgical excision of a neoplasm. The surgery resulted in a significant defect in the forehead exposing 20 × 10 cm of muscle. A 70 × 100 mm sheet of SIS was tacked into place over the entire wound which exposed muscle fascia. Granulation tissue was observed within the first week over at least 50% of the wound, mostly at the periphery. The following week the entire wound was covered. Over the course of several weeks later, these layers thickened and ultimately pigmented tissue was laid down over the entire site.

#### Aquatic Turtles: Shell Lesions

In most shell lesions, long term bandaging techniques (as with epoxy) are frequently employed. By placing SIS in freshly debrided lesions, moistening with sterile saline and then sealing either with epoxy or bone wax (Ethicon, Johnson and Johnson, Somerville, NJ 08876-0151 USA), defects seem to heal several weeks sooner than animals where the product was not utilized.

#### Select Cases

Ten juvenile red-bellied short-necked turtles (*Emydura subglobosa*) developed significant bacterial shell abscesses secondary to overcrowding and lack of basking. These lesions resulted in large defects often extending to the coelomic membrane. Defects in four turtles affected 30-40% of the plastron, smaller abscesses were found in the carapace. The lesions were managed medically until the infectious process seemed to be resolved. After light debriding, SIS was used as described above followed by an epoxy 'bandage'. Animals undergoing this procedure had calcification within 2 mo after treatment. It is possible that healing occurred more rapidly, but the epoxy was not removed until a scheduled 2 mo evaluation.

#### Aquatic Birds

The product has been used on a penguin with pododermatitis at the Aquarium. Surgery was performed on the animal's plantar surface. The SIS was placed deep within a debrided area and sealed over by apposing surgical edges. The animal recovered well in a dry enclosure and has had no recurrence of pododermatitis lesions.

Successful use of SIS in aquatic animals is contingent on the following:

- Appropriate preparation of wounds

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- Resolution of any infectious or inflammatory process
  - Avoiding disruption of the SIS while tacked onto lesion (as with nets or handling)
  - Under some circumstances, a second covering may prolong contact and decrease disintegration rate (note that these may become foreign bodies if they fall off)
  - Reapplication may be necessary
  - Close attention to the osmoregulatory status of teleosts is needed

## **Summary**

All of the described cases are highly subjective based on the author's observations. Most lesions had an incredible expanse or severity that was not, in the author's opinion, conducive to healing. In these cases, the author felt BioSisT™ was extremely important for the full recovery of these animals. To further document the effectiveness of BioSisT™ in aquatic animals, a future direction will include a controlled study examining wound healing with and without the product.

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## ORAL LESIONS AND THEIR TRANSFORMATION TO SQUAMOUS CELL CARCINOMA IN ATLANTIC BOTTLENOSE DOLPHINS, *Tursiops truncatus*

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### Abstract

Four Atlantic bottlenose dolphins (*Tursiops truncatus*) housed together for 18 yr developed oral lesions over a period of 16 yr. Two of the four animals (case 1 and case 2) were diagnosed with malignant oral squamous cell carcinoma (SCC) in 1999, 4 yr prior to their deaths. However, mouth lesions were first observed in these dolphins in 1987. The lesions likely underwent transformation in 1996 when rapid growth was observed. In the first case, treatments included laser, cryotherapy, intra-lesional chemotherapy, and brachytherapy.<sup>14</sup> The tumor invaded the entire inter-mandibular space and was too extensive at the time of diagnosis to attain a cure. However, partial remission of the primary tumor was achieved. In the second case, the lingual SCC tumor was excised and did not reappear.<sup>15</sup> Multi-focal 1-2 mm reddened, raised lesions did recur, however, and were frozen using liquid nitrogen. On necropsy both dolphins had extensive metastasis. In case 1, metastasis were found in numerous foci of the oral cavity, lung, urinary bladder, lymphatic vessels of the bladder, kidney, lymph nodes of the kidney, adrenal gland, pericardial sac, and oviduct and associated lymphatics. In case 2, metastasis was evident in the lung, lymph nodes of the lung, pleura, diaphragm, pre-scapular lymph nodes, and mediastinal lymph nodes. The remaining two cases (case 3 and case 4) were biopsied. Histopathology results in case 3 showed papillary hyperplasia with cellular atypia, while case 4 demonstrated multifocal mild acanthosis with pseudoepitheliomatous hyperplasia. The dolphins oral lesions were treated using laser or cryotherapy.

Neoplasia has been increasingly documented in cetaceans. Benign as well as malignant tumors of the skin, oral cavity, gastro-intestinal tract, pancreas, liver, lung, kidney, adrenal glands, bladder reproductive organs, spleen, lymph nodes and brain have been documented.<sup>2-4,6,7,9-12,14-17,22</sup> SCC of the skin, oral cavity, and lung have been previously reported in dolphins.<sup>3-5,14,16</sup> Initiators and promoters of squamous cell carcinoma are likely multi-factorial and complex. Genetics, immune status, UV radiation, hormonal influences, irritants, environmental contaminants and viruses have been implicated.

Papillomaviruses and possibly herpesviruses are possible causative agents in the development of oral tumors in case 1 and case 2. In Situ hybridization was positive for the human papillomavirus antiserum in case 2. In case 1, In Situ hybridization tests were equivocal. The virus was not observed on electron microscopy. Other molecular pathologic studies are on-going

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to confirm the presence of papillomavirus in the tumors. The papillomavirus has been isolated in harbor porpoises (*Phocoena phocoena*), dusky dolphins (*Lagenorhynchus obscurus*), long snouted common dolphins (*Delphinis capensis*), bottlenose dolphins (*Tursiops truncatus*), Burmeisters porpoises (*Phocoena spinipinnis*), and in the killer whale (*Orcinus orca*).<sup>1,19,20,21</sup> In humans, domestic cats and dogs, cattle, rabbits and snow leopards, there is a clear correlation between the papillomavirus and the development of SCC.<sup>8,13,18,22</sup>

The oral lesions observed in these two dolphins were first seen 16 yr prior to their death. They were slow growing and went through cycles of waxing and waning before becoming chronic in nature. Ulceration, bleeding, infection and discomfort were minimal until the last several years of their lives. It is important to immediately biopsy any oral lesions that persist longer than several weeks to look for malignant change. Following diagnosis, lesions should be excised and carefully monitored for reoccurrence.

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**SURVEY FOR NASAL CRYPTOCOCCOSIS IN A HERD OF CHINESE GORAL  
(*Nemorhaedus goral*)**

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**Abstract**

The San Diego Wild Animal Park maintains a herd of 11 Chinese goral (*Nemorhaedus goral*). They are housed in a single species outdoor exhibit 2.5 acres in size. A single adult male in the herd was noted to have a unilateral nasal discharge. Based on physical examination, hematology, serology, histology and fungal cultures from nasal lesions a diagnosis of nasal cryptococcosis was confirmed. This male is currently under medical treatment for *Cryptococcus neoformans* at the Harter Veterinary Medical Center. A survey was conducted to screen the remaining 10 goral for the presence of nasal cryptococcosis. The survey included physical examination, complete blood count, chemistry panel, bilateral nasal cytology, bilateral nasal fungal culture and latex cryptococcal antigen serologic testing. The survey showed that all 10 animals had favorably low or negative latex cryptococcal antigen titers. All of the animals appeared healthy with no nasal lesions observed. Two of the 10 animals cultured positive for *Cryptococcus neoformans* on nasal swabs. The results suggest that we currently do not have any goral in the exhibit with active nasal cavity disease. However, nasal colonization with this organism as demonstrated in two of the goral, can be considered a precursor for potential future nasal cavity disease. Historic pathology records from the San Diego Wild Animal Park document previous cases of *Cryptococcus neoformans* disease in southern pudu (*Pudu puda*), Hartmann's mountain zebra (*Equus zebra hartmannae*), cheetah (*Acinonyx jubatus*) and tufted deer (*Elaphodus cephalophus*).

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## POLYARTHRITIS IN MAXWELL'S DUIKERS (*Cephalophus maxwellii*) AT THE BRONX ZOO FROM 1982-2003

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### Abstract

A retrospective study on polyarthritis in Maxwell's duikers, *Cephalophus maxwellii*, was conducted at the Bronx Zoo from 1982-2003. Medical records from a total of 96 Maxwell's duikers were reviewed. Forty-eight of those animals reached at least 1 yr of age and remained at the Bronx Zoo (i.e., not transferred to another institution). Of the 48 animals, 13 animals, (four males and nine females) between the ages of 2 and 7 yr, presented with polyarthritis.

The polyarthritis manifest in the carpi and tarsi. No other joints were affected. Clinically, animals presented with carpal and/or tarsal swelling with minimal lameness until late in the disease process. In severe cases, luxations of the carpi developed that could not be stabilized with external coaptation. Radiographically, the disease was characterized by soft tissue swelling, joint space narrowing, erosion of articular surfaces, and new bone formation (periosteal reaction) along the joint margins. There were no differences in complete blood counts, serum biochemical analyses, mineral panels and serology, including tests for lentiviruses, *Borrelia burgdorferi*, and rheumatoid factor, between affected and non-affected animals. In most cases, arthrocentesis was performed for joint cytology and microbiologic culture with sensitivity. *Mycoplasma* sp. was cultured from one animal. Treatments included antibiotics, analgesics, and, in some cases, steroid therapy. No treatment proved successful. One affected animal currently remains in the collection, and all previous cases were humanely euthanatized due to the severity of the disease.

The cause of the polyarthritis in these cases remains undetermined. Results of radiographic examinations are consistent with immune-mediated polyarthritis/rheumatoid arthritis; however, this has not been verified by laboratory investigations.

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## MANAGEMENT OF ENTEROLITHIASIS IN A SOMALI WILD ASS (*Equus africanus somalicus*) AT THE SAN DIEGO WILD ANIMAL PARK

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### Abstract

Enterolithiasis has been reported in domestic horses in California and has been documented in several non-domestic equids at the San Diego Wild Animal Park (WAP). A laparotomy was performed on a pregnant 4.5-yr-old Somali wild ass (SWA, *Equus africanus somalicus*) with acute onset of severe abdominal pain. An enterolith, 10 cm in diameter, was removed from the transverse colon. Post-operatively, the animal experienced decreased appetite, decreased fecal output, dehydration, brief diarrhea, and a stillbirth. Following passage of the stillborn foal, the SWA improved rapidly and was released to the exhibit 60 days after surgery. Enterolithiasis has been documented in approximately 15 non-domestic equids at the WAP, and preventive measures such as dietary changes, water modifications and radiographic screening have been implemented.

### Introduction

Equine enteroliths are most common in California<sup>2</sup> and the southwestern US, including Texas.<sup>1</sup> Equine enteroliths are usually comprised of struvite (magnesium ammonium phosphate) crystals deposited in concentric layers around a non-digestible nidus.<sup>3</sup> In a review of 900 equine patients treated for enterolithiasis at the University of California Davis Veterinary Teaching Hospital,<sup>2</sup> enteroliths were present and associated with disease in 15% of all horses admitted for colic, and Arabians and Arabian crosses appeared over-represented. Stones were found most often in the right dorsal colon, the descending colon, and the transverse colon, and were both solitary and found in large numbers. While no dietary predisposition prior to admission was identified, recurrence of stones occurred significantly less frequently in horses that were fed less alfalfa hay. With a high magnesium component, alfalfa is theorized to increase magnesium content in the colon, as well as to alkalize colonic contents, thereby favoring the formation of struvite. Thus far, an adequate experimental model for enterolith formation has not been developed, and much information regarding etiology, prevention, and treatment remains unknown.

Enteroliths have also been reported in non-domestic Perissodactylids. Struvite enteroliths were successfully removed surgically in two female Grant's zebras (*Equus burchelli bohmi*) showing signs of colic in northern California after enterolithiasis was demonstrated at necropsy in other

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zebras of the same herd.<sup>4</sup> Enteroliths from South American tapirs (*Tapirus terrestris*) and a Malayan tapir (*Tapirus indicus*) were analyzed and were found to be primarily composed of vivianite and newberyite.<sup>5</sup>

In 1989, at the San Diego Wild Animal Park (WAP), an enterolith was surgically removed from an eastern kiang (*Equus kiang holdereri*) that was demonstrating signs of colic. Approximately 15 more cases of enterolithiasis have been identified between 1996 and 2003, predominantly in kiangs, but also in Somali wild asses (SWA, *Equus africanus somalicus*), Przewalski's horses (*Equus przewalskii*), a Persian onager (*Equus hemionus onager*) and a Grant's zebra.

### Case Report

A 252-kg, 4.5-yr-old female SWA presented with acute onset of abdominal discomfort in November 2002. She had no significant prior medical history. Anesthesia was induced with etorphine hydrochloride (0.02 mg/kg, i.m.) and acepromazine maleate (0.04 mg/kg, i.m.) via remote injection. Glycerol guaiacolate was administered i.v. to effect to facilitate intubation with a 26 mm endotracheal tube. Isoflurane and oxygen were administered and the patient was placed on a mechanical ventilator for the 2 hr procedure. An i.v. catheter was placed in the right jugular vein, and the patient was positioned and aseptically prepared for a ventral midline laparotomy. Abdominal exploratory revealed an enterolith, approximately 10 cm in diameter, in the transverse colon. Two enterotomies were performed to remove fecal material and the enterolith. The enterotomies were closed in two layers with 00-polydioxinone (PDS®) suture using a simple continuous pattern followed by a continuous Cushings pattern. A viable third trimester foal was palpated in the uterus. No other abnormalities were observed. The abdominal wall was closed in three layers. The abdominal fascia was apposed using #2 polyglactin 910 (Vicryl®) in an interrupted cruciate pattern. The subcutaneous tissue was apposed using 0-polyglactin 910 and the skin edges were apposed using stainless steel staples. Intraoperative treatments consisted of a balanced electrolyte solution (Lactated Ringers Solution [LRS], 8 liters, i.v.), flunixin meglumine (1.6 mg/kg, i.v.), ampicillin sodium (16 mg/kg, i.v.), and gentamicin sulfate (8 mg/kg, i.v.). Anesthesia was antagonized with diprenorphine (0.04 mg/kg, i.v.) and naltrexone (1 mg/kg, i.v.). The SWA appeared quiet and responsive the following morning (day 2). Post-operative phenylbutazone (6 mg/kg, p.o.) was prescribed once daily for 5 days.

Food was withheld for the initial 24 hr post surgery, but water was offered free choice. The SWA was placed on a standard WAP post-enterotomy dietary schedule (Table 1), to return to a regular diet by day 16 post-op. On day 4, she appeared moderately depressed, and had failed to eat or defecate since prior to surgery. Flunixin meglumine (1.2 mg/kg, i.m.) was administered, with no improvement noted following treatment. On day 5, she was anesthetized with etorphine hydrochloride (0.02 mg/kg, i.m.) and detomidine hydrochloride (0.04 mg/kg, i.m.) by remote injection. Propofol was administered in 3 separate 500 mg i.v. boluses to maintain anesthesia. On examination, mucus membranes were bright red and the SWA appeared 5% dehydrated. Fetid diarrhea was noted in rectum. Three fecal cultures were negative for *Salmonella* sp., and the diarrhea resolved quickly over the next 2 days. Abdominal ultrasonographic examination

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revealed a strong fetal heartbeat. An i.v. catheter was placed in the right jugular vein, 11 liters of a balanced electrolyte solution (LRS) were administered over 40 min, then administered at a rate of 750 ml/hr overnight. Flunixin meglumine (1 mg/kg, i.v.) was administered twice daily for 2 days. On day 6, the SWA had eaten a small amount of pellets. On day 7, she appeared brighter and more alert. On day 8, she was observed eating pellets and hay, and the catheter was removed under manual restraint. On day 9, labor was observed mid-day, and a stillborn foal was delivered. Following the stillbirth, the SWA improved steadily, with increasing appetite and fecal production noted.

The SWA was anesthetized again on day 45 for a hoof trim and recheck of the suture line. Anesthesia was induced with etorphine hydrochloride (0.023 mg/kg, i.m.) and detomidine hydrochloride (0.06 mg/kg, i.m.), and maintained with propofol (1 mg/kg, i.v.), and inhalation anesthesia using isoflurane and oxygen. Other than a firm swelling at the ventral abdominal suture site, no abnormalities were noted. Staples were removed, and anesthesia was antagonized with naltrexone (1.45 mg/kg, i.v.) and yohimbine (0.1 mg/kg, i.v.). The SWA was released from the hospital to a holding pen on day 46, and was released back to the exhibit on day 60. No problems have been noted since release, and the SWA recently gave birth to a healthy female foal.

## **Discussion**

The acute onset of severe abdominal discomfort in the SWA in this report is consistent with the majority of enterolith-associated colics observed in non-domestic equids at the WAP. With the history at the WAP, rapid surgical intervention is often selected, in place of diagnostics such as a complete blood cell count, a serum biochemistry panel, abdominocentesis, or abdominal radiographs. However, elective radiographic examination has become the cornerstone of the preventive screening program developed to manage enterolithiasis in non-domestic equids at the WAP. Between December 2002 and January 2004, 7 SWA and 10 kiangs were screened for the presence of enteroliths, using a four-quadrant approach described for domestic horses (Figure 1).<sup>6</sup> View 3 visualizes the right dorsal colon/ampulla coli, transverse colon, and proximal small colon, and is the most beneficial view in domestic horses.<sup>6</sup> Unlike diagnostic abdominal radiographic studies in domestic horses, the studies performed at the WAP on non-domestic equids are done with the animals in lateral recumbency during general anesthesia, making stone location and accurate anatomic identification more challenging. In 2003, four asymptomatic kiangs (including a female that had enteroliths surgically removed in 1989) were determined radiographically to have enteroliths large enough to potentially cause full or partial obstructions, and underwent elective laparotomies without complications. In several asymptomatic animals where radiographic findings were considered equivocal, repeat radiographs were performed 6 mo later, to evaluate size and movement of suspected enteroliths. Adequate hospital facilities and availability of veterinary and support staff are paramount to being able to implement and adhere to an aggressive preventive health program such as this one. Having recently completed the screening all of the equids at the WAP that are considered “at-risk”, WAP veterinary services is

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considering repeating radiographs for each animal within 2 to 3 yr, depending on development of clinical cases.

The enterolith removed from the SWA in this case report was a solitary stone located in the transverse colon, one of the three most common locations reported in domestic horses.<sup>2,6</sup> Analysis of previous stones recovered from WAP equids has identified struvite, or magnesium ammonium phosphate, as the predominant component, identical to domestic equid enteroliths. Possible causes of enterolithiasis in WAP equids are similar to those proposed for domestic horses and include dietary contributions, nidus ingestion, mineral content in drinking or ground water, and breed or species predisposition. It has been reported that California may far exceed recommended magnesium requirements in both alfalfa and water.<sup>2</sup> Dietary changes were made at the WAP in November 2002, and modification of the watering system for these animals is currently underway.

Post-operative complications in enterolith-associated colic surgery are reportedly low, compared to other forms of intestinal surgery in domestic horses, and include postoperative diarrhea, incisional infection, incisional hernia formation, and positive fecal *Salmonella* sp. culture.<sup>2</sup> Decreased appetite, decreased fecal output, diarrhea, and mild dehydration were considered mild to moderate post-operative complications in this case. Stillbirth of a previously healthy third trimester foal was also a complication, and may have been related to maternal stress, dehydration, anesthetic or therapeutic agents, or other unknown etiologies. Gross and histologic examination of the placenta and foal was unremarkable, however tissue cultures were not performed. Fortunately, this event did not appear to affect the female's long-term reproductive health, as she gave birth to a healthy female foal in March 2004, 1 yr and 4 mo following her stillbirth.

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**Table 1.** Standard post-operative management of non-domestic equids at the San Diego Wild Animal Park following abdominal surgery to remove enteroliths.

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Medication:

Omeprazole 2 mg/kg PO SID X 4 wk (while animal is hospitalized)

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Diet:

Day 1: Water only.

Day 2-4: Total daily ration divided and administered in four meals throughout day.

Day 5-8: Total daily ration divided and administered in three meals throughout day.

Day 9-15: Total daily ration divided and administered in two meals throughout day.

Day 16: Resume normal once-a-day feeding.

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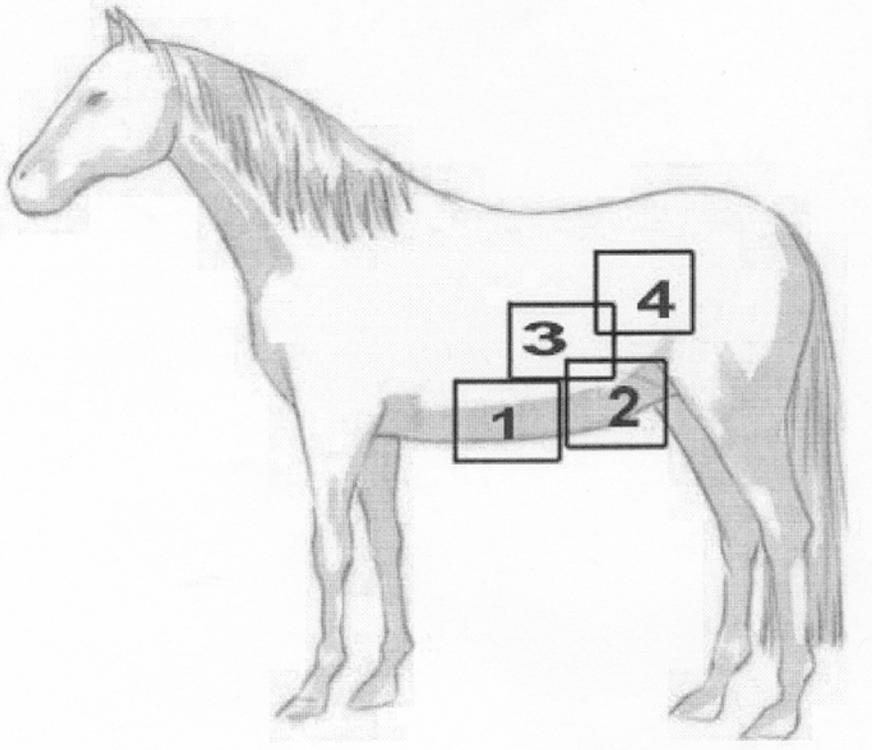
Housing:

Weeks 1-4: Maintain in hospital pen.

Weeks 5-8: Maintain in holding pen (slight increase in exercise, still controlled).

Day 56: Release back to exhibit.

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**Figure 1.** Standard four-quadrant radiographic technique utilized by San Diego Wild Animal Park to screen non-domestic equids for presence of enteroliths. Adapted from domestic horse literature.<sup>6</sup>

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## THIRTY IMMOBILIZATIONS OF CAPTIVE GIRAFFE (*Giraffa camelopardalis*) USING A COMBINATION OF MEDETOMIDINE AND KETAMINE

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### Abstract

Immobilization of giraffe and the associated risks have been previously described in the literature.<sup>1,2,5</sup> The use of medetomidine and ketamine for immobilization of free-ranging giraffe has also been described.<sup>3</sup> This report summarizes the immobilization of captive giraffe using this same drug combination.

Fifteen captive giraffe (*Giraffa camelopardalis*) were immobilized on thirty occasions using a combination of intramuscular medetomidine and ketamine. All but one very ill animal recovered from anesthesia. Two animals died the following day from presumed anesthetic-related causes. One animal was euthanatized four days following immobilization due to severe ileus and clinical deterioration. Accurate weights were not obtained on the majority of animals; and therefore, doses are summarized by age group (Table 1).

The first drug effect (time from initial injection to initial drug effect) ranged from 2-6 min (average = 4 min). Twenty-six giraffe became recumbent following the initial dose of medetomidine and ketamine. The time from the initial injection to recumbency ranged from 4-38 min (average = 13 min). Increasing doses of medetomidine did not necessarily decrease the time to recumbency nor did it ensure animals became recumbent. Four giraffe received additional intramuscular ketamine and/or medetomidine prior to becoming recumbent. All four animals were leaning against a wall or were head-pressing and this posture likely prevented recumbency. Once recumbent, supplements included intravenous medetomidine, intravenous ketamine, intravenous guaifenesin (GGE), intravenous propofol, or isoflurane via facemask or endotracheal intubation. Intravenous GGE or propofol is preferred although transient apnea was observed in a few cases following rapid propofol administration.

The duration of the anesthetic events (time from initial drug dosing to the administration of the antagonist) ranged from 21-145 min with an average of 82 min. Atipamezole or a combination of atipamezole and yohimbine were used to antagonize the effects of medetomidine. The preferred route for atipamezole administration is intramuscular, and it is recommended that giraffe be held in lateral recumbency for approximately 15 min prior to allowing attempts to stand. The

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recommended dose is approximately 200 µg/kg or up to five times the amount of medetomidine in milligrams. Several animals stood and fell during the recovery period. Animals usually remained calm in a sternal position before re-attempting to stand. A few animals required additional atipamezole due to incomplete reversal. Some animals showed evidence of resedation or drug recycling 10-28 hr following atipamezole administration. Signs of medetomidine resedation included decreased awareness of the animal's surroundings, dull eyes, inappetance, salivation, tongue drooping, excessive licking, ataxia, leaning against walls, and even recumbency. Animals responded positively to the administration of intramuscular atipamezole.

#### **ACKNOWLEDGMENTS**

The authors gratefully acknowledge the contributions of Dr. Mitch Bush and the participation of the veterinarians and staff members from the Fossil Rim Wildlife Center, National Zoo, Riverbanks Zoological Park and Botanical Garden, San Diego Zoo's Wild Animal Park, and Zoo Atlanta.

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**Table 1.** Summary of doses of medetomidine and ketamine used to immobilize captive giraffe at various ages.

# Events	Age group	Estimated weight range	Initial drug doses	Comments
6	2.5-6.5 mo	100-160 kg	8-9 mg med <sup>a</sup> 100-200 mg ket <sup>b</sup>	This dose range worked well for this age group.
4	8-12 mo	272-295 kg	18-21 mg med 220-400 mg ket	This dose range worked well for this age group.
3	20-28 mo	370-540 kg	20-25 mg med 300 mg ket	This dose worked well for this age group but would recommend 20 mg med and 300-500 mg ket.
17	8-22 yr	700-1100 kg	40-90 mg med 450-1500 mg ket	Recommend 40-50 mg med and 800-900 mg ket for an adult female; 50-70 mg med and 1000-1200 mg ket for an adult male; however, lower doses may also be effective and should be considered for smaller, ill or compromised animals.

<sup>a</sup>Medetomidine.

<sup>b</sup>Ketamine.

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**CHARACTERIZATION AND CRYOPRESERVATION OF SEMEN COLLECTED BY ELECTROEJACULATION FROM CAPTIVE EASTERN GIANT ELAND (*Taurotragus derbianus gigas*)**

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**Abstract**

Eastern giant eland (*Taurotragus derbianus gigas*) are the largest antelope species in the world and may weigh up to 1000 kg. These unique antelope have bred well in captivity in North America (now numbering approximately 60 individuals in 7 institutions), a success that has resulted in a degree of inbreeding. In order to maintain genetic diversity in this population, the introduction of new genes would be beneficial. The risk and cost involved in the importation of new eland into North America makes the importation of cryopreserved semen a more attractive alternative. Semen was collected by electroejaculation from eastern giant eland under anesthesia. Semen and blood (for testosterone levels) were collected at 2 different times throughout the year to investigate seasonal effects. Testicular firmness was subjectively assessed and testicular length and width were measured. Testicular volume was calculated from length and width measurements. Sperm morphology, percent (%) motility, progressive motility, and viability were measured on raw semen. Three cryoprotectants (Tris, TEST, and BF5F) were compared for their effects on % motility, progressive motility, and viability following cryopreservation. Eastern giant eland semen is similar in its characteristics to domestic hoofstock. BF5F was a significantly better cryoprotectant than Tris and TEST. While raw semen quality was similar in fall and spring, thawed semen quality was significantly better from fall BF5F-cryopreserved semen than spring BF5F-cryopreserved semen. Serum testosterone levels were significantly higher in fall than spring. In North America, it is recommended that giant eland semen be collected in the fall for cryopreservation and that BF5F be used as the cryoprotectant.

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# RECOMMENDED PHLEBOTOMY GUIDELINES FOR PREVENTION AND THERAPY OF CAPTIVITY-INDUCED IRON-STORAGE DISEASE IN RHINOCEROSSES, TAPIRS AND OTHER EXOTIC WILDLIFE

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## **Abstract**

Captive conditions, most likely related to non-native diets, induce progressive iron accumulation in a wide range of exotic wildlife, and iron toxicity contributes to high morbidity and mortality in affected species. Preventive and therapeutic strategies focus on dietary manipulations to limit iron uptake and on physical removal of excess iron by pharmacologic chelation or phlebotomy. Until nutritional management becomes an effective alternative, repetitive phlebotomies provide the simplest and most practical method to prevent and reduce iron overburden and its adverse effects. Guidelines are presented for a phlebotomy protocol applicable to rhinoceroses, tapirs and other animals based on the broad clinical experience in humans affected by iron storage disorders.

## **Introduction**

Over the past decade, convincing evidence has accumulated that African black (*Diceros bicornis*)<sup>4,7,12,15</sup> and Sumatran (*Dicerorhinus sumatrensis*)<sup>7,8,10,11</sup> rhinoceroses, as well as tapirs of all four species (*Tapirus* spp.),<sup>13</sup> develop pathologic overloads of iron under a variety of captive conditions. In rhinoceroses, blood and necropsy studies confirm that (a) iron accumulates progressively in multiple organs in proportion to time in captivity, (b) that iron stores in captive animals reach levels hundreds or thousands of times greater than found in the wild, and (c) that iron overload is frequently associated with histopathologic and biochemical evidence of severe cellular damage and dysfunction.

Although iron is an essential element, its presence in excess is invariably deleterious. Free iron actively catalyzes generation of highly toxic hydroxyl free radicals that in turn cause oxidative damage to cell and organelle membranes, to nucleoproteins, and to structural, functional and enzymatic proteins at multiple molecular sites.<sup>1,3,6</sup>

One of the principal clinical effects of excess iron is increased virulence of microorganisms and increased host susceptibility to infections of all types,<sup>5,14,17,18</sup> therefore iron overload may be largely responsible for the high incidence of diverse and exotic infectious diseases known to affect black and Sumatran rhinoceroses and tapirs in captivity. Additional evidence linking iron overload to other disorders has also been accumulating,<sup>7,12</sup> although direct cause-and-effect

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relationships have not been unequivocally established. Nonetheless, an enormous body of experience with iron storage disease in humans and other animals has demonstrated that iron overburdens of the magnitude observed in these species can contribute to and/or directly cause severe, life-threatening, multi-system disorders, some of which are clinically similar to dyscrasias affecting them in captivity.

### **Rationale for Repetitive Phlebotomy**

To avoid the well known deleterious effects of chronic iron toxicity, several strategies have been proposed and tested over the past few years to prevent and/or reduce iron overburdens in affected rhinoceroses and other species. Decreasing the bioavailability of dietary iron has received the greatest attention, including formulation of low-iron feeds and addition of iron-binding components (such as tannins) to standard captive diets. These important approaches deserve continued investigation since they could substantially benefit both captive-born calves and wild-caught animals, both of which have normal iron stores initially. Unfortunately, even if 100% effective, interdiction of iron uptake would have no effect on the pathologic overloads already existing in most of the captive populations.

Since mammals cannot excrete iron physiologically, even when present in great excess, preexistent iron overburdens can only be corrected by physical removal of the metal.

One standard therapeutic approach is pharmacologic chelation with desferrioxamine (Desferal, Novartis Pharmaceuticals, NJ). Chelation is the preferred treatment to correct iron excess in avians, in many other small captive animals, and in children with chronic anemias who accumulate iron from multiple blood transfusions. We have found that desferrioxamine actively mobilizes storage iron for urinary excretion in rhinoceroses, but it remains prohibitively expensive to consider its routine use in large animals.

The second effective approach to prevention and/or correction of iron overload is repetitive phlebotomy, the standard of practice for humans with highly common genetic predispositions to overload iron. Since hemoglobin contains a fixed amount of iron (0.34% by weight), removal of a liter of blood with 15 g/dl hemoglobin content physically reduces an individual's iron load by 0.5 g. Thus, the iron removed by repetitive phlebotomy can be precisely calculated by measuring the volume and hemoglobin content of the blood removed

### **Guidelines for a Repetitive Phlebotomy Program**

Historically, the seemingly draconian practice of blood-letting was one of the earliest known attempts to treat human ills and was also used in veterinary medicine as far back as ancient Egypt. Although its justification was often dubious, a sound scientific basis for this practice emerged in modern times to treat a number of iron-overload syndromes affecting humans<sup>2</sup> and other animals. In veterinary practice, therapeutic phlebotomy has been documented by numerous case reports in the literature, but seldom with consensus on optimal procedures for any given

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species. In humans, however, experience with this technique has been much more extensive due to the high frequency of iron-storage disorders, particularly hereditary hemochromatosis caused by mutations of the *HFE* gene, the most common genetic defect in the U.S. population.

Definitive guidelines for diagnosis and management of human hemochromatosis have been published by the Practice Guideline Development Task Force of the College of American Pathologists (CAP)<sup>19</sup> and by the U.S. Centers for Disease Control and Prevention (CDC).<sup>16</sup> The following recommendations for rhinoceroses and tapirs are based largely on extrapolation of these guidelines modified by consideration of the practical aspects of working with exotic wildlife in captive settings. It is expected that further modifications will be individually tailored to the special characteristics of each animal and to accommodate circumstances unique to each institution.

### **Candidate Populations, Selection Criteria and Goals**

The browser rhinoceros species appear to be at higher risk than grazers for development of pathologic iron overloads in captivity, but under certain circumstances even the latter can accumulate iron excessively. We therefore recommend that the iron loads of all captive rhinoceros and tapir species be assessed at earliest opportunity and periodically thereafter. In humans, the CDC recommends transferrin saturation (serum iron concentration divided by total iron binding capacity) as a simple inexpensive screening test. CAP guidelines cite persistent elevations of transferrin saturation (>60%) and serum ferritin concentrations (>200-400 ng/ml for women and men, respectively) as thresholds for diagnosis of pathologic iron loads in hereditary hemochromatosis. Serum ferritin concentration provides the single most reliable non-invasive indicator of total body iron stores. (Ferritin assays require species-specific reagents, and these are available for rhinoceroses and tapirs, as well as other species, at the Laboratory of Comparative Hematology, Kansas State University College of Veterinary Medicine.)

In humans, the primary therapeutic indication for phlebotomy or iron-chelation therapy is the demonstration of significantly increased iron stores even in the absence of any clinical manifestations of iron-storage disease. On the basis of our experience, we would recommend threshold values of 65-70 % transferrin saturation and 500 ng/ml serum ferritin, above which therapeutic intervention in rhinoceroses or tapirs would be clinically justified. These thresholds represent, respectively, double and triple the mean values we have measured in black rhinoceroses free-ranging in the African wild (34 % transferrin saturation and 180 ng/ml ferritin). Animals that are already overtly anemic should be excluded as candidates for a phlebotomy program.

Within each candidate species, two subpopulations merit distinction: (1) those with transferrin saturation and ferritin values consistently above the reference ranges, and (2) captive-born calves and animals of any age that have been recently translocated from the wild into captivity, (all of which initially have iron analyte values within normal ranges). For the first group, the long-term goal of a phlebotomy program is therapeutic, to remove the pathogenic iron as rapidly as

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possible.<sup>19</sup> By contrast, periodic phlebotomy for the second group would be a preventive measure with the goal of maintaining normal iron stores despite captive conditions that otherwise would inexorably lead to progressive iron accumulation. (In rhinoceroses, body iron stores can increase as much as tenfold in as little as 3 yr in captive newborns.)

## Procedures

The essential qualification for inclusion of animals in a repetitive phlebotomy program would be their ability to tolerate such procedures without undue stress or endangerment to the animals themselves or to the keeper and veterinary staffs. An entire session of presentations at the 2002 Annual AAZV Conference amply demonstrated the value and effectiveness of operant conditioning in animals ranging from small primates to large carnivores and megavertebrates. Training animals to allow routine care and clinical management without sedation or anesthesia is now widely practiced, and venipuncture is one of the most common procedures performed.

Ear and forefoot interdigital veins are most conveniently used with rhinoceroses, but these vessels may be prone to thrombosis under the trauma of frequent venipuncture. In addition, they are generally too small to obtain large volumes of blood quickly, so they would not be optimal for repetitive phlebotomies. The medial radial vein of the rhinoceros foreleg, however, provides the most accessible channel for rapid removal of sizeable volumes through large-bore needles (18 gauge or greater), and rhinoceroses at several different institutions have already been conditioned to tolerate this procedure.

CAP guidelines emphasize that the rate of phlebotomy in both frequency and volume must be established for patients individually, and it is axiomatic that each animal in a phlebotomy program will dictate its own tolerance limits. Humans are generally bled 450-500 ml once or twice per week. For a 1,000-kg black rhino, that would be equivalent to ~7-15 liters weekly, clearly not a feasible amount. Experience with rhinoceros donors for transfusions, however, suggests that 1.5-3 liters or more are reasonable and achievable target volumes for typical phlebotomy sessions. *It should be emphasized that any amount of blood removed from an iron-overloaded animal would be beneficial in that it contributes to negative iron balance and reduction of excess storage iron.*

In humans, phlebotomy programs are initiated slowly, (for example, once every 2 wk over the first 2 mo) since this stimulates erythroid marrow to proliferate toward maximal rates of production. Subsequently, the frequency can be progressively increased to once or twice weekly. Ultimately, the goal of a repetitive phlebotomy program is to mobilize storage iron by inducing a slight blood-loss anemia. This can be accomplished by reducing packed red-cell volumes (PCV) by as little as 5% below their normal baselines. To quote the CAP guidelines, "...if the hematocrit before any phlebotomies is 45%, maintaining it at 40% provides an adequate challenge to the marrow without provoking symptoms of anemia."<sup>19</sup> Lowering each animal's baseline PCV by ~5% would thus serve as a valid objective, and no additional advantage is gained by decreasing it further.

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Progress in reducing iron stores can be monitored by measuring serum iron, transferrin saturation, and ferritin every 3-6 mo. These values, however, can be altered by concurrent infectious and inflammatory processes, making trends shown by periodic assays far more reliable than isolated values alone. Serum iron typically remains elevated until the storage pool nears depletion. As tissue iron stores are mobilized to replace hemoglobin lost by phlebotomy, ferritin concentrations in the plasma gradually decline. If repetitive phlebotomies eventually return the storage pool to normal, both the serum ferritin and red cell MCV would decline, reflecting the decreased availability of storage iron for enhanced erythropoiesis. In some species (such as rhinoceroses), reticulocytes mature before leaving the marrow, therefore erythropoietic responses are better assessed by changes in MCV than by reticulocyte counts.

### **Cost/Benefit Assessment and Secondary Benefits**

Many captive black rhinoceroses already have extreme elevations of storage iron, dramatically reflected by serum ferritin concentrations in the thousands, tens of thousands, and greater (compared to a normal range of 100-200 ng/ml). Phlebotomy programs will require long-term commitments to contribute substantively to the welfare of these animals. Even if iron stores were eventually returned to normal, less frequent phlebotomies (perhaps one every 3-4 mo) would still be of value to prevent reaccumulation of the metal. These would not be necessary, however, if current dietary research studies result in measures that effectively reduce enteric iron uptake.

Any phlebotomy program would demand significant time commitments from personnel involved in animal training and performance of the procedure. Additionally, institutional administrators must be persuaded to commit staff time and other costly resources to institute remedial programs for problems that are not clinically obvious. (Rhinoceroses in particular seem to have evolved a capacity to remain outwardly stoic despite the presence of extensive internal damage or disease.) In humans, phlebotomy programs have been shown to be unequivocally cost-effective by avoiding the huge future expense of treating chronic and terminal multi-system disorders that are the inevitable consequences of prolonged iron overburden. Since chronic iron toxicity is as insidious as it is pernicious, it generally remains undetected until terminal failure of some critical organ system occurs. Nonetheless, progressive dysfunction of various organs contributes to deteriorating quality of life in affected animals as well as shortened life spans.

Operant conditioning of animals to tolerate venipuncture has at least two potentially important fringe benefits. It allows the veterinary staff easier access to obtain diagnostic blood samples for any disorder that might develop, and it provides a ready route for therapeutic parenteral administration without sedation, should that become necessary. Additionally, for a relatively small investment in a core central facility, blood removed by phlebotomy could be fractionated for long-term preservation and storage of red cells, leukocytes, platelets, and plasma as a resource to treat future hemolytic, hemorrhagic, infectious, or other disorders without jeopardizing additional rhinoceroses as donors of fresh blood. Potential sites and funding

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sources for a centralized national blood bank for rhinoceros blood components are currently being investigated.

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## COMPARATIVE STUDY ON RHINOCEROS HEAD ANATOMY USING ENDOSCOPY, COMPUTED TOMOGRAPHY (CT) AND GROSS MORPHOLOGY

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### Abstract

Studies of rhinoceros head anatomy are rare in the literature.<sup>1,2,4-6</sup> In order to elucidate the normal anatomy and morphology of the rhino head, five adult zoo animals were examined by different methods. The study was carried out for three reasons: First, considering the paucity of information about rhino head anatomy further investigation was indicated. Second, the characterization of the dental apparatus was a primary interest in order to improve the evaluation and current therapies for recurring dental problems in captive animals. Finally, the characterization of the upper respiratory tract was performed in order to describe intubation techniques for improved inhalation anesthesia. Inhalation anesthesia may be necessary if long medical interventions are performed.<sup>3</sup>

The heads of five captive animals of two species (Asian rhinoceros, *Rhinoceros unicornis* and white rhinoceros, *Ceratitherium simum*) that died or were euthanatized for various medical reasons were examined by three methods: endoscopy (live animal), computed tomography and classic preparation techniques (post mortem preparation, band-saw preparation with the frozen head, maceration) (Table 1).

Endoscopic examination gave a detailed insight into the *cavum oris proprium* of the living animal with severe dental problems.<sup>5</sup> The classic preparation techniques provide a detailed analysis of the entire anatomic structures of the head. However, these classic mechanical preparation techniques are destructive as important details are lost during the examination. Therefore computed tomography proved an excellent tool by imaging the anatomic structures *in situ* in a non-destructive way. For example, the osseous structures in a three-dimensional model could be imaged followed by addition of the soft tissue in a definite, preplanned way using the option of “windowing”. In this technical process, selected parts of the dataset can be imaged, while other parts can be faded out, depending on the radiographic density (measured in Hounsfield units, HU). The subject head can be planarly or curvilinearly dissected in a virtual manner. Multiple measurements were easily obtained including the diameters and distances of the oral cavity, the epiglottis and the trachea. A virtual endoscopy within the upper respiratory tract was also performed to review intubation techniques. Specific problems with the computertomographic examination were the large size (the horn) and weight of the rhino heads

combined with the high radiographic density of the integument. Therefore in one specimen, the main part of the horn and associated skin were removed in order to obtain improved image quality of the other head structures. The resulting images showed a significant enhancement of image quality with a decrease in artifact compared to the scan of the intact head. All methods were considered to be of value for the study with the combination of techniques enabling a detailed investigation of rhino skull and head morphology.

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**Table 1. Specimen and method of examination.**

Species (#)	Sex	Endoscopy (live animal)	Computed Tomography	Post Mortem Preparation	Band-Saw Preparation	Maceration
<i>Cerat. simum</i> I	male			x		x
<i>Cerat. simum</i> II	female				x	
<i>Cerat. simum</i> III	female			x		x
<i>Cerat. simum</i> IV	female		X	x		
<i>Rhino. unicornis</i> I	female			x	x	
<i>Rhino. unicornis</i> II	female	x	X		x	

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## VETERINARY OBSERVATIONS OF WILD GREATER ONE-HORNED RHINOCEROS (*Rhinoceros unicornis*) IN THE ROYAL CHITWAN NATIONAL PARK: IMPLICATIONS FOR CAPTIVE MANAGEMENT

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### Abstract

Recent health surveys of greater one-horned rhinoceros (*Rhinoceros unicornis*) held in zoological facilities in both North America and Europe highlight the prevalence of a significant medical condition, chronic pododermatitis (CP). CP, characterized by nail overgrowth, non-healing fissures and ulcers located between the sole of the central toe and the adjacent pad and by pad overgrowth, bruising and chronic infection, occurs predominantly in adult male rhinos. The condition has not been recorded in wild animals. This paper describes a study that has utilized field observations of wild *R. unicornis* in the Royal Chitwan National Park (RCNP), Nepal to further elucidate the causes of CP as they relate to captive management. Our observations indicate that unusually large body size of captive animals, particularly males, and suboptimal husbandry are likely to be the most influential etiologic factors in the development of CP. Treatment of advanced CP has been shown to be largely ineffective<sup>1</sup> and appropriate changes in our approach to the husbandry and management of this species in captivity are essential if we are to control or prevent this debilitating disease.

### Introduction

As zoo veterinarians and animal managers we recognize our responsibility to ensure the best possible healthcare, housing, and husbandry conditions for the animals in our charge. In order to maximize sustainability of captive endangered species programs, improved knowledge of behavior, anatomy, physiology, nutrition and general health in normal individuals is desirable. In the authors' experience, the most effective way of accumulating this knowledge base is through the establishment of professional relationships with animal managers in the field, participation in *in situ* research programs and observation of the physiologic and anatomic features of free-ranging and recently captured wild animals.

The RCNP is located in south-central Nepal at 120-200 m above sea level and occupies an area of 932 km<sup>2</sup>. A recently established buffer zone provides an additional 750 km<sup>2</sup>. In 2002 the *R. unicornis* population in the park and surrounding area was estimated to be 544 animals. Eighty-seven animals have been captured from this park and translocated to other protected reserves in

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western Nepal since 1986.<sup>2,4</sup> The population of *R. unicornis* in RCNP is generally well protected, and has been comprehensively studied over the past 30 yr.

Due to thermoregulatory and nutritional requirements, the distribution of wild *R. unicornis* in RCNP tends to be limited to narrow strips of riverine habitat characterized by alluvial *Saccharum spontaneum* grassland, significant silt deposits and soft, sandy soils with a year-round average moisture content of 30-40%.<sup>4</sup> Home ranges tend to be small (3.4 – 4.3 km<sup>2</sup>) and foraging on hard, rocky ground tends to be limited by the presence of non-palatable ‘sal’ forest (*Shorea robusta*), a habitat not considered to be suitable for grazing rhino.<sup>4</sup> Animals have been frequently observed to utilize mud wallows and pools apparently as a means of thermoregulation. Wallowing frequency increases during periods of high humidity. It has been demonstrated that the extent and duration of wallowing correlates strongly with vapor pressure density, precipitation and mean maximum monthly temperature.<sup>4</sup> Male dominance appears to be primarily determined by incisor length and intra-specific aggression and sparring is frequent, severe and occasionally fatal.<sup>4</sup> Based on field observation and examination of weight records of animals captured for translocation, substantial size dimorphism between sexes is not seen in wild *R. unicornis*.

Conversely, captive male *R. unicornis* in North American and European zoos may be up to 1,500 kg heavier and 25 cm taller (shoulder height) than females.<sup>3,4,9</sup> Primary flooring substrates tend to be concrete and access to pools and wallows is limited in most zoological facilities, particularly during the winter. Males tend to be maintained separately, especially once they reach maturity (6+ yr), have few opportunities for exercise and are fed diets high in energy and protein content.<sup>3,10</sup> Growth rates are rapid and males frequently attain a massive body weight by an early age. For example, an adult male *R. unicornis* weighing 3,800 kg was recently transferred between zoological facilities in Europe. At another facility a 2-yr-old male weighed 1,500 kg. In contrast, the body weight of most adult males captured in RCNP seldom exceeds 2,000 kg. Males born in captivity may become much larger than captive-born females after only 4 yr; in contrast, 4-yr-old males in the wild are always substantially smaller than adult females.<sup>4</sup> It has been observed that severe lesions of CP are primarily noted in adult animals.<sup>8-10</sup>

### **Observations to Date**

Multiple etiologies and pathogenesis of CP as well as preventive, therapeutic and management options have been proposed.<sup>1,5,10</sup> However, in order to better understand the specific changes occurring in the feet of affected *R. unicornis*, and to determine why adult males are predominantly affected, it is critical to gain a better understanding of what constitutes ‘normal’. Observation of wild *R. unicornis* in RCNP before, during and after capture/translocation,<sup>2,4,7</sup> in conjunction with examination of relevant scientific literature and evaluation of historic data, has provided the authors an opportunity to assess size, condition and health status and visually examine and in some cases, measure, various parameters of foot anatomy from more than 30 individuals. This information has been compared to our observations of captive animals.

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All adult rhinos examined in RCNP appear to exhibit similar podiatric features: each hoof (toe nail) has an oval to semi-circular shape; the central toe tends to be larger and longer with a more pronounced semi-circular shape than the medial and lateral toes; the palmar/plantar aspect of the sole of the central toe merges with the structures of the foot pad while the lateral and medial hooves remain more mobile with a more distinct dorsal edge and obvious inter-digital separation; the horn wall of all toes appears to be long, dense and structurally very hard and forms an elevated 'rim' distinct from the sole, which tends to be markedly concave; the foot pad is roughened and hard with multiple superficial cracks and fissures present in the horny tissue and the hard horn wall 'rim' serves as the major weight-bearing surface of the foot during ambulation. As a result, these animals have been classified as "toe walkers."<sup>10</sup>

The majority of captive *R. unicornis* feet examined in zoos in North America and Europe exhibit significantly different anatomic characteristics than those noted in their wild counterparts: thin, smooth and flattened footpads, pale coloration with frequent cracks, fissures and hematoma; short horn wall with indistinct dorsal edges; toes indistinct from the sole; soles flat (no longer concave) with even transition to the adjacent pad and hoof walls (particularly lateral toes) which are frequently abraded and have a pale colored, thin, flattened wall structure. These animals are classified as "pad walkers."<sup>10</sup> Adult *R. unicornis* suffering from CP also exhibit nail overgrowth, non-healing fissures and ulcers located between the sole of the central toe and the adjacent pad and pad overgrowth, bruising and chronic infection.<sup>1</sup>

## Hypothesis and Discussion

Although there are exceptions, adult male and female *R. unicornis* tend to be of similar body size in the wild. Animals forage and maintain small home ranges in areas limited by their proximity to rivers. Local terrain is characterized by moist, soft, sandy substrates. Consistent with a dominance hierarchy, mature males interact with each other frequently, defending their home ranges vigorously and seeking out breeding opportunities. Dominance appears to be related to mandibular incisor length. (It is hypothesized that the size and condition of the tusks in breeding age *R. unicornis* help determine dominance, access to estrus females, and ultimately reproductive success.<sup>4</sup>) In captivity where sedentary young males receive large quantities of nutritious feed from an early age, extremely rapid growth rates seem to occur. These animals are protected from frequent intra-species aggression, are seldom exercised and are generally housed on concrete or a similar unforgiving substrate allowing abrasive damage to occur to the toes and feet, often leading to CP. This is likely to occur more frequently in males because they get comparatively much larger and heavier than similarly raised females. Infrequent access to ponds or wallows throughout the year, particularly during the winter months, limits thermoregulatory control, likely encourages skin desiccation and cracking and further exacerbates the traumatic effects of prolonged weight bearing on joints and feet.

Chronic pododermatitis is a severe, difficult to control but ultimately preventable disease of *R. unicornis* which has a very high incidence in the captive population. The husbandry implications for captive rhino managers and veterinarians are significant and readers are referred to the EEP 2002 *R. unicornis* Husbandry Manual where many of these concerns are addressed.<sup>6</sup> In light of

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extensive field observation and our experiences with maintaining this species in captivity, we recommend serious consideration of the following when developing management plans, preventive health protocols and new facilities:

- Dietary restriction to limit rapid growth, massive body size and obesity especially in young males.
- Flexible, shock-absorbent and non-abrasive flooring. Avoid concrete or sand as a primary surface, utilize rubberized/urethane surfaces or alternative substrates such as wood chips or clay-soils.
- Year-round access to a pond or mud wallow.

In light of the severity of chronic pododermatitis in this species and the apparent link to husbandry practices, it is important we remain aware of our management responsibilities when recommending importation of wild-caught animals to augment the captive population.

#### ACKNOWLEDGMENTS

The assistance and support of WWF-US, WWF-Nepal, King Mahendra Trust for Nature Conservation and the International Rhino Foundation (IRF) is gratefully acknowledged.

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## BOVINE TUBERCULOSIS SURVEY IN AFRICAN BUFFALO (*Syncercus caffer*) IN THE NORTHERN HALF OF THE KRUGER NATIONAL PARK

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### Abstract

### Introduction

The second non-lethal bovine tuberculosis (BTB) detection survey was conducted on all significant (>150 animals) buffalo herds (*Syncercus caffer*) north of the Olifants River in the Kruger National Park from 4 July – 27 July 2003. A total of 651 buffalo from 30 herds were sampled. The previous survey was conducted in various months in 2000 and coincided with the capture of pregnant cows and a few breeding bulls for the Koos Bekker and Skukuza disease-free breeding projects. No animals were captured for breeding purposes during the 2003 survey.<sup>1</sup>

BTB was detected in all herds tested south of the Letaba/Shingwedzi watershed and confirmed in one herd north of this watershed. Eight herds north of the watershed had suspicious results on either the gamma-interferon assay or tissue samples and we await the mycobacterial culture results to confirm the TB status of these herds. Confirmed BTB negative herds (from consecutive negative results in previous surveys as well) only occur north of the line running east-west through Dzundzini hill in the far north of the Kruger National Park. These results indicate a marked increase in the spatial spread of TB in the northern herds when compared to the 2000 survey and supports the theory that BTB will eventually spread to all buffalo herds in contact with each other in the Kruger National Park.

### Methodology

The 2003 sampling of buffalo herds started on 4 July in the Letaba area and moved progressively northward up to the most northern herds in the Limpopo valley. The Olifants/Letaba watershed was the southern most geographic limit of the survey. One to two herds were sampled per day. Herds were found either by fix wing aircraft the previous afternoon and sampled the next day by helicopter or found directly by helicopter and sampled. A split subgroup was separated from the main herd and randomly selected animals were then darted from the helicopter using aluminum darts. Etorphine (M99) with azaperone and hyalase were used in the initial stages of the survey and M99 combined with A30-80 and azaperone was used in the latter part of the survey.

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The sample size of animals selected per day depended on the estimated size of the herd: between 16 and 30 animals were captured per herd (10-16 at a time, therefore more than one group were immobilized per day or on different days from larger herds). Blood samples were collected from each animal for the gamma-interferon tuberculosis assay, foot & mouth disease serology and for corridor disease research.<sup>2</sup> Serum samples and random DNA samples were collected for banking purposes and future reference. Probang samples were collected from most buffalo for F&MD virus isolation.

A radio collar was fitted to an adult cow in each herd tested, so that the group could be located 36 hr later. Each animal was identified on the back, with a painted letter of the alphabet allocated to that herd, and a specific number correlating to its blood samples for immediate future recognition. These identifying marks persisted for up to five days after which the paint was either rubbed off or obscured by dirt and mud. In addition to the paint they also received a general 'X' hot brand ensuring that the animals can be identified in future years as animals tested in 2003. After all the procedures were completed, a specific antidote was administered to the immobilized animals, thereby reversing the anesthetic agent. Most immobilized groups were found close together after being revived and no indications of post capture predator related mortalities (or any other form of mortality) were recorded.

The gamma-interferon assay preparation was done immediately on return from the capture. Stimulated plasmas were incubated overnight and results were available within 36 hr. If an animal tested positive or suspicious for TB then an attempt was made to get at least one positive animal from a herd, euthanize it and perform a detailed necropsy. Head and thoracic lymph nodes were excised and carefully examined for macroscopic tuberculous lesions. Lungs were carefully palpated and the thoracic lymph nodes were excised and examined.

## **Discussion**

Macroscopic lesions were found in all but one animal with a strong positive gamma interferon test, confirming the disease in that herd. That single macro-negative animal was however positive on culture. The gamma interferon test in free-ranging Kruger buffalo appears to have excellent sensitivity and specificity.<sup>2</sup>

The 2003 survey results indicate that there has been a significant increase in the number of positive herds in this region of the KNP, compared with the 2000 survey. All herds south of the Shingwedzi/Letaba watershed tested positive in 2003, whereas only four out of 12 herds were positive in this area in 2000. Disease detection in buffalo herds north of the watershed was much lower than herds south of this watershed, with only one animal having a confirmed positive result on BTB. This animal that tested positive was captured at Malahlapanga, approx 20 km south of Punda Maria – this is the most northern point where BTB has been detected in the Kruger National Park to date. This animal tested positive on the gamma interferon assay and had a macroscopic lesion confirmed at necropsy. There were a number of herds north of the watershed with suspect reactions and we await the culture results from samples of euthanized

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animals. With only one positive result but several suspicious reactions, as well as several previous confirmed cases at Nkokodzi, Tussen In and Biesiesvlei (lion), it is suggested that BTB is present at a very low prevalence in the greater part of the far north districts.

We can accept for management purposes that all herds south of the line running east-west through Dzundzini hill are infected at a very low prevalence with BTB. Herds south of the Shingwedzi/Letaba watershed are infected at low to medium prevalence. As of 2003, herds north of the east-west line through the Dzundzini hill are still considered negative for BTB since multiple buffalo capture and sampling opportunities, over the past 4 yr, have failed to detect any TB in this area.

The next non-lethal BTB survey planned for 2005 will only be conducted north of the Shingwedzi/Letaba watershed as we have now shown that all herds south of this line are infected. There is still merit on doing the non-lethal sampling in the low incidence herds in the north of the park to avoid killing healthy buffalo in this way. Should there be a need to determine more precise prevalence of TB in the infected herds, between the Letaba and Shingwedzi Rivers, a lethal sample is recommended as this will be more cost effective.

The continued monitoring of the TB status in the KNP buffalo herds cannot be over emphasized as the knowledge gained is unparalleled elsewhere in wild populations. We have a commitment to our neighbors (both livestock and wildlife interface) to inform them of the risks and only with up to date information of the disease and its status in the Park, can rational management decisions regarding possible containment, control and eradication measures be made.

#### **ACKNOWLEDGMENTS**

This survey would not have been possible without the excellent support of the district rangers, section rangers and field rangers of the northern regions of the park. The technical staff from the regions also provided support without which we could not have managed. Special thanks must go to Louis Olivier for his clinically perfect organization of staff, observers and general logistic support!

Anita Michel from the OVI and Cornelia Gerstenberg from the National Directorate of Animal Health supported our work from their Institutes / Departments for which we are very grateful. The State Veterinary Team (Dewald, At, Schalk, Kenneth and Johan) was an integral part of the exercise and is thanked for efficient professional assistance. Eunice, Jenny, the students (Monika and Dean) and our own staff (Marius, Hoepel, David, Ernest, Sollie and Amos) were highly efficient and we could not have done it without their loyal support and effort.

Judith Kruger and Sandra MacFadyen provided prompt GIS back up and provided the maps attached to this document, many thanks to them as well.

We would all like to thank the three pilots involved (Martin, Hennie and Fanie) who made the actual sampling possible in their typically professional way. All in all it was a great team effort, and foreign colleagues visiting the operation remarked positively on the planning, efficiency and co-operation that existed between all role players.

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**A NEWLY RECOGNIZED NEUROLOGIC DISEASE ASSOCIATED WITH *Parelaphostrongylus odocoilei* IN EXPERIMENTALLY INFECTED THINHORN SHEEP**

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**Abstract**

Recently, the muscle-dwelling protostrongylid nematode *Parelaphostrongylus odocoilei* was discovered in Dall's sheep (*Ovis dalli dalli*) in the Mackenzie Mountains, Northwest Territories, Canada. Subsequently, a survey of thinhorn sheep (*Ovis dalli*) and mountain goats (*Oreamnos americanus*) in northern North America has revealed that this parasite is widely distributed in the Subarctic.

In thinhorn sheep both naturally and experimentally infected with *P. odocoilei*, eggs and larvae caused granulomatous interstitial pneumonia and lung hemorrhage, while adult nematodes were associated with localized myositis and muscle hemorrhage. Experimentally infected sheep showed a consistent pattern of weight loss and decreased muscle mass. At 2 wk prior to patency, two of five experimentally infected sheep developed hind end ataxia, loss of conscious proprioception, hypermetria and an eosinophilic pleocytosis in cerebrospinal fluid. Antibody to *Parelaphostrongylus* spp. was detected in the cerebrospinal fluid and serum of infected, but not control, sheep. Neurologic signs stabilized at the time of patency and subsequently disappeared until recurrence following treatment with ivermectin. Uninfected control sheep showed no weight loss or clinical abnormalities at any time.

In five thinhorn sheep each experimentally infected with 200 third-stage larvae of *P. odocoilei*, pre-patent periods ranged from 68-74 days. Shedding of first-stage larvae peaked at >10,000 larvae per gram of feces between 90 and 110 days post infection. The identity of first-stage larvae was confirmed by comparing sequence of the ITS-2 region of nuclear DNA with known sequence for *P. odocoilei*. Adult *P. odocoilei* were recovered from three experimentally infected sheep, but not the two sheep that developed neurologic signs, which are currently being monitored.

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While other researchers have recovered *P. odocoilei* adults from the epidural space of experimentally infected mule deer (*Odocoileus h. hemionus*), we did not find adult *P. odocoilei* in the spinal canals or cords of ten naturally infected or three experimentally infected thinhorn sheep. Therefore, this is the first evidence of a neural migration for *P. odocoilei* in experimentally infected thinhorn sheep, and the first description of a clinical neurologic syndrome caused by this parasite in any host species. These findings indicate that this host-parasite relationship is more complex than previously believed. Considering the susceptibility of protostrongylid life cycles and northern hosts to climate change, *P. odocoilei* may constitute a significant emerging disease risk for thinhorn sheep.

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***Frenkelia*-LIKE ENTERIC COCCIDIA IN COOPER'S HAWKS (*Accipiter cooperii*)**

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**Abstract**

During a study of mortality in raptors, sporulated coccidian oocysts were noted in the lamina propria of the small intestine of 47 of 86 (54.7%) Cooper's hawks examined. No pathology was associated with the presence of these oocysts. On subsequent examination of fresh feces from seven birds, sporocysts with mean dimensions of  $13.4 \times 8.9 \mu\text{m}$ , a shape index of 1.3 (1.4-1.6), and diffuse residuum were observed. These sporocysts were morphologically similar to *Frenkelia* and *Sarcocystis* spp. To determine the phylogenetic relationship of this *Frenkelia* sp. to other *Frenkelia* and *Sarcocystis* spp., a fragment (~700-bp) of the 18S rRNA gene was amplified from three samples and sequenced. This *Frenkelia* sp. was most closely related to *F. buteonis* (*S. microti*) and *F. glareoli* (*S. glareoli*), both of which use hawks in the genus *Buteo* as definitive hosts and various rodents as intermediate hosts.

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## INFECTIOUS DISEASE EXPOSURE IN ENDANGERED ISLAND FOX (*Urocyon littoralis*) POPULATIONS: IMPLICATIONS FOR SPECIES CONSERVATION MANAGEMENT

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### Abstract

The island fox (*Urocyon littoralis*) is only found on six Channel Islands off the coast of Southern California and is the largest terrestrial carnivore on the islands. Since 1994, island fox populations have experienced severe declines (up to 95%), which have resulted in the recent listing of the four most affected subspecies as federally endangered on March 4, 2004. An outbreak of canine distemper virus in 1999 is believed to be responsible for the dramatic decline of the Santa Catalina Island fox population, but little is known about current pathogen exposure in the entire fox population at risk. Prior to the decline, fox populations on all six islands had no evidence of exposure to canine distemper virus (CDV), while exposure to other canine viral pathogens, *Toxoplasma gondii* and *Leptospira interrogans* serovars varied among islands. Our study investigates what role infectious diseases like distemper may have had in the declines by estimating the exposure prevalence of infectious diseases in the post-decline island fox population. To date, 218 island fox serum samples collected from 2001 through 2003 on all six islands have been analyzed for exposure to canine distemper virus, canine adenovirus-1, canine parvovirus, canine coronavirus, canine herpes virus and six *Leptospira* serovars at Cornell University Veterinary Diagnostic Laboratory. Our results indicate that canine parvovirus and adenovirus exposure is still prevalent on most islands, and that Santa Catalina Island remains naive to adenovirus exposure. In contrast to pre-decline serology, foxes on all six islands now have evidence of exposure to CDV (11.8% on San Miguel Island, 4.8% on Santa Rosa Island, 63.6% on Santa Cruz Island, 33.3% on Santa Catalina Island, 27.7% on San Clemente Island and 68.9% on San Nicolas Island). On islands where some or all of the current population is being held in captivity, stratification of CDV antibody titers by fox birth location (captivity vs. wild-born) reveals that titers are only present in wild-born foxes. These results suggest that wild fox populations on all six islands have been exposed to CDV or a closely related morbillivirus in the past, but it is not known why Santa Catalina Island foxes appeared to have high mortality while the fox population on San Nicolas Island has remained stable despite having the highest CDV antibody prevalence. These initial antibody titer results have been used by managers making

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decisions regarding vaccination programs for wild and captive island foxes and will be used to help assess the risk of moving island foxes to and from the mainland or between islands for captive breeding.

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## EXAMINING THE HEALTH RISKS TO WILDLIFE ASSOCIATED WITH INTRODUCTIONS OF DOMESTIC AND EXOTIC SPECIES IN THE NORTHWEST TERRITORIES, CANADA

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### Abstract

The introduction and translocation of both domestic and wild animals are key factors in the emergence of infectious diseases. Throughout North America, interactions between domestic livestock and wildlife have often resulted in pathogen exchange and disease outbreaks; for example, contact between domestic sheep/goats and bighorn sheep has resulted in pneumonia epizootics and decimation of bighorn sheep populations. In the Northwest Territories (NT), large epizootics have not been reported and as yet there has been no contact between domestic sheep, goats, or llamas and wildlife such as thornhorn sheep and mountain goats. The equilibrium of the wildlife host-environment-pathogen system, particularly during this period of accelerating climate change, may be precarious and susceptible to additional stressors, such as contact with domestic animals. It is, therefore, very important to pro-actively assess and minimize these potential stressors.

In the Northwest Territories (NT), healthy populations of Dall's sheep, mountain woodland caribou, moose, and mountain goats are the foundation for subsistence hunting, tourism, and outfitted sport hunting. At the same time, there is a growing movement towards promotion and development of an economically sustainable agricultural industry in the NT, including raising of domestic and exotic species for meat, milk, and wool/hair. Additionally, both domestic goats and llamas are becoming increasingly popular as pack animals for back-country expeditions.

To protect the wildlife of the NT while at the same time developing the agriculture industry, it is critical to understand: 1) the risk of disease introduction from domestic livestock and exotic species, 2) the risk of disease transmission between wild and domestic/exotic animals, and 3) how these risks can be mitigated with minimal impact on both sectors. To this end, we conducted a literature-based risk assessment and developed a framework for legislators, wildlife managers, and domestic animal producers to pro-actively make informed decisions that minimize risks to wildlife health.

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## INFLUENCE OF HOST HABITAT ON THE HELMINTH COMMUNITIES IN BLUE-WINGED TEAL

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### Abstract

Blue-winged teal are exposed to a wide array of habitat types during migration. The consequence of which is exposure to helminth species that may or may not be found on the breeding grounds. To learn more about the relationships between hosts, habitats, and helminths, we examined helminth communities of blue-winged teal collected from brackish and freshwater habitats. Thirty blue-winged teal were collected from each of the two habitat types. Blue-winged teal carcasses were placed on ice, viscera were fast frozen in the field, and both were stored in freezers. At necropsy, helminths were removed, identified, and counted. In blue-winged teal collected from brackish habitats, prevalence of trematodes, cestodes, nematodes, and acanthocephalans was 100, 100, 100, and 23%, respectively; whereas in hosts collected from freshwater habitats prevalence was 100, 90, 100, and 43%, respectively. At least one species, *Psilochasmus* sp., only occurred in birds collected from brackish habitats, whereas *Echinostoma* sp. occurred only in hosts collected from freshwater habitats. *Trichobilharzia* sp. occurred in all hosts examined. This study will aid in understanding how host habitat selection affects helminth communities in blue-winged teal.

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## AVIAN INDICATORS OF WEST NILE VIRUS IN GEORGIA IN 2002 AND 2003

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### **Abstract**

West Nile virus (WNV) was first detected in the state of Georgia in the summer of 2001. Since then, dead bird surveillance, human and equine cases, and live bird serology have illustrated a nearly complete spread of WNV across the state. As amplifying hosts of WNV, avian species play an important role in the distribution and epidemiology of the virus. The objective of this study was to identify avian species that could serve as indicators for WNV over the physiographic and land use variation present in the southeastern United States.

A total of 6,750 avian serum samples from birds captured throughout Georgia during the summers of 2002 and 2003 were tested by plaque reduction neutralization test (PRNT) for antibodies to WNV and a closely related *Flavivirus*, St. Louis Encephalitis virus. Four hundred and fifty of these samples were found positive for antibodies to WNV. WNV antibody positive Northern Cardinals (*Cardinalis cardinalis*) and Northern Mockingbirds (*Mimus polyglottos*) were distributed across all land use types and physiographic regions, with wetland areas being least represented. Positive Rock Doves (*Columba livia*) had high antibody titers against WNV, however sampling sites for positive birds was not well distributed across all physiographic regions and land use types. Northern Cardinals appear to be the best indicators of WNV in the state of Georgia.

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## ASSESSING RELATIVE VULNERABILITY OF CHRONIC WASTING DISEASE INFECTED MULE DEER TO VEHICLE COLLISIONS

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### **Abstract**

Since 1996, tissue samples have been collected from deer killed in vehicle collisions throughout Colorado as part of a monitoring program for detecting chronic wasting disease (CWD) in free-ranging populations. We estimated CWD prevalence among vehicle-killed mule deer statewide and compared the estimate to CWD prevalence among the surrounding mule deer population to determine if CWD-infected mule deer are more vulnerable to vehicle collision. Overall prevalence was 66% higher in the vehicle-kill population; prevalence for vehicle-killed deer was 0.101 (95% confidence interval [CI] = 0.064–0.139) compared to 0.061 (95% CI = 0.051–0.072) for mule deer harvested or culled in the vicinity of vehicle-kills. The probability of detecting a CWD-infected, vehicle-killed deer, given that there is at least one other CWD-infected deer within a 3-km radius of the vehicle-kill site, was 16.67%. Our data suggest increased susceptibility of CWD-infected individuals to vehicle collisions. It follows that using vehicle-kill mule deer may be exploited in designing surveillance programs for detecting new foci of infection, but that this differential vulnerability also may bias estimates of CWD prevalence in natural populations. Evidence of increased susceptibility to vehicle collisions may aid in understanding vulnerability of CWD-infected individuals to other forms of death, particularly predation.

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## USE OF MAGNETIC RESONANCE IMAGING TO INVESTIGATE NEUROLOGIC DYSFUNCTION IN A SOUTHERN HAIRY-NOSED WOMBAT (*Lasiorhinus latifrons*)

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### Abstract

A captive adult female Southern hairy-nosed wombat (*Lasiorhinus latifrons*) was discovered recumbent and unable to rise. Small patches of blood were present in her pelage and around her muzzle. She had appeared completely normal when last seen the previous evening. Physical examination revealed mild scrapes and lacerations, predominantly down the left side, consistent with convulsive or struggling self trauma. She also displayed rigid paralysis, an inability to raise herself or stand (with stimulation producing only uncontrollable rolling to the left), evenly dilated pupils which were responsive only to intense light, continuous nystagmus, tachypnoea and anxiety. Urine and CSF samples were normal and serial testing for toxoplasmosis proved negative. Blood samples revealed lymphocytosis and azotaemia. Supportive care was provided for the next 17 days during which the lymphocytosis and azotaemia resolved. Her neurologic condition improved slightly, however her physical condition deteriorated over this period.

Detailed neurologic examination at this point revealed mildly rigid paresis with significant muscle wastage, nearly absent postural reactions, depression (strong stimulation producing some mild paddling only), constant chewing, right head tilt, significant struggling if placed on left side, right side of head and ears cooler than left, profound blindness and deafness, and absent pupillary, menace and startle responses (to light, pain, movement and arousal) although pupils were even. Ophthalmoscopic examination, nystagmus, strabismus, cranial motor and sensory functions, olfaction and spinal reflexes all appeared normal. The problem could then be categorized as left central vestibular disturbance with possible partial involvement of cranial nerves II and VIII (producing visual and auditory deficits). However, there was still no indication of the cause of the disturbance and, thus, no way to determine treatment or prognosis.

It was decided to attempt magnetic resonance imaging (MRI) as this technique produces excellent soft tissue definition, particularly in the cranial region where soft tissue is difficult to visualise. Prior to transport to the MRI unit, the wombat was briefly anaesthetized (isoflurane in oxygen via a face mask) to place a catheter in each cephalic vein. At the unit, anaesthesia was induced using IV propofol. The animal was intubated and anaesthesia maintained using isoflurane in oxygen. As no metallic objects (or people) can remain inside the shielded MRI room during imaging, anaesthesia was maintained remotely using manual IPPV. The wombat was strapped into the MRI machine in dorsal recumbency with anaesthetic and oesophageal

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stethoscope extension tubing running into the next room. There was no visual contact with the wombat, so the anaesthetist relied on the oesophageal stethoscope alone to judge anaesthetic depth. A special MRI pulse oximeter, present in the MRI room, was used periodically to support the stethoscopic monitoring. A 20-min regular MRI series was followed by a further 20-min contrast MRI series. Had either of these series been interrupted due to anaesthetic difficulties it would have been necessary to repeat them from the beginning. The procedure and recovery went extremely smoothly.

The MRI clearly revealed a focal oedematous lesion in the upper aspect of the cerebellum on the left side with a small area of extension to the left dorsolateral aspect of the brain stem. The lesion strictly respected the midline and vascular boundaries and was consistent with an area of subacute infarction in the region of the left superior cerebellar artery. The nature of the embolus was unknown. Nor was any embolic source ascertained on later cardiac ultrasound or blood culture. No other cranial abnormalities were detected and no cause for the deafness or blindness could be found. In humans and domestic species it is not uncommon that cerebellar infarction can occur spontaneously, unassociated with any event or underlying / pre-existing illness. As the wombat appeared normal the day before she became ill, this spontaneous sequence seemed quite feasible in her case.

Prognostically, many humans and domestic animals with spontaneous cerebellar infarction eventually recover to almost normal function. Once a plateau is reached during convalescence, it is likely that this will be the permanent state of the patient. There is no treatment other than supportive care. In the case of the wombat it was therefore decided to continue supportive care and monitor progression. After a further 2 wk the neurologic examination was repeated by a human neurologist producing findings consistent with multifocal CNS disease, bilateral optic atrophy and profound bilateral deafness. At this time her condition had plateaued at a level that was considered unacceptable in terms of quality of life and she was euthanatized.

Upon sectioning the fixed brain, a large malacic focus was identified within the left caudal cerebellum extending into the left dorsal region of the posterior colliculus. Histologically there was multifocally extensive encephalomalacia in the cerebellum and posterior colliculus, and mild, non-suppurative, focally eosinophilic encephalitis. The foci of malacia found within the cerebellum and posterior colliculus were consistent with the lesions seen on the MRI.

There is one further curious point that may or may not have had any bearing on part or all of this case. The wombat had recently recovered from a 2-mo period of profound sedation following administration of 20mg of flufenazine (1mg/kg) some 3 mo prior to her illness.

MRI has very rarely been used on non-domestic species in Australia due to prohibitive costs, restraint / anaesthetic difficulties, lack of suitable facilities and ethical concerns regarding human waiting lists (there are no purely veterinary CT or MRI machines in Australia). As a result, cranial infarctions (and other cranial soft tissue lesions) have not previously been able to be confirmed ante mortem in these species. To our knowledge, this was the first wombat to undergo

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advanced imaging in this country. The diagnosis provided by MRI and the subsequent progression provided a solid foundation for treatment decisions and enough prognostic information to ultimately indicate the need for euthanasia.

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## WEST NILE VIRUS DETECTION IN AVIAN CARCASSES IN THE WEST CENTRAL UNITED STATES, 2003

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### **Abstract**

We tested 343 dead birds of 58 species for West Nile virus (WNV) infection from mid-July to September 2003 at the Centers for Disease Control and Prevention in Fort Collins, Colorado. The vast majority of bird carcasses tested was from Colorado (19 counties), with a relatively small number of birds from Wyoming (2 counties) and Nebraska (3 counties). The sample set consisted of dead birds found by the public, as well as birds that died or were euthanatized at wildlife rehabilitation centers. The gold standard test in our study was TaqMan RT-PCR of heart samples with two sets of primers. Additional tests included VecTest® WNV Antigen Assay of oral swabs and plaque assay of heart samples. Thirty-two percent of birds tested positive by TaqMan RT-PCR of heart; concurrent experimental evaluation of VecTest® WNV Antigen Assay of oral swab indicated an overall sensitivity of 70%. When birds were separated into groups, VecTest® sensitivities were 85% (n = 60) for corvids, 44% (n = 27) for raptors, and 62% (n = 26) for other (non-corvid, non-raptor) bird species. The sensitivity of plaque assay of heart tissue was 89% as compared to TaqMan RT-PCR of heart tissue, with 88% sensitivity for corvids, 89% for raptors, and 92% for other species. We recognize that none of these tests are 100% sensitive for WNV, but based on our results, we believe that VecTest® WNV Antigen Assay of oral swab is a reasonable test for use in corvids, and plaque assay is a relatively useful test in all bird species to detect the presence of WNV when RT-PCR is not available. Our results may be useful for WNV surveillance as well as diagnostic purposes.

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## BATS, RATS AND CIVET CATS: POPULATION DYNAMICS, MAINTENANCE AND RESERVOIR CHARACTERISTICS OF TWO EMERGING INFECTIOUS DISEASES

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### Abstract

Hendra virus (HeV) is a recently emerged paramyxovirus in the genus *henipavirus*. HeV came to our attention in 1994 and 1999 when three separate outbreaks caused the death of fifteen horses and two people. The fruit bat (genus *Pteropus*) has been identified as the natural wildlife reservoir of HeV. All four mainland species of Australian pteropid bats have antibodies to HeV but the seroprevalence differs significantly between species. In this study we use mathematic models to investigate the maintenance strategies of HeV in populations of *Pteropus poliocephalus*, *P. scapulatus*, *P. conspicillatus*, and *P. alecto*. We identify mechanisms that may lead to species differences in seroprevalence and explore how these differences could affect the comparative risk of spillover from pteropid species to domestic animals and humans. Our results indicate that HeV cannot be maintained in bat populations with simple SIRS-like dynamics. However, incorporating metapopulation dynamics, latency or loss of resistance into the model led to maintenance of infection over time. Furthermore, our model indicated that *Pteropus scapulatus* is the species most likely to act as a reservoir for HeV in wild populations. We contrast the dynamics of HeV with that of Sudden Acute Respiratory Syndrome (SARS) in proposed wildlife hosts. The dynamics of this coronavirus are vastly different to that of HeV and we reflect on the different reservoir characteristics required to maintain these 2 viruses.

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## THYROID HISTOLOGY AND HORMONE CONCENTRATIONS IN THE BOWHEAD WHALE INTERPRETED WITH RESPECT TO HISTOLOGIC, SEASONAL AND CONTAMINANT-RELATED FACTORS

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### **Abstract**

Thyroid activity was investigated in the bowhead whale (*Balaena mysticetus*) during the spring and fall phases of their annual migration from the Bering Sea to the Beaufort Sea. Histologic sections from thyroid glands (n = 27) were examined in conjunction with serologic thyroid hormone analyses (n = 50). Serum was assayed for triiodothyronine (total {tT<sub>3</sub>}, free {fT<sub>3</sub>}) and thyroxine (total {tT<sub>4</sub>} and free {fT<sub>4</sub>}) via radioimmunoassay. Thyroid tissue was assessed via light microscopy and the utilization of an epithelial-follicular index (EFI, via methodology developed by Sorkin, 1971).

Results show no effect of age, sex or season on serum hormones. However, a seasonal effect was noted on the epithelial follicular index (EFI), with a significant difference in the height of the follicular lining being noted in spring (versus fall) samples. All results were compared to additional data collected in these whales, including vitamin A and E and organochlorine concentrations in the liver, serum and blubber.

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**EVALUATION OF THE WESTERN IMMUNOBLOT FOR USE IN DIAGNOSING *Brucella abortus* INFECTIONS IN ELK**

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**Abstract**

As the prevalence of wildlife and cattle brucellosis in the United States continues to fall, the effects of false positive serology become increasing detrimental to both wildlife and agricultural managers. Cross reactions between commensal bacteria that share surface proteins with *Brucella* on standard serology have continued to frustrate the efforts of regulatory agencies to control *Brucella* and increase the need for more reliable methods of diagnosing infection. Because of these difficulties, the western immunoblot was evaluated for use in diagnosing *Brucella abortus* infections in elk. 133 samples encompassing 4 different elk herds were tested using management standard buffered antigen and CARD serology as well as western immunoblots. Samples were analyzed from Wyoming animals experimentally challenged with *Brucella abortus* strain 2308 and animals naturally infected with *B. abortus* biovar 1 as positive controls. For negative controls, sera from two different CA elk herds were used. Negative serologic tests, negative cultures, and no history of herd infection or disease support non-infected status. By comparing sensitivities and specificities among the tests used, the western immunoblot indicates higher reliability than standard serology in establishing disease status in elk.

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**PREVALENCE AND ANTIBIOTIC SENSITIVITY OF *Campylobacter* AND *Salmonella* spp. FROM THE GASTROINTESTINAL TRACT OF WILD AND STRANDED NORTHERN ELEPHANT SEALS (*Mirounga angustirostris*)**

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**Abstract**

*Salmonella* and *Campylobacter* spp. are zoonotic, pathogenic bacteria that can cause gastrointestinal disease. *Salmonella* has previously been reported in multiple marine mammal species, although not in northern elephant seals (*Mirounga angustirostris*). *Campylobacter* has not previously been reported in marine mammals. The Centers for Disease Control is finding increasing antibiotic resistance in both of these bacterial species.<sup>1</sup> Increasing antibiotic resistance in both of these bacterial genera is a concern for human and veterinary medicine because it causes an increase in mortality, morbidity, and cost of treatment. There are also increasing reports of antibiotic resistance in marine wildlife.<sup>2-3</sup> The source of the resistance in marine wildlife is not known, however they may be exposed to bacteria and/or antibiotics when near high population coastal areas which may be contaminating the marine environment. Once these marine animals are exposed, they can continue to shed antibiotic resistant bacteria in the environment. The prevalence and antibiotic sensitivity of *Salmonella* and *Campylobacter* have not been previously established in northern elephant seals.

In this study, *Campylobacter* and *Salmonella* species were isolated from juvenile wild northern elephant seals at two different colonies in California, Point Reyes and Año Nuevo, and from seals presenting for rehabilitation at The Marine Mammal Center (TMMC) in the months of February through June in 2003. Rectal swabs were performed on the seals, selective culture techniques were used, and isolates were then identified as *Campylobacter* and *Salmonella* through standard identification techniques. Antibiotic sensitivities were obtained by either broth microdilution or agar dilution methods.

In the Point Reyes colony, *Campylobacter* was detected in only one animal (n = 32) with no evidence of *Salmonella* infections. In the Año Nuevo colony, *Salmonella* prevalence was 8.8% and *Campylobacter* prevalence was 32.4% (n = 34). The difference in *Campylobacter* prevalence between the two colonies was found to be statistically significant. In elephant seals stranded and admitted to rehabilitation, the prevalence of *Salmonella* spp. was 37.3% (n = 102), *Campylobacter jejuni* was 33.7% (n = 101), *Campylobacter lari* was 6.9% (n = 101) and a novel *Campylobacter* sp. was 11.9% (n = 101). *Salmonella* serotypes were Typhimurium, Newport, Saint-Paul, Montevideo, or Reading. Seals which were stranded and being admitted to TMMC

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showed a higher prevalence of both *Salmonella* and *Campylobacter* when compared to wild seals which was statistically significant. *Salmonella* and *Campylobacter jejuni* isolates were sensitive to all antibiotics tested for, with a few exceptions in stranded seals. This study demonstrates that *Campylobacter* and *Salmonella* are common in seals that are in relatively close association with humans, and that prevalence of antibiotic resistance exists but is low in these seals.

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**SPATIAL ANALYSIS OF THE DISTRIBUTION OF *Ehrlichia chaffeensis*, CAUSATIVE AGENT OF HUMAN MONOCYTOTROPIC EHRLICHIOSIS, ACROSS A MULTI-STATE REGION**

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**Abstract**

*Ehrlichia chaffeensis*, the causative agent of human monocytotropic ehrlichiosis (HME), is maintained in a zoonotic cycle involving white-tailed deer (WTD; *Odocoileus virginianus*) as a vertebrate reservoir and the lone star tick (*Amblyomma americanum*) as the principal biologic vector. Using data from a prototypic white-tailed deer *Ehrlichia chaffeensis* surveillance system, we modeled the probability of *E. chaffeensis* occurrence using geostatistic analyses (kriging) and logistic regression. The analyses included the *E. chaffeensis* serostatus of 563 counties from 18 south-central and southeastern states. Cross-validation showed that kriging accurately predicted counties with high HME risk (87%). Large clusters of negative counties were accurately identified, but negative counties surrounded by large numbers of positive counties tended to be misclassified as high risk. Logistic regression modeling of the entire region and three subregions detected climatic and land cover variables significantly associated with *E. chaffeensis* occurrence. The accuracy of each subregion model (78-85%) was higher than the regional model (75%). Use of subregions also greatly increased the specificity from 39% for the regional model to 48-68% for the subregional models. The predicted *E. chaffeensis* distribution had good concordance with human case data. The integration of a WTD surveillance system with geostatistic and logistic regression analyses was useful in developing HME risk maps.

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## CYSTINE UROLITHIASIS AND CYSTINURIA IN CAPTIVE SERVALS (*Leptailurus serval*)

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### Abstract

Cystinuria is caused by hereditary defects in the renal basic amino acid transporter. The lack of appropriate resorption leads to varied losses of cystine, ornithine, lysine, and arginine, however, it is the insolubility of cystine in acid urine which leads to cystine crystaluria and urolithiasis. Cystinuria has been reported in humans, many canine breeds, and several cats.<sup>3</sup> Cystinuria and cystine uroliths have also been reported in a captive caracal (*Caracal caracal*, formerly *Felis caracal*)<sup>5</sup> and captive and free-ranging maned wolves (*Chrysocyon brachyurus*).<sup>1,2</sup> In humans cystinuria is inherited as an autosomal recessive trait. Similarly an autosomal recessive mode of transmission has been documented in Newfoundland dogs with severe cystinuria due to a missense mutation in the rBAT gene.<sup>3,4</sup> A diagnosis of cystinuria can be made based upon a urinary nitroprusside screening test, the presence of hexagonal cystine crystals in urine sediment, urolith crystal analysis, and urinary amino acid analysis (Josephine Deubler Genetic Disease Testing Laboratory (PennGen) at the University of Pennsylvania).

We report here on two captive male servals (*Leptailurus serval*) with obstructive cystine urolithiasis as well as the urinary nitroprusside test results on a captive serval population at the same facility (n = 24). Clinically affected servals were 16 and 4 yr old; the first was the grand sire of the second serval. Both presented with a brief history of anorexia and acute signs of urinary obstruction. Radiographically, cystic calculi were identified as radio-opaque structures in the urethra. During surgery it was noted that the bladder was ruptured in one case and severely inflamed in the second. There were numerous calculi in the bladder and urethra (50-100). The calculi were yellow brown in color and ranged between 2 and 5 mm in size. Based upon crystallographic analysis the calculi contained purely cystine. Surgical correction was attempted in the second case (perineal urethrostomy). However, intraoperative findings and serum biochemistry results indicated a poor prognosis for these two animals and euthanasia was elected. All the servals at the facility are maintained on the same diet (fresh/frozen rodents) and water *ad libitum*.

Furthermore, the urine of several related servals kept in the same facility tested positive for cystine by the nitroprusside test, but have not developed any calculi based upon radiographs and

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clinical observation. As dietary measures seem not to be rewarding in other species emphasis on diuresis, alkalinizing, and chelation may be considered in cystinuric servals.

Cystinuria and cystine calculi should be included as a differential diagnosis for servals with urinary tract problems. Further studies are needed to define the renal transport defect and mode of inheritance. It is important to determine the heritability of diseases since heritable diseases are a key factor in captive breeding programs.

#### **ACKNOWLEDGMENTS**

Supported in part by a grant from the National Institutes of Health (RR #002512).

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## ORAL EOSINOPHILIC GRANULOMAS IN TIGERS (*Panthera tigris*): A COLLECTION OF FOUR CASES

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### Abstract

Eosinophilic granulomas (EG) were diagnosed in four adult tigers (*Panthera tigris*) from three separate collections. Age at initial diagnosis ranged from 8-16 yr. All lesions were located on the hard or soft palate. The gross appearance of the lesions ranged from small flat ulcers or plaques (1 × 2 cm) to large pedunculated masses (6 × 4 × 4 cm) hanging into the oral cavity. Diagnosis was made via histopathologic examination of biopsy specimens. The histopathologic features in all cases were similar to those seen in domestic cats diagnosed with eosinophilic granulomas.<sup>1</sup> In three cases, there were no clinical signs related to these lesions at the time of diagnosis. In Case 4, bleeding from the mouth and visualization of a mass during vocalizations were noted. Clinical signs coinciding with the progression of the oral lesions included inappetence, salivation, and regurgitation. In domestic cats, EGs are most often found on the thigh and are generally thought to be a result of a hypersensitivity reaction.<sup>2</sup> Hypersensitivity to seasonal allergens was demonstrated via skin testing in Case 1. No seasonality or specific underlying hypersensitivity was noted in the other three cases.

All cats were treated with corticosteroids either orally, intramuscularly, intralesionally or via a combination of routes. Other treatments included antibiotics, chlorpheniramine, ranitidine, sucralfate, flax seed oil, and a vitamin supplement. In Case 4, corticosteroid treatment was initiated after surgical debulking of two pedunculated masses. Most lesions initially responded to corticosteroids by becoming smaller or less inflamed, but would worsen after cessation of treatment. Problems suspected to have been related to treatment included inappetence, aspiration pneumonia, hepatopathy, adrenal cortical atrophy, oral botryomycosis, and cryptococcal pneumonia.

Eosinophilic granulomas may be underreported in large felids and further investigation into their etiology and treatment is warranted.

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***Yersinia pseudotuberculosis* IN A CLOSED COLONY OF EGYPTIAN FRUIT BATS  
(*Rousettus aegyptiacus*)**

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**Abstract**

*Yersinia pseudotuberculosis*, a gram negative coccobacillus with a worldwide distribution, is an important cause of disease in captive animals.<sup>1,2,4</sup> In June and July 2003, an outbreak of *Y. pseudotuberculosis* occurred in a colony of Egyptian fruit bats at the Rosamond Gifford Zoo in Syracuse, NY. Over a 6-wk period, 10 bats either died or were euthanatized due to severe clinical disease. Seven of these bats cultured positive for *Y. pseudotuberculosis*. Two of the *Yersinia*-positive bats exhibited the acute form of pseudotuberculosis, characterized by sepsis and multi-organ failure with rapid progression to death, while the remainder of the bats exhibited the chronic and debilitating form of the disease, characterized by necrotizing abscessation involving one or more organs, especially the liver, spleen, and mesenteric lymph nodes.

It is highly suspected that a wild rodent reservoir was the source of the *Yersinia* outbreak. The colony of bats, which contained 125 animals at the time of the outbreak, had been closed since its establishment from thirteen animals in 1986. In addition, primates in nearby exhibits had numerous enteric cultures for *Yersinia*, which were negative. Several mice trapped from the region of the exhibit have cultured negative for *Y. pseudotuberculosis*, however they are still considered to be the most likely origin of the infection in this colony. Reported species differences in susceptibility to clinical disease from *Y. pseudotuberculosis* may indicate that fruit bats are predisposed.<sup>3</sup> Furthermore, stress, which has been implicated as being an important element in precipitating outbreaks of *Y. pseudotuberculosis*,<sup>1,4</sup> may have been a significant contributing factor in this colony, which was overpopulated and may have also had an inappropriate gender ratio (D. Heard, pers. comm.). This hypothesis is supported by the simultaneous diagnosis of several other diseases often associated with general debilitation and immunosuppression, including microsporidiosis, generalized cutaneous demodicosis, and mycobacteriosis.

Due to the suspected high prevalence of *Y. pseudotuberculosis* in the remaining bats in the colony, the close proximity of other animal exhibits, and the zoonotic potential of this pathogen, the entire colony was depopulated. Gross necropsy was performed on the 115 euthanatized bats, revealing that 80 (70%) of the bats exhibited gross evidence of potential infection with *Y. pseudotuberculosis*, especially hepatic abscessation, splenomegaly, and mesenteric lymphadenopathy.<sup>2,4</sup> Studies are ongoing to document the prevalence of *Y. pseudotuberculosis*

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in this colony, characterize the pattern of gross lesions typical of this disease in Egyptian fruit bats, and describe the epidemiologic patterns involved in this outbreak.

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## A RETROSPECTIVE STUDY OF MORBIDITY AND MORTALITY OF CAPTIVE NORTH AMERICAN JAGUARS (*Panthera onca*): 1982-2002

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### **Abstract**

This study determined common causes of morbidity and mortality, and the affect that age, sex, and melengestrol-acetate (MGA) implants had on morbidity and mortality of captive jaguars (*Panthera onca*) in North America. The most common causes of morbidity in captive jaguars were found to be dental, gastrointestinal, integumentary, and musculoskeletal diseases. Prevalence of disease was shown to vary with age. However, tooth fractures, endoparasites, pododermatitis, conspecific and self-trauma, and arthritis predominated for this captive jaguar population. Other diseases or clinical signs that seemed remarkable were a high prevalence of episodes of epistaxis among jaguars. The data suggest that MGA implants increased the risk of developing female reproductive disease, and that females develop reproductive disease more frequently than males. The most common causes of mortality were reproductive disease in females and euthanasia due to musculoskeletal disease in males. Based on our findings, we present management suggestions for the captive jaguar population.

### **Introduction**

The jaguar (*Panthera onca*) is a spotted South American cat, the third largest cat in the world after the lion (*P. leo*) and the tiger (*P. tigris*). Although the jaguar was once distributed from the southwestern United States through southern Argentina, its home range and population have diminished in the past century due to habitat destruction and hunting. In 1973, jaguars were placed on CITES Appendix I. The captive jaguars housed at American Zoo and Aquarium Association (AZA) institutions offer an invaluable resource for education on jaguar conservation and protection.

The primary objective of this study was to determine the most common causes of morbidity and mortality of captive jaguars held at AZA-accredited institutions in North America between 1982 and 2002. Additionally, we examined age and sex as factors of morbidity and mortality. In females, we recorded melengestrol-acetate (MGA) implant history to determine the association with reproductive disease.

### **Materials and Methods**

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We examined the medical records of 172 (84.82.6) jaguars and the necropsy reports of 84 (36.42.6) jaguars housed at 30 AZA institutions.<sup>4</sup> Data were tabulated via body system, age, sex, and for adult females the MGA implant history was recorded. Prevalence of body system diseases was determined within each age group by tallying each individual that displayed a body system disease during that age range. For body system categories in which >20% of the population was diseased, the most commonly reported types of disease were determined.

Sex comparisons were made by comparing total number of males with disease of a body system to total number of females with disease of that body system. To determine the impact of implants on female reproductive disease, we recorded the number of implants received by females older than 2 yr old, and number of cases of reproductive disease. Causes and ages of mortality were recorded for males and females. Diseases that resulted in euthanasia were tallied based on the clinical signs that led the animal to be euthanized.

### **Morbidity Within Age Groups**

Prevalence of disease within each body system was determined (Table 1). For body systems in which > 20% of the age group population displayed a disease, the most commonly reported diseases in that body system are listed below.

0 to <2 yr (n = 85 jaguars)

*Gastrointestinal*—83% (15/18 cases)-Endoparasites or bacterial infection. One case each of gastric mucosal calcification, necrotic enteritis, and pancreatitis.

2 to <5 yr (n = 53 jaguars)

*Gastrointestinal*—75% (9/12 cases)-*Endoparasites or bacterial infections. One case each of chronic vomiting, GI ulcerations, and pancreatic exocrine insufficiency.*

*Integument*—28% (5/18 cases)-Foot pad lesions (pododermatitis, cracked pads). 28% (5/18 cases)-Skin lesions due to conspecific trauma. 11% (2/18 cases)-Skin lesions due to self-trauma. 11% (2/18 cases)-Idiopathic abrasions. 11% (2/18 cases)-Dermatitis. One case each of ingrown nails and a nonspecific subcutaneous mass.

*Neurologic/Behavioral*—100% (11/11 cases)-Self-mutilating behaviors, primarily tail sucking.

5 to <16 yr (n = 99 jaguars)

*Dental*—49% (23/47 cases)-*Tooth fractures. 38% (18/47 cases)-Moderate to severe calculus or dental caries. 9% (4/47 cases)-Periodontal disease.*

*Gastrointestinal*—59% (22/37 cases)-Endoparasites and bacterial infections. 16% (6/37 cases)-Inflammatory diseases. One case each of ulcerations, chronic idiopathic vomiting, foreign body ingestion and pancreatic adenocarcinoma.

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Integument—20% (12/61 cases)-Foot pad lesions. 18% (11/61 cases)-Conspecific trauma. 15% (9/61 cases)-Self-trauma. 15% (9/61 cases)-Inflammatory skin reactions (e.g., dermatitis, MGA implant reactions). 10% (6/61)-Ingrown nails. One case each of subcutaneous masses, lipomas, unexplained abrasions, hyperkeratosis, granulomas, and lymphosarcoma.

16 to 25 yr (n = 42 jaguars)

Dental—41% (14/34 cases)-Tooth fractures. 32% (11/34 cases)-Calculus and caries. 24% (8/34 cases)-Periodontal disease.

Gastrointestinal—35% (6/17 cases)-Endoparasites and bacterial infections. 24% (4/17 cases)-Inflammatory processes (e.g., peritonitis, gastroenteritis). 18% (3/17 cases)-Pancreatic carcinomas.

Hematologic—46% (6/13 cases)-Epistaxis. 23% (3/13 cases)-Anemia. 15% (2/13 cases)-Splenic disease. 15% (2/13 cases)-Prolonged bleeding.

Hepatic—21% (3/14 cases)-Hepatic lipidosis. 14% (2/14 cases)-Hepatitis. 14% (2/14 cases)-Primary hepatic neoplasia. One case each of cysts, cholestasis, hepatomegaly, steroid hepatopathy, and idiopathic liver failure.

Integument—27% (9/33 cases)-Ingrown nails. 21% (7/33 cases)-Inflammatory processes (e.g., dermatitis, reactions to MGA implants). 18% (6/33 cases)-Self-trauma (especially to tail). One case each of foot pad lesions, sebaceous cysts, lipomas, and hyperkeratosis.

Musculoskeletal/Neuromuscular—36% (9/25 cases)-Joint diseases (e.g., arthritis, degenerative joint disease, trauma). 36% (9/25 cases)-Generalized signs (e.g., ataxia, progressive hindlimb weakness, unexplained lameness). 12% (3/25 cases)-Bone lesions (e.g., fractures, osteomyelitis). 16% (4/25 cases)-Diseases of spinal processes (e.g., intervertebral disc disease, spondylosis).

Neurologic/Behavioral—71% (10/14 cases)-Behavioral (e.g., psychogenic feline alopecia, tail sucking, pacing). One case each of encephalitis, head tremors, seizures, and encephalomalacia.

Renal—29% (6/21 cases)-Idiopathic “renal disease” or “renal failure”. 24% (5/21 cases)-Glomerulonephritis or interstitial nephritis. 14% (3/21 cases)-Renal cysts. 10% (2/21 cases)-Hydronephrosis. 10% (2/21 cases)-Cystitis. 10% (2/21 cases)-Pyelonephritis. 5% (1/21 cases)-Renal hypertension.

Reproductive (Female)—71% (15/21 cases)-Neoplasia. 19% (4/21 cases)-Hyperplasia or cysts. One case each of pyometra and mineralization of the uterus.

Respiratory—36% (4/11 cases)-Pneumonia. 18% (2/11 cases)-Rhinitis. 18% (2/11 cases)-Atelectasis. One case each of emphysema, sinusitis, and granulomas.

### **Morbidity Associated with Sex and MGA Implant History**

Our data suggest that females have a significantly higher prevalence over males of reproductive disease (33% vs 5%). Sixty percent of females that had received at least one MGA implant (n =

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25) developed some form of hormonally-linked reproductive tract or mammary disease, compared to 32% of non-implanted females (n = 37). The diseases that were most frequently represented in this population include endometrial hyperplasia, pyometra, ovarian cysts, ovarian papillary cystadenocarcinoma, and mammary adenocarcinoma.

### **Mortality**

Reproductive disease (18%), musculoskeletal disease (13%), and stillbirths/perinatal deaths (10%) were the top 3 causes of mortality in captive jaguars. For males, the most common cause of death was euthanasia due to musculoskeletal disease (22%). For females, reproductive disease was the most common cause of mortality, accounting for 36% of deaths. Interestingly, 47% of the females that died from reproductive disease had never received an MGA implant. Causes of death for eleven jaguars were unspecified in the records.

### **Discussion**

Gastrointestinal disease represented a significant portion of jaguar morbidity in all age groups. The primary cause of gastrointestinal health problems was parasite infestations. In most instances, these infestations were treated with anthelmintics and cleared before clinical signs became apparent. The prevalence of inflammatory gastrointestinal diseases such as peritonitis and gastroenteritis increased with age.

Integument diseases, frequently seen in jaguars over 2 yr old, included cracked pads, pododermatitis, dermatitis, abscesses, trauma from cage-mates, ingrown nails, and self-trauma. Cracked pads may be related to concrete enclosures, and pododermatitis can be caused by *Staphylococcus aureus* infections or immune-mediated processes.<sup>1</sup> Inflammatory responses to MGA implants were reported fairly frequently in adults. Lacerations and bite wounds from cage-mates may have occurred due to territorial disputes, and could be prevented by housing jaguars separately or providing more individual space. In older cats, ingrown nails became a problem. Nails should be trimmed regularly. Self-trauma, classified as a neurologic/behavioral disease in this study, often resulted in secondary integumentary damage, causing a raw, inflamed tail. Enrichment devices and activities should be supplemented and the cats should be provided adequate space and areas to hide to prevent self-trauma from boredom or stress.

Dental disease was very common in jaguars 5 yr and older. Fractured canines were the most common dental lesion, followed by moderate to heavy tartar. Periodontal disease was seen in older jaguars. Annual exams should always include a dental exam and treatment when deemed necessary. Chewing on bars and fencing promotes tooth fractures, and enrichment devices should be given to encourage jaguars to chew dental-friendly items.

Musculoskeletal, reproductive, renal, hematologic, hepatic, and respiratory diseases were important causes of morbidity in animals 16-25 yr old. Musculoskeletal diseases observed included arthritis, spondylosis, and intervertebral disc disease. Arthritis can be immune-

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mediated or degenerative. Arthritis may also be related to cement housing.<sup>1</sup> Obesity should be prevented as it can also be a contributing factor to developing arthritis and other musculoskeletal diseases. Reproductive diseases were highly prevalent in females between 16-25 yr, well beyond the age of last reproduction of wild jaguars (8 yr).<sup>2</sup> We suggest that MGA contraceptive implants, as indicated in other studies,<sup>3</sup> may increase the risk of reproductive disease. Additionally, females who received at least one MGA implant appeared to be more likely to acquire reproductive disease than those who had never received an MGA implant. MGA implants appear to increase risk of developing reproductive tract disease, and should be seriously considered before being used as a contraceptive device. The most common report of renal disease in geriatric jaguars was renal failure of unknown etiology. Cystitis and glomerulonephritis were also reported in several jaguars. Hematologic diseases, such as anemia and epistaxis, became more prevalent in geriatrics. Epistaxis was a surprisingly common problem in these jaguars, and was present through all age groups. Due to the high frequency of epistaxis in the population, and because epistaxis can indicate a primary bleeding disorder, future cases of epistaxis should be worked up for etiology. Hepatic lipidosis was the most frequently reported liver problem, possibly related to problems of obesity in captive jaguars. Other hepatic diseases included hepatitis and primary hepatic neoplasia. Pneumonia was a common respiratory diagnosis. Of the infectious pneumonias, *Actinomyces israelii*, *Flavobacterium* spp., *Pasturella* spp., *Penicillium* spp., *Candida* spp., and *Aspergillus* spp. were cultured from the lungs.

Episodes of mortality in jaguars less than 2 yr old tended to be due to stillbirths, trauma, or pneumonia. Jaguars older than 5 yr tended to die from reproductive tract disease or musculoskeletal/neuromuscular disease.

Based on the findings of this study, we present the following management suggestions:

Full dental exam and treatment performed at every annual exam. Avoid types of fencing or bars in enclosure that may contribute to fractured teeth.

Early diagnosis and aggressive treatment for pododermatitis and cracked pads.

Thorough workups of epistaxis and anemia cases.

Solitary housing with enrichment for aggressive animals.

Use a softer substrate rather than housing animals on cement.

Avoid using MGA implants—Ovariohysterectomies recommended instead.

Keep complete medical records. Include pathology and necropsy reports for every animal.

#### ACKNOWLEDGMENTS

The authors thank the Friends of the National Zoo, Bob Wiese, Abilene Zoological Gardens, Akron Zoological Park, Audubon Zoo, Caldwell Zoo, Denver Zoo, Elmwood Park Zoo, Erie Zoological Gardens, Fort Worth Zoo, Gladys Porter Zoo, Granby Zoo, Henry Doorly Zoo, Houston Zoo, Jacksonville Zoological Gardens, Knoxville Zoo, Lee Richardson Zoo, Louisville Zoological Gardens, Memphis Zoo, Milwaukee County Zoo, Smithsonian's National Zoological Park, Oklahoma City Zoo, Palm Beach Zoo, Philadelphia Zoo, Rio Grande Zoo, Sacramento Zoo, Saint

Louis Zoological Park, Salisbury Zoo, San Antonio Zoo, San Diego Zoo, Tulsa Zoo, and Woodland Park Zoological Gardens.

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**Table 1.** Prevalence of disease (%) within age groups of captive jaguars (*Panthera onca*).

Body system	0 - <2yrs (n = 85)	2- <5yrs (n = 53)	5-<16yrs (n = 99)	16-25yrs (n = 42)	All ages (n = 172)
Aural	0	1.9	1	0	1.14
Cardiac	1.2	0	13.1	14.3	10.2
Dental	3.5	11.3	41.4	52.4	35.2
Endocrine	1.2	3.8	5.1	9.5	6.8
Gastrointestinal	21.2	22.6	28.3	35.7	36.4
Hepatic	2.4	5.7	15.2	21.4	15
Hematologic	1.2	0	9.1	26.2	10.8
Integumentary	11.8	32.1	50.6	59.5	45.5
Musculoskeletal	7.1	9.4	17.2	47.6	25
Neurologic/Behavioral	1.2	20.8	15.2	31	18.8
Ocular	3.5	7.6	4.0	16.7	9.1
Renal	3.5	1.9	13.1	45.2	17.6
Reproductive	1.2	1.9	17.2	35.7	17
Respiratory	8.2	0	4.0	26.2	12.5

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## DIAGNOSIS AND TREATMENT OF LYMPHOMA IN SELECTED EXOTIC FELIDS

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### Abstract

Lymphoma is the most common neoplasm diagnosed in domestic cats.<sup>1</sup> Further, lymphoma is becoming increasingly more common in exotic felids. Approximately 70% of domestic cats with malignant lymphoma are feline leukemia virus (FELV) positive, but the rate of positives varies with the anatomic form of lymphoma.<sup>3</sup> In contrast, FELV is rarely detected in malignant lymphomas in exotic feline species. Treatments for lymphoma have been attempted in domestic cats and in general their lymphomas respond poorly to chemotherapies and have shorter remissions than other domestic animals.<sup>1,2</sup> Treatments for lymphoma in exotic felids are uncommon, usually attributed to the challenge of handling the animals, and to typically late diagnosis. Recently, however, chemotherapeutic regimens have been attempted. The actual response to the chemotherapeutic protocols is still largely unknown due to a limited sample size. This study explores 27 cases of lymphoma in exotic felids collected through zoological institutions and those published in the literature. Factors such as species, sex, age at diagnosis, methods of diagnosis, lymphoma treatment(s), and period of survival after diagnosis were compared. Species diagnosed with lymphoma included: 12 (44.4%) African lions (*Panthera leo*), 5 (18.5%) tigers (3 Amur tigers (*Panthera tigris altaica*), a bengal tiger (*Panthera tigris tigris*), and a white tiger (*Panthera tigris*)), 2 (7.4%) cheetahs (*Acinonyx jubatus*),<sup>4</sup> 2 (7.4%) mountain lions (*Felis concolor*), and one case each in a bobcat (*Felis rufus*), a clouded leopard (*Neofelis nebulosa*), a leopard (*Panthera pardus*), a pampas cat (*Felis colocolo*), a Siberian lynx (*Felis lynx*), and a snow leopard (*Uncia uncia*). Methods of diagnosis ranged from physical examination, ultrasound, radiographs, complete blood count (CBC), biopsies, fine needle aspirates, ocular examinations, and necropsy. The diagnosis of malignant lymphoma was confirmed in all cases by microscopic evaluation. Treatments consisted of no treatment, supportive care treatments, splenectomy, ovariohysterectomy and enterotomy, and various chemotherapies. Survival after diagnosis ranged from zero days to 7 mo. One case, an African lion, was treated by a splenectomy, followed by chemotherapy with doxorubicin HCl (doxorubicin hydrochloride injection, USP, Ben Venue Labs, Bedford, OH 44146 USA; 30mg/m<sup>2</sup> i.v. once), prednisone (Roxane Laboratories, Inc., Columbus, OH 43216 USA; 0.55

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mg/kg p.o. every other day), and lomustine (CCNU<sup>®</sup>, Bristol-Myers Squibb Co., Princeton, NJ 08543 USA; 60 mg/m<sup>2</sup> p.o. every 21 days for four treatments, then every 6 wk). Complete clinical remission was confirmed 2 mo after treatment was initiated through bone marrow biopsy results and a CBC. Treatment is ongoing and no clinical relapse has been noted since February 2004.

#### **ACKNOWLEDGMENTS**

The authors would like to thank the following institutions or organizations for their participation in this study: Audubon Zoo, Binder Park Zoo, Buffalo Zoo, Cincinnati Zoo and Botanical Garden, Granby Zoo, Hogle Zoo, Indianapolis Zoo, Knoxville Zoo, Minnesota Zoo, National Zoo, Philadelphia Zoo, Potter Park Zoo, San Francisco Zoo, Toledo Zoo, Toronto Zoo, Tulsa Zoo, Wildlife Way Station, and Woodland Park Zoo.

The authors would also like to thank: Potter Park Zoo's animal keepers, Gerry Brady, Dennis Laidler, and the rest of the zoo and society staff and volunteers; Ramona Kwapisyewski, CVT Surgeons of Mid-Michigan, James Ford and crew; MSU CVM: George Bohart, Lori Bidwell, Heather Flaherty, Diana Rosenstein, Bryden Stanley, Brian Teunissen, Jennifer Thomas, Cheryl Swenson, Vanessa Rengstorf, and all of the MSU CVM students who helped.

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## COMPARATIVE INVESTIGATIONS ON REPRODUCTION BIOLOGY IN DIFFERENT BEAR SPECIES

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### Abstract

### Introduction

To improve breeding success it is imperative to gain more knowledge about the reproduction biology and refine assisted reproduction technologies in species where there is a likelihood of success. The bear family (Ursidae) is an appropriate example. Some bear species, kept in few numbers in European zoos, are on the verge of extinction and efforts to conserve them are being undertaken.<sup>4,43</sup> Experimental research on suitable model species, which are more frequently distributed in zoos, is necessary to develop new reproduction technologies (International Panda Conference, San Diego 2000). New strategies of reproduction management based on these model species can be developed using refined methods of estrus detection, pregnancy monitoring and improved techniques for assisted reproduction. Later these results can be transferred to target species such as the giant panda (*Ailuropoda melanoleuca*), Malayan sun bear (*Helarctos malayanus*), and spectacled bear (*Tremarctos ornatus*). In this study the taxonomic relationship and the reproduction biology were examined within the Ursid family to detect suitable models for endangered target species. New approaches on reproduction management were presented and results from our recent investigations are compared with those in selected print sources.

What do we know about reproduction in bears from literature?

In the bear family (Ursidae) there are eight species of bears in three different subfamilies (Ursinae, Tremarctinae, Ailuropodinae).<sup>12</sup> The status of the giant panda is widely discussed but it is believed to be an early divergent from the bear family.<sup>3,36,49,51</sup> Due to genetic studies on the structure of hemoglobin, the South American spectacled bear was described to be the giant panda's closest relative and the link between the *Ailuropodinae* and the *Ursinae*. In the Ursine sub-family the polar bear (*Ursus maritimus*) is the closest relative of the brown bear (*Ursus arctos*).<sup>11,46</sup> Breeding between these two species has been reported.<sup>44</sup> The two species of black bears (*Ursus americanicus*, *Ursus thibetanus*) share a common ancestor from about 4 million yr ago but phylogenetic studies revealed that the closest relative of the American black bear is the

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Malayan sun bear.<sup>12,50</sup> The sun bear is thought to be the most ancient type of bear.<sup>28,47</sup> Its hemoglobin structure is the same as that of the polar bear and the American black bear.<sup>17,18</sup>

The habitats of the brown bear and the American black bear overlap in northern America, but inter-specific breeding has not been reported from the wild.<sup>9</sup> The giant panda and the Asiatic black bear have much in common concerning habitat.<sup>33</sup> They show a moderate ecological overlap in activity schedules and use of space but differ markedly in their feeding strategy.<sup>41</sup> The body size of the giant panda, the Asiatic black bear, the American black bear, and the spectacled bear are similar.<sup>12,51</sup>

The reproduction biology of most bears is very similar, although the giant panda and the Malayan sun bear are extraordinary within the Ursid family. The reproduction biology of the Ursids is characterized by two main traits—seasonality and delayed implantation.<sup>10,19</sup> Seasonality is largely dependent upon environmental conditions and food supply.<sup>5</sup> Most bears are seasonal with a mating period ranging from spring to early summer.<sup>15,38</sup> The only exceptions are the Malayan sun bear and the sloth bear from the island of Sri Lanka (*M. ursinus inornatus*) which are both non-seasonal.<sup>7,34</sup> All bears except the Malayan sun bear have a delayed implantation.<sup>6,33</sup> The prolonged gestation can be more or less reduced since the implantation of the embryo is regulated by the photoperiod.<sup>2</sup> As a result, the birth of bears takes place during winter hibernation. Species without hibernation, like the giant panda, deliver in late summer, or throughout the year like the Malayan sun bear.<sup>1,12,24</sup> Bear cubs are always small and underdeveloped. Among all bears, giant panda cubs are the smallest and can be bottle-fed with a sun bear formula.<sup>32</sup> The mating behavior of most bears is rather unspectacular. The giant panda is the most unusual, displaying vocalization and predominant scent marking.<sup>21,31,39,40,45</sup> In the wild bears do not interbreed but in captivity there are reports about inter-specific sexual affinities.<sup>6</sup> The American black bear was described as a model species for oocyte recovery and maturation for endangered Ursids.<sup>20</sup> The structure of the placenta is very similar in the spectacled bear and the brown bear.<sup>30</sup>

## Recent Comparative Investigations

### Morphology and Sonomorphology

To describe the sonomorphology of the male and female genital tract and to monitor the variations of the reproductive organs in detail, we performed 117 ultrasound examinations in 17.39 bears of eight species.<sup>13,23</sup> The sonographic measurements were proven by post mortem preparations in 2.5 bears of four species. The size and weight of the sexual organs varied with the body size. Our results also revealed seasonal changes in the size of the reproductive organs (Table 1). The male giant panda was outstanding in the size of its testes, especially during breeding season as described previously.<sup>8</sup> The accessory sex glands of the giant panda most resemble those of the spectacled bear, no matter what the reproduction status. The size of the testes of the brown bear, Malayan sun bear, and sloth bear are quite similar. The size of the accessory sex glands is related to the body size.

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In the females the length and weight of the reproductive tract are also related to the body size of the animals. However, the diameters of the uterus and the ovarian size vary more with the reproductive status (e.g., pregnant, pseudo-pregnant, non pregnant) than with the reproduction season.

### Endocrinology

Investigations were carried out on non-invasive estrus detection and pregnancy monitoring in three bear species (giant panda, brown bear, and spectacled bear). Hormonal profiles of gonadal steroid metabolites were established at IZW from urine and feces by enzyme immuno-assays (EIA).<sup>14,29</sup> In the Giant panda, urinary estradiol (E2) was determined and used to monitor the increase in estrogens, which was usually followed by estrous behavior and reached a maximum prior to high peak receptivity. In the brown bear and the spectacled bear several E2 peaks were measured but did not correlate with observed mating behavior. In the giant panda a second EIA was performed to show that the baseline values of urinary gestagens followed a steady increase at the assumed time of implantation. So far in the other bear species no urinary gestagen metabolites corresponding to the panda results have been found. In brown bears an EIA measuring fecal gestagens made it possible to differentiate between pregnant and pseudo-pregnant females after implantation.<sup>13</sup> Inclination of fecal gestagens were also described in Malayan sun bears after estrus.<sup>42</sup>

In addition, seasonal changes of urinary volatile substances (volatiles) were investigated by solid-phase micro-extraction in combination with gas chromatography and mass-spectra (GCMS).<sup>22</sup> A group of volatiles belonging to a single chemical family could be discovered in the giant panda and the brown bear. This estrogen-linked group appeared not only during the rise of E2 but also at the peak of E2. This led to the conclusion that for the increase of the estrogen-linked group of volatiles a certain E2-threshold had to be exceeded. The same group of volatiles was also found in the giant panda using a colorimetric enzyme assay. However this enzyme assay showed several false-positive results, which could be excluded by using extraction. In the spectacled bear, the estrogen-linked group also appeared but did not correlate with E2. A second urinary volatile, a steroid-like substance, which could only be found in the giant panda, displayed increasing oscillations towards ovulation and afterwards correlated with urinary gestagen metabolites of the giant panda.

### Conclusions

The collected data is still incomplete, but it contributes to basic research and a better understanding of comparative investigations in living animals. In the future, methods to predict estrus and to detect pregnancies will be much more precise. Concerning possible model species for endangered Ursids, our preliminary results led us to the following conclusions:

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Giant panda: Suitable model species for the Giant panda would be the Spectacled bear due to its genetic relationship and the Asiatic black bear because it occupies the same habitat and feeds on a similar diet. Each of these model species is the most equal in size and anatomy to the giant panda.

Malayan Sun bear: The Malayan sun bear is the most divergent of all the bears regarding reproduction. Due to their body size the two species of black bears are regarded as model species.

Spectacled bear: The spectacled bear's reproduction biology is similar to all members of the ursine sub-family, but because of its body size the two species of Black bears would be more suitable model species.

## Summary

The reproductive tracts of male bears (six species) and female bears (eight species) were investigated and compared in morphologic and sonomorphologic measurements. The size of the organs relied heavily on the body size of the individual and on seasonality. Female bears (three species) were tested for excretion of gonadal steroid metabolites and urinary volatile substances. A peak of an estrogen-linked group of volatiles was detected prior to estrus and pregnancies could be detected after implantation of the embryo by fecal hormone metabolites. All gained results were combined and proven by investigation of selected print sources to represent model species for endangered Ursids. For the giant panda the Asiatic black bear and the spectacled bear were found to be suitable model species; for the Malayan sun bear and the spectacled bear the more suitable model species would be the two black bears. It will be necessary in the future to undertake more research to close existing gaps comparing the species.

## ACKNOWLEDGMENTS

We would especially like to thank the Berlin Zoological Garden for sponsoring this study in form of a scholarship for a PhD student. Also we are very grateful to all Zoos and their staff who contributed in terms of making examinations possible, who collected samples or sent us material for post mortem analyses. Special thanks to Dr Lydia Kolter, Zoological Garden Cologne, for constructive comments.

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**Table 1.** Testis diameters (mm) in different bear species during breeding and non-breeding seasons measured ultrasonographically.

Species	Body weight (kg)	Breeding season	Non-breeding season
Brown bear	200-250	26.1 ± 7.1 (n = 4)	17.9 ± 2.2 (n = 2)
Malayan sun bear	70-85	24.5 ± 3.8 (n = 4)	Non seasonal
Sloth bear	74-156	No data	17.8 ± 8.4 (n = 4)
Spectacled bear	150-160	26.6 ± 3.1 (n = 8)	25.7 ± 2.1 (n = 3)
Giant panda	110	51.3 ± 3.7 (n = 6)	25.5 (n = 1)

[DJB1]

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## TRYPANOSOMA INFECTION IN SUGAR GLIDERS (*Petaurus breviceps*) AND A HEDGEHOG (*Atelerix albiventris*) FROM TEXAS

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### Abstract

Trypanosomiasis is generally considered an exotic disease of tropical Africa and India. American trypanosomiasis (Chagas' disease) which is caused by *Trypanosoma cruzi*, affects dogs, cats, armadillos, monkeys, and small wild animals.<sup>1-3,5</sup> *T. cruzi*, unlike its relatives, multiplies within the cytoplasm of the mammalian host's cells. It has a predilection for cardiac and skeletal muscle where transformation to amastigotes, a form closely resembling *Leishmania*, occurs within the cells and the collections form pseudocysts. The vector is the reduviid or 'kissing' bug (*Triatoma*). *T. cruzi* is known to be common in the triatomid bugs of south central Texas, and has caused chronic heart disease in dogs.<sup>4,6</sup> It is not considered a risk to the human population, as the triatomid bugs' behavior does not favor transmission of the protozoans (the bugs do not tend to defecate in the vicinity of the bite wound).

Marsupials in South America have been intensively studied in relationship to triatomid/trypanosoma life cycles.<sup>7</sup> Most New World species appear to be highly resistant to pathologic disease, and function primarily as reservoirs of trypanosomes. Sugar gliders (*Petaurus breviceps*) appear to be peracutely affected and infection results in death. This may be the first report of Chagas' disease in Old World marsupials.

Five sugar gliders and an African hedgehog (*Atelerix albiventris*) died over 1 mo. Most specimens showed little physical change or outward abnormalities. On post mortem examination, grossly enlarged and thickened myocardium and hyperemic lungs were common findings. Several sugar gliders had periocular swelling and hemorrhagic gastrointestinal contents. The most significant histologic lesion was a diffuse lymphoplasmacytic myocarditis with intralesional protozoa. Immunohistochemistry was negative for *Toxoplasma gondii*, *Neospora* sp., and *Sarcocystis neurona*. On thin sectioning, the protozoa were determined to have a distinct kinetoplast. Electron microscopic examination demonstrated the salient features of flagella (some in and some out of cytoplasm) and kinetoplasts supporting an identification of *Trypanosoma* sp.

*Triatoma* sp. bugs were found on the premises. The sugar gliders are fond of insects as a supplement to their diet and actively seek arthropods of many species in their nightly forays. It is suspected that *Triatoma* were not feeding on the Gliders, but rather were themselves being eaten.

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This is a known route of infection (often non-fatal and inducing a carrier state) in South American didelphid opossums.

#### **ACKNOWLEDGMENTS**

Dr. Bradd Barr and Mr. Robert Nordhausen provided invaluable assistance with additional testing.

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## AMYLOIDOSIS IN THE BLACK FOOTED FERRET (*Mustela nigripes*)

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### Abstract

The black-footed ferret (*Mustela nigripes*) is among the most critically endangered mammals in North America.<sup>1,12</sup> Populations of this highly specialized predator were adversely impacted when its primary prey the prairie dog (*Cynomys* spp.) was targeted as a nuisance species and largely eradicated from some of its home ranges by human development and land conversion.<sup>1</sup> Sylvatic plague has also played a role in decimating large populations of prairie dogs.<sup>1</sup> These factors combined to produce a well documented genetic bottleneck in the wild black-footed ferret population in the 1980's.<sup>1,12</sup> The last isolated wild ferret population also experienced epidemics of plague and distemper that further restricted the gene pool.<sup>9-11</sup> The last wild population was subsequently captured for captive breeding programs. Although captive propagation has been successful, the bottleneck has resulted in decreased genetic diversity in the remaining population.<sup>12</sup>

A number of infectious processes have been reported in wild or captive black footed ferrets, including distemper,<sup>3,7,10,11</sup> plague,<sup>9,11</sup> gastritis due to *Clostridium perfringens* type A,<sup>8</sup> and enteritis due to enterotoxigenic *E. coli*.<sup>2</sup> A few cases of neoplasia<sup>4,6</sup> and a single case of diabetes mellitus<sup>5</sup> have also been reported. In none of these studies has amyloidosis been reported as a primary or concurrent lesion in affected animals. A literature search could find no reference to amyloidosis occurring in mustelids. Furthermore, aside from black-footed ferrets, amyloidosis has rarely been diagnosed among mustelid cases submitted to Northwest ZooPath in the past 10 yr. (Garner, unpublished data). This study describes amyloidosis occurring in 26 black-footed ferrets from eight U.S. zoological institutions.

Of the 26 study ferrets, six were females, 18 were males and sex was unknown for two. Age of affected ferrets ranged from 0.5 yr to 8 yr, with average age = 5.1 yr. The most common clinic signs were inappetance (11), diarrhea (5), "renal disease" or azotemia (4), and lethargy (4). Ten were euthanatized, 13 died, and manner of death was not known for three. The most common gross findings were hepatic cysts (14), abnormalities in renal morphology (11), hemorrhage in various organs (9), and thin or emaciated body condition (8).

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Histologically, homogenous amphophilic deposits in all tissues were positive for amyloid using the Congo red stain. The most common sites for amyloid deposition were kidney (26), intestine (15), stomach (11), gall bladder (9), blood vessels (8), and pancreas (8). In 25/26 animals, the most severe amyloid deposition occurred in the kidney, and the glomeruli and tubular basement membranes were equally affected. In the kidney, severity of amyloid deposition in the glomeruli positively correlated with number of tubules that contained protein casts. The most common concurrent disease processes were miscellaneous inflammatory conditions (16), miscellaneous neoplastic conditions (13), biliary cysts or tumors (12), cholecystitis/cholangiohepatitis (10), bacterial sepsis (7), and chronic gastroenteritis (7).

The common occurrence of an otherwise uncommon mustelid disease in a bottlenecked population of black-footed ferrets suggests that these animals may be genetically predisposed to development of amyloidosis. Because all animals had concurrent disease processes, particularly inflammatory or neoplastic diseases, it is considered possible that amyloid deposition was precipitated by other disease processes. Further characterization of the type of amyloid may provide more insight into the pathogenesis of this condition. Additional cytochemical, immunohistochemical and electron microscopic characterization of the amyloid deposits in these ferrets is currently being performed. Because glomerular amyloidosis with protein casts is a consistent finding in affected ferrets, urinalysis that includes measurement of protein:creatinine ratio may be a helpful test for identifying affected animals.

#### ACKNOWLEDGMENTS

The authors acknowledge the cooperation of the following institutions regarding submission of cases for this study: The Hogle Zoo, the San Antonio Zoo, The Cheyenne Mountain Zoo, The Akron Zoo, The Lee Richardson Zoo, the Fort Worth Zoo, The Louisville Zoological Gardens, and the Elmwood Park Zoo.

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## FREQUENT AND WIDESPREAD SIMIAN RETROVIRUS INFECTION IN PERSONS EXPOSED TO NONHUMAN PRIMATES

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### Abstract

The recognition that AIDS originated as a zoonosis heightens concerns associated with human infection with simian retroviruses (SRs) endemic in nonhuman primates (NHPs), including simian immunodeficiency virus (SIV), simian type D retrovirus (SRV), simian T-cell lymphotropic virus (STLV), and simian foamy virus (SFV).<sup>1,6,7</sup> Although few SR infections in persons occupationally exposed to NHPs have been reported, the prevalence and significance of these zoonoses are not fully defined.<sup>3-5</sup> In addition, infections with SRs in persons who hunt, butcher, or keep NHPs as pets has not been documented. Consenting participants (n = 418) from 15 North American research centers and zoos provided a serum sample for serologic testing for SIV, SRV, STLV, and SFV. Matching plasma and peripheral blood lymphocyte (PBLs) from an anonymous survey of primate hunters in Cameroon (n = 1,099) were also available for SFV testing. Testing of sera from NHP workers identified no STLV infection. Two sera from persons with known SIV infection were positive for SIV.<sup>3</sup> Sera from 2 persons were positive for SRV (0.48%) but PCR testing and virus isolation were negative in both cases.<sup>4</sup> In contrast, sera from 14 workers (3.35%; 12 males, 2 females) and 10 Cameroonians (0.9%; 7 males, 3 females) were found to be SFV positive using validated serologic assays.<sup>2</sup> SFV integrase sequences were PCR-amplified from the PBL DNA from 13 workers and 3 Cameroonians. Phylogenetic analysis showed SFV infection originating from African green monkey (n = 1), baboons (n = 4), and chimpanzees (n = 8) in the occupationally exposed persons and from mandrill (n = 1), gorilla (n = 1), and DeBrazza's guenon (n = 1) in the primate hunters.<sup>8,9</sup> Our study documents SFV infection originating from six NHP species in persons exposed to NHPs and suggests that such zoonoses are more frequent, widespread, and contemporary than previously thought. These findings highlight the importance of defining the public health significance of these emerging zoonotic infections.

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## OVERVIEW OF THE OCCUPATIONAL PRIMATE DISEASE SAFETY GUIDELINES FOR ZOOLOGICAL INSTITUTIONS

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### Abstract

Zoonotic diseases are a concern when working with nonhuman primates (NHPs) in zoological institutions. These concerns for employee health can have implications in the management of these animals. The American Association of Zoo Veterinarian's (AAZV's) Infectious Disease Committee formed an ad hoc committee to address current industry safety practices and to provide information on management techniques to minimize the risks of disease transfer to and from NHPs. A set of guidelines was produced which provide a framework of recommendations for managing NHPs in zoological collections. These guidelines aim to reduce human exposure to zoonotic diseases, as well as to reduce human-to-animal disease transmission, while still maintaining a high quality of care and existence for the animals. These guidelines can be used to develop specific institutional policies dealing with NHPs. The information assists staff in assessing and implementing its individual occupational nonhuman primate safety policy.

The guidelines include sections on personnel responsibilities, material required, definitions, various husbandry and veterinary procedures, staff training, policy development and enforcement, public protection, special procedures, and necropsy guidelines. Since this document only addresses safety issues related to disease transmission, it should be part of a more comprehensive occupational health and safety program (OHSP) that includes all hazards: physical, chemical, and biologic. Two recent publications, "Occupational Health and Safety in the Care and Use of Nonhuman Primates" and "Occupational Health and Safety in the Care and Use of Research Animals" published by and available from the National Research Council are valuable resources in developing an OHSP.<sup>1,2</sup>

A primary goal of this document is to provide zoo personnel with information they may use to make informed animal and personnel management health risk decisions. Unfortunately, current knowledge does not allow quantitative risk assessments to be performed in many zoological settings. The level of risk associated with each primate taxon and each institution will vary depending on a number of factors including: the history of the collection, individual animal medical history, facilities and equipment available for working with the animals, and staff training and expertise. One of the appendices in the guidelines includes a retrovirus information

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sheet that also covers recommendations for testing and working with positive animals; part of that information is included in this abstract as an example (Appendix 1). Steps being taken to address the current deficit in knowledge include development of medical surveys and recommendations for serologic and other testing to determine presence of disease concerns in captive NHP zoological populations and within individual institutions to aid in assessing risk factors.

After developing the initial set of guidelines, the document has been modified through review by the American Zoo and Aquarium Association's Animal Health Committee (AZA-AHC), various primate taxon advisory groups, AZA Wildlife Conservation and Management Committee (WCMC), and has been submitted for consideration to the AZA's Board of Directors. A current version of the guidelines can be viewed at [www.aazv.org](http://www.aazv.org). It is hoped that this document will assist AZA member institutions as one tool in their programmatic approach to developing and implementing an effective health and safety program.

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#### **Appendix 1: Retrovirus Information Sheet**

This document is intended to provide both information about, and guidelines for, the care of captive nonhuman primates infected with retroviruses. These viruses may be significant for the health of individual primates and collections as a whole. These viruses present an extremely low, but documented risk of transmission to humans. No human disease has been associated with infection with NHP retroviruses at the time of the writing of these guidelines.

What are retroviruses, and what types are found in nonhuman primates?

Retroviruses are a large group of RNA viruses that replicate in a unique way, using an enzyme called reverse transcriptase. They are divided into 3 groups: the oncornaviruses, the lentiviruses, and the spumaviruses. Retroviruses are found in all animal species tested to date, and do not always cause disease. The NHP retroviruses that may represent significant zoonotic concerns are listed below:

#### **Oncornaviruses**

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*Simian T-lymphotropic virus (STLV)*

- Closely related to Human T-cell leukemia virus (HTLV), which is prevalent in many human populations in Asia, Africa and the Americas. HTLV can cause adult T-cell leukemia or lymphoma in a small proportion of infected humans and has also been associated with rare neurologic disorders. There is evidence that HTLV originated from ancient cross-species transmission of STLV.
- There are several distinct but related viruses in this group.
- Seroreactivity has been seen in more than 33 species of Old World primates, both captive and wild. Mode of transmission is thought to be through sexual contact and from dam to infant in breast milk.
- Usually does not cause clinical signs, but has been associated with disease in baboons, African green monkeys, and gorilla.
- A related virus has been found in spider monkeys, and is the only STLV-like virus found in New World primates, but no disease has been associated with it at this time.

*Gibbon ape leukemia virus (GaLV)*

- Isolated from many captive gibbons (in Asia, USA and Europe) with leukemia.
- Virus is shed in urine and feces, and sexual transmission is also suspected.
- Chronically infected, apparently healthy, antibody negative, virus positive gibbons have been reported.
- The host range for GaLV has not been well explored.

*Simian sarcoma virus*

- Known from a single isolate from a fibrosarcoma in a woolly monkey which was housed with a gibbon (suspect mutant of GaLV).

*Simian retrovirus Type D (SRV)*

- Several different serotypes, all unique to macaques.
- Causes acquired immune deficiency and is associated with opportunistic infections and cutaneous and retroperitoneal fibromatosis in captive macaques.
- Transmitted readily through sexual contact, bite wounds and from dam to infant, both pre- and post-natally.
- Apparently healthy carrier animals have been recognized, particularly in cynomolgus macaques. These virus positive animals may be seronegative, making their identification by serology alone difficult.
- Antibodies to type D retrovirus have been reported in 2 of 247 persons who were occupationally exposed to nonhuman primates. No disease has been identified in these individuals.

Lentiviruses

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*Simian immunodeficiency virus (SIV)*

- Very closely related to human immunodeficiency virus (HIV); in fact HIV-1 originated from a strain of SIV in chimpanzees. HIV-2 originated from SIV of sooty mangabeys.
- A large percentage of African monkeys, both wild and captive that have been tested, are seropositive for SIV. Each species appears to be infected with its own strain of SIV.
- Clinical signs of immunosuppression due to SIV is rare in African species, but have been recognized in some individuals.
- Asian primates are not natural hosts of SIV and are very susceptible to immunodeficiency disease when they contract SIV.
- Susceptibility of New World primates and prosimians is unknown.
- Natural transmission is thought to be through sexual contact, although bite wounds are also suspected.
- 2 of 3123 (0.06%) samples from humans with occupational exposure to NHPs have tested positive for SIV. These tests, however, represent an unknown number of repeat tests for some of the same individuals, so the prevalence may actually be a bit higher. One of those persons has since reverted to seronegative status. No clinical disease has been noted in either positive person.

Spumaviruses

*Simian foamy virus (SFV)*

- Complex retroviruses that have been identified with high prevalence in many Old and New World primate species. Foamy viruses have not yet been identified in prosimian species but are suspected to exist.
- A foamy virus genetically closely related to chimpanzee foamy virus has been isolated from a human. No disease association with foamy virus infection in humans has been established.
- No known disease associated with these viruses in their natural NHP hosts.
- 11 of 296 (3.7%) blood samples from humans with occupational exposure to NHPs have tested positive for SFV. At least 4 of these were associated with deep bite wounds. No disease has been noted in any of these individuals.

Nonhuman Primate Testing and Collection Management Recommendations

Although at this time there is little retroviral associated disease in NHPs and no apparent disease from NHP retroviruses in humans, it is recommended that the retroviral status of NHPs collections be determined for reasons of animal health and occupational safety. This can be accomplished by serologic screening of all animals for antibodies to the retroviruses discussed on the retrovirus information sheet (Appendix 1). Animals should be tested at two time points, 1

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yr apart. Serologic testing alone is sufficient for detection of SIV and SFV-infected animals. For STLV, a prolonged interval to seroconversion may require repeated testing - over several years, or use of molecular techniques for viral detection at initial screening. For SRV, initial testing by both serology and virus detection methods are required to identify all infected animals. Testing for GaLV is currently not routinely available, but may be available in the near future. Both serology and virus detection methods will need to be employed to detect all infected gibbons.

Once an individual nonhuman primate has been confirmed to be test-positive for any retrovirus, it should be considered infected for life, and retesting for that virus is not necessary. (It should be emphasized that an initial positive test result should be confirmed through follow-up testing before considering the animal “positive”.) Serum banking at the time of annual examination is still recommended, for surveillance of other diseases. If an animal is test-negative, but housed with positive animals, retesting on an annual basis is recommended. If all animals in the collection are negative after repeat testing, and no new animals are introduced, alternate or every third year testing, with serum banking in the off years, is justifiable.

The retroviral status of new acquisitions should be determined prior to introduction. Whenever possible, positive animals should only be introduced into groups with positive animals. Introduction of positive animals into known all-negative groups may result in retrovirus-related disease in the naïve animals. The documented differential pathogenicity of some retroviruses between Asian and African species should reinforce the standard practice of preventing direct contact between members of these two groups of nonhuman primates. The pathogenic potential of variants of these viruses among different species of African primates is largely unknown. There is currently insufficient information to make recommendations for individual risk assessment for movement of NHPs infected with retroviruses. The primate TAG and SSP veterinary advisors should be consulted for specific advice.

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**SURGICAL AND MEDICAL MANAGEMENT OF NECROTIZING FASCIITIS IN THE THROAT SAC REGION OF AN ADULT MALE SUMATRAN ORANGUTAN (*Pongo pygmaeus abelii*)**

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**Abstract**

A 30-yr-old, captive-born, male Sumatran orangutan (*Pongo pygmaeus abelii*) with no prior medical history presented on the afternoon of day 1 with a plum-sized dependent swelling of the throat sac region. Medical history was unremarkable. The animal was quieter than usual and appeared sweaty and feverish. A preliminary diagnosis of early/mild throat sacculitis was made based on species predilection and presenting symptoms. Ceftazidime (2 gm i.m.) was administered and a medical work-up planned for the following day. In the early morning of day 2 the animal was found recumbent, unresponsive to voice or touch, but breathing steadily and blinking his eyes. Septic shock was suspected. The throat sac swelling was the size of a small basketball and was dense rather than fluid filled. The epidermis had sloughed, leaving a white, devitalized, glistening surface.

Physical examination, bacterial culture and biopsy of the affected region confirmed necrotizing fasciitis caused by beta-hemolytic streptococcus, Group A. Surgical intervention was performed the same day and included aggressive debridement/resection of all portions of the necrotic skin and underlying subcutaneous tissue. The throat sac mucosa was not involved with the necrotizing disease process and was left intact throughout the surgery. The resulting suture line extended approximately 40 cm in a crescent shape from below one ear, across the anterior thorax, to just below the opposite ear. It was not possible to excise all affected tissues and still achieve complete primary closure. Since management of a post-operative wound was not possible in this patient, primary closure necessitated leaving small portions of devitalized tissue along portions of the suture line.

Post-operative medical care and husbandry focused on aggressive antibiotic therapy and deterrents to dehiscence of the suture line by the orangutan. Antibiotic therapy included parenteral clindamycin (8 mg/kg i.m., b.i.d.), amikacin (7 mg/kg i.m., b.i.d.) and enteral amoxicillin (7 mg/kg p.o., t.i.d.). The animal was a highly motivated suture picker. Techniques employed to deter this behavior included 24 hr observation with positive and negative reinforcement, the sleep aid zolpidem tartrate (Ambien, Sanofi-Synthelabo, Inc., New York, NY, 10016, 10 mg p.o. in the evening), acepromazine (0.22 mg/kg p.o., t.i.d.), food stuffs braided into

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the animals hair and other enriching/distracting items. Diazepam (0.25 mg/kg p.o.) was tried but had little effect on the suture picking behavior.

A single follow-up procedure to further debride and close a partial dehiscence of the surgical site was performed on day 4. The animal recovered uneventfully and was returned to his family group on day 33. The removal of affected tissue resulted in the loss of all pendulous portions of the throat sac, which creates a significant change in the physical appearance of an adult male orangutan. Follow-up observations revealed that the diminished capacity of the throat sac space had no effect on the animal's ability to carry out normal male orangutan vocalizations. It is uncertain, however, whether the dramatic change in this animal's secondary sex characteristics has changed the social dynamics of the group. Since the time of the surgery a 6-yr-old male in the group has developed aggressive behavior toward this dominant male (his sire).

Necrotizing fasciitis, also referred to as "flesh-eating bacteria" is a rare but life-threatening infection. It is often associated with group A streptococcal infections, but can be seen with many other bacteria including other streptococcal serotypes, polymicrobial infections and clostridial infections.<sup>1</sup> Once the infection is seeded it proceeds with rapidly progressive inflammation and subsequent necrosis of the muscle fascia and surrounding tissues.<sup>2</sup> Mortality can be high. Early diagnosis followed by aggressive surgical debridement is essential to a successful outcome. In the case of this orangutan, immediate diagnosis of necrotizing fasciitis was hindered somewhat by the predilection of orangutans to throat sac infections. However the rapid progression from onset to a life-threatening situation (less than 24 hr) facilitated our diagnosis and encouraged us to proceed with immediate and life-saving surgical intervention.

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## HEALTH AND REPRODUCTIVE ASSESSMENT IN THE YUNNAN SNUB-NOSED MONKEY (*Rhinopithecus bieti*)

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### Abstract

The black-and-white, or Yunnan snub-nosed monkey (*Rhinopithecus bieti*) is among the world's top 25 most endangered primates. It occurs only in forests above 2,700 meters in a tiny range in Southeast Tibet and Northwest Yunnan, between upper Yangtze and Mekong. Only 14 groups remain with the whole population consisting of about 1,500 individuals. The Yunnan snub-nosed monkey was mistaken as a subspecies of *Rhinopithecus roxella*, when a French missionary, Biet, brought the first skull and body specimen to Paris in 1890. In 1962, eight pelts bought by professor Hongshou in a Tibetan village again confirmed the existence of the Yunnan snub-nosed monkey. In 1979, the Chinese scientists proved the existence of this very shy primate species living in the rough Tibetan forest. Since the rediscovery of this magnificent monkey, an intensive research project was initiated by the Chinese Academy of Sciences. Shortly thereafter, captive breeding centers were established at the Zoological Institute of Kunming (KIZ), at the Kunming Zoo and at the Beijing Zoological Park. Currently there are 3.5 adult snub-nosed monkeys with 4 offspring at the Breeding Center of Endangered Primates of KIZ and the Kunming Zoo. As part of a Sino-German research program on reproductive biology in Yunnan snub-nosed monkeys, first time ultrasonographic examinations were performed in males and a new electro-ejaculation technique was developed. One of the major goals of this program is the establishment of a sperm bank for this highly endangered species.

The investigations were performed in December 2002 and 2003 which is the peak of the breeding season. Breeding season lasts from the end of November through the beginning of March. A total of nine examinations and semen collections were performed in three adult males over the 2-yr period. The dominant sire was examined twice in 2002 and three times in 2003 while the two subdominant adults were each examined twice in 2002 and 2003. Former difficulties with the semen collection were overcome by applying a new electro-stimulation technique with a customized transrectal probe which was developed based on the sonomorphologic findings of the ultrasound investigations. The entire procedure was performed under general anesthesia. Animals were anesthetized by darting with ketamine hydrochloride (10 mg/kg) (Ketamine 10%, Essex GmbH, Germany). Body weights ranged from 17.0-19.5 kg. Total time of anesthesia ranged from 30-40 min. The preparations for the transcutaneous

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ultrasound exam (heart, liver, kidney, spleen, testis) and transrectal ultrasonography (accessory sex glands) included shaving specific scanning windows and the genital area, as well as a rectal enema with lukewarm water. To prepare the animal for semen collection, the urinary bladder was emptied by catheterization and refilled with cell culture medium M199 (Sigma GmbH, Germany).

Due to the extensive digestive tract, typical of leaf-eating monkeys, the transcutaneous ultrasonography was limited. Heart and liver were imaged but the kidneys and spleen were easier to visualize by transrectal ultrasound. One of the subdominant males showed a pseudo-membrane formation in the urinary bladder which split the bladder nearly completely into two parts, a larger cranial part (2/3), and a smaller caudal part (1/3). The cranial compartment seemed static without inflow from the ureters or draining through the urethra. Even under ultrasound-guided catheterization it was impossible to drain this compartment. Medical records from this male were not indicative of a former urinary bladder infection. A teratogenic malformation of the bladder could not be excluded but seemed unlikely. Even with this dramatic reduction of bladder capacity, there was no obviously different urination behavior observed. However, the extensive pathologic alteration detected led to the recommendation to exclude this male from the breeding program.

Ultrasound findings on the genital tract reflected the social rank of the three males. The dominant male showed the largest testicles with a moderate echogenic parenchyma indicating an active spermatogenesis. This interpretation was supported by color-Doppler investigations showing several intra-parenchymal blood vessels. The testes of the two subdominant males were smaller but showed equal tissue activity. In contrast to equal-sized macaque species or baboons, the dimensions of the internal accessory sex glands (bulbo-urethral gland, prostate, seminal vesicle) were relatively small which resulted in a smaller amount of the typical coagulum in the ejaculate of this species. The liquid phase of the ejaculate contained a total sperm cell number comparable to other primate species.<sup>1,2</sup> However, the spermatogenic recovery time seemed to be extremely long in snub-nosed monkeys. Second and third electro-ejaculations in the same male with a time interval of 3-5 days resulted in a nearly aspermatic ejaculates with almost similar ejaculatory volumes even in the proven breeder. This suggested that the time of the spermatogenic cycle is extended in this primate species. The very rigid social structure of small family groups with one male and 2-5 females may play a role in this phenomenon. Further investigations of spermatogenesis are planned including future ultrasound-guided testicular biopsies and flow cytometric analyses.

#### **ACKNOWLEDGMENTS**

The authors thank the animal keepers from the Kunming Breeding Center of Endangered Primates.

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## **LINKS BETWEEN HUMAN AND MOUNTAIN GORILLA HEALTH: AN EXAMPLE FROM THE MOUNTAIN GORILLA (*Gorilla beringei beringei*) VETERINARY PROJECT IN RWANDA**

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### **Abstract**

### **Introduction**

Potential disease transmission from humans has been identified as a key threat affecting population viability of the mountain gorillas (*Gorilla beringei beringei*). In 2000, the Mountain Gorilla Veterinary Project's (MGVP) human health working group identified strategies that could be incorporated into future programming to reduce the risk of disease transmission between humans and gorillas, while improving both the health of humans living in close proximity to the gorillas and relations with local community members. One strategy, an employee health program (EHP), targets park conservation employees as the human group with the most frequent close contact with mountain gorillas.

### **Methods**

In 2001, MGVP initiated an EHP for personnel in the Parc National des Volcans, Rwanda, which involves clinical history and physical examination, laboratory tests, follow-up care for health problems, vaccinations and health education. Socio-demographic data provide a basis for developing disease risk profiles. Standardized data collection allows for comparative analyses of health indicators over time to strengthen understanding of the relationship between human and gorilla health. Testing consisted of parasite, bacterial and viral tests on feces, malaria screening, human immunodeficiency virus (HIV) screening, packed cell volume (PCV) sedimentation, an automated complete blood count (CBC) analyses, blood chemistry tests and dip stick tests on urine.

### **Results**

A high prevalence of potentially transmissible pathogens was found. For example, 48.8% of personnel were infected with at least one type of gastrointestinal parasite, and fecal culture results indicated 37.3% had *Campylobacter* sp., 4.6% had *Salmonella* sp., and 0.7% had *Shigella* sp. The type of latrine used and education level were the variables most strongly associated with pathogen prevalence.

### **Conclusion**

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Results of the EHP indicate that conservation personnel carry pathogens that can be transmitted to the mountain gorillas. Focusing on human health as a wildlife disease prevention strategy is a novel approach, and is particularly relevant at interfaces between human and nonhuman primate populations.

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## ELIMINATION OF TRICHURIASIS IN A GROUP OF COLOBUS MONKEYS (*Colobus guereza*)

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### Abstract

#### Case Report

Kikuyu colobus monkeys (*Colobus guereza*) at the Los Angeles Zoo have a long history of parasite-associated gastrointestinal problems and poor health. Chronic trichuriasis was present in the group and was documented on many fecals, even though eggs are shed in small numbers and at irregular intervals. One animal died of hemorrhagic gastroenteritis, and two other animals had similar lesions in gastric biopsies. Clinically these animals had chronic weight loss, inappetance, vomiting, and regurgitation. *Trichuris* sp. worms were seen in the complex stomach on gastroscopy.

Colobus monkeys are folivorous with an enlarged sacculated stomach which houses a multitude of microbes, very similar to the structure and function of a rumen. The normal pH in the sacculated stomach ranges from 5.5-7, an environment that is fairly similar to the cecum and colon.<sup>1,4</sup> Both gastric amebiasis and gastric trichuriasis have been documented in the howler monkey.<sup>2</sup> Concurrent infection may add to the pathogenicity of the individual organisms.

Numerous treatments were attempted while the group was on exhibit in an effort to reduce the parasite load. There were many challenges with medication administration and patient compliance. In addition, the exhibit was dirt-floored and heavily contaminated. *Trichuris* eggs can survive in the environment for up to 5 yr<sup>3</sup> resulting in continual reinfection.

Due to the impending acquisition of a breeding male it was decided to try and resolve the trichuriasis infection. The colobus monkeys were moved to a cement floored enclosure at the Health Center for a period of 6 mo. During that time, the monkeys were treated with ivermectin monthly at 0.2 mg/kg for three treatments by subcutaneous or intramuscular injection (Ivomec 1% solution, Merial, Iselin, NJ), fenbendazole at 50 mg/kg p.o. for 3 days once (Panacure granules 22.2%, Intervet, Millsboro, DE) and milbemycin oxime 5.75 mg p.o. monthly for 3 mo (Interceptor, Novartis, Greensboro, NC). Attempts at oral administration of albendazole (Valbazen, Pfizer, Exton, PA) were unsuccessful. The treatment choices were based on efficacy and patient compliance. Some of the courses of oral medication were incomplete due to compliance problems. Concurrently, 6 inches of dirt was removed from the floor of the exhibit and replaced.

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Multiple negative fecals were obtained prior to the monkeys being returned to their exhibit in December 2001. The group has remained parasite free to date (2004).

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## NONHUMAN PRIMATE QUARANTINE, PRACTICE AND PROBLEMS

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### Abstract

There are significant issues challenging the institutions that operate a Centers for Disease Control and Prevention (CDC) quarantine facility. Transfer of nonhuman primates from one country to another is an important activity in providing nonhuman primates for captive breeding, exhibition in zoological parks and use in research. It is also a practice that is facing increasing complexity and challenges from emerging diseases and a changing regulatory environment. These challenges fall into three categories. The first is represented by pathogens that have been long recognized as a threat to both human and nonhuman primates. Screening for tuberculosis continues to be a significant challenge to quarantine facilities and outbreaks of tuberculosis continue to occur post quarantine screening.<sup>1</sup> The second challenge is represented by new potential pathogens that have not been recognized previously or may be sub-clinical infections. The occurrence of the Reston Ebola infection in the late 1980's represented a new virus from a different geographic region. This agent had dramatic impacts on quarantine practices as well as regulatory oversight of quarantine. Simian retroviruses also represent infectious agents that can have a negative impact on breeding programs and can be difficult to detect.<sup>2</sup> The final challenge to nonhuman primate quarantine is from infectious agents that may not naturally occur in nonhuman primates, but may still impact the transport of nonhuman primates. This last situation occurred in 2003, when the spread of the SARS virus in China resulted in temporary suspension of all animal transport in and out of China.<sup>3</sup> The quarantine of newly imported nonhuman primates will continue to be an essential part of good primate colony management, as well as a regulatory requirement of the CDC.

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**EVALUATION OF A GAMMA INTERFERON ENZYME IMMUNOASSAY, A MULTIPLE-ANTIGEN ELISA, AND AN ANTIGEN 85 IMMUNOASSAY FOR SCREENING FOR *Mycobacterium tuberculosis* INFECTION IN ORANGUTANS (*Pongo pygmaeus*)**

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**Abstract**

Orangutans (*Pongo pygmaeus*) are susceptible to *Mycobacterium tuberculosis* (*M. tuberculosis*) infection and infected animals pose a health risk to other animals, caretakers, and the public. Knowledge of the tuberculosis status of an individual is crucial to prevent spread of infection. The intradermal tuberculin test is the traditional method used for screening captive primates for *M. tuberculosis* infection. However, this test is problematic in orangutans because interpretation of results is subjective and there is a high incidence of false positive or nonspecific reactors.<sup>2</sup> Although mycobacterial culture is considered the gold standard for determining tuberculosis status, false negatives can occur and results often take many weeks to obtain. New testing modalities promise rapid turn-around time with increased sensitivity and specificity.

In this study of 17 captive orangutans, baseline values were determined for several diagnostic tests that are designed to detect and identify mycobacterial infections. We also attempted to assess the potential value of these tests as screening tools for *M. tuberculosis* infection in orangutans. These tests included a *M. tuberculosis* gamma interferon test, a multiple antigen serum ELISA, an Antigen 85 (Ag 85) immunoassay, and the mammalian old tuberculin intradermal test. The results of these tests were compared to the tuberculosis status of each animal which was evaluated using individual and group histories, physical examinations, complete blood counts (CBC), serum biochemistry profiles, and thoracic radiographs, as well as acid-fast staining and mycobacterial cultures of tracheal and gastric lavage samples.

The *M. tuberculosis* gamma interferon immunoassay quantifies and compares the amount of gamma interferon that is produced by sensitized lymphocytes after incubation of blood with

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bovine purified protein derivative (PPD), avian PPD, and a nil antigen, thereby measuring a component of cell-mediated immune reactivity to mycobacteria.<sup>1,5</sup> The serum ELISA uses multiple antigens to detect antibody responses to infection with mycobacteria, thereby measuring a component of humoral immune reactivity. The optical density values are then compared to positive control values and the ratio of these results is reported.<sup>7</sup> Antigen 85 complex proteins are major secretory products of actively replicating mycobacteria and are typically produced in the early stages of infection.<sup>8</sup> Measurement of serum Ag 85 by monoclonal antibody immunoassay could provide a method for identifying active mycobacterial infections that is less dependent on host immunity. This test has previously been shown to detect serum Ag 85 in orangutans, although correlation with clinical disease or mycobacterial infection has not been demonstrated.<sup>6</sup>

In this study, physical examination findings, CBC results, biochemistry panel results, thoracic radiographic findings, and clinical signs of the study animals were not suggestive of infection with *M. tuberculosis*. No tracheal or gastric lavage cultures grew *M. tuberculosis*. Eight of the 17 animals reacted to intradermal tuberculin tests. Five animals in the study had current or historic positive atypical mycobacterial cultures and all of these animals had positive intradermal tests.

Based on cut-off levels determined for macaques,<sup>1</sup> gamma interferon test results were positive for *Mycobacterium avium* (*M. avium*) complex in nine of the 17 animals. Of these nine, one had an historic gastric culture of a *M. avium* complex bacterium, but the other eight had no positive mycobacterial cultures. One of the 17 animals was positive for *M. tuberculosis* based on the gamma interferon test; this animal had a historic positive gastric culture for *Mycobacterium fortuitum*, which shares antigenic determinants with *M. tuberculosis*.<sup>4</sup>

The optical density ratios for the multiple antigen ELISA were highly variable between individuals. There was generally high seroreactivity to PPD and Ag 85, and less seroreactivity to modified protein 70, purified from *M. bovis* strain AN5 (MPB), early secreted antigen target of *M. tuberculosis* (ESAT), antigen 64 secretory protein of *M. tuberculosis* (Ag 64), and antigen 32 secretory protein of *M. tuberculosis* (Ag 32). Optical density ratio values showed no significant differences when animals with positive intradermal tests were compared to those with negative skin tests. Similarly there were no significant differences when animals with a history of positive mycobacterial cultures were compared to those with no such history, and there were no differences between those that were positive for *M. avium* on the gamma interferon test when compared to those that were negative.

All 17 of the orangutans in the study showed reactivity in the Ag 85 immunoassay. Most reactions were mild to moderate, but three had relatively strong reactions. Two of these three had positive atypical mycobacterial cultures and all three had positive intradermal tests. On the gamma interferon assay, one was negative, one was *M. avium* complex positive, and one was *M. tuberculosis* complex positive.

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This study established baseline values for the gamma interferon, multiple antigen ELISA, and Ag 85 immunoassay for orangutans that were presumed to be negative for *M. tuberculosis* infection. However, interpretation of these results is difficult since no animals in the study were found to have *M. tuberculosis*. In a clinical trial involving 135 macaques, the gamma interferon test was reported to be 92% sensitive and 100% specific for *M. tuberculosis* complex infection.<sup>1</sup> The gamma interferon test may show some promise for ruling out *M. tuberculosis* infection in orangutans because only one animal in our study was defined as positive on this test. However, validation of the gamma interferon assay will require positive results with blood from an *M. tuberculosis*-infected orangutan.

A previous study using a different ELISA protocol with *M. avium* and *Mycobacterium bovis* antigens showed an association between ELISA reaction and evidence of mycobacterial infection on gastric lavage, but an inability to differentiate between responses to different mycobacterial species.<sup>3</sup> Results from the multiple-antigen ELISA in our study appeared highly variable and non-specific for infection; serologic reactions to PPD and Ag85 appeared to be particularly non-specific. There was generally less reactivity to MPB, ESAT, Ag64, and Ag32, so these antigens may be useful for detecting *M. tuberculosis* infection, assuming that an infected animal would have markedly greater seroreactivity than the orangutans in this study.

Previous investigators have questioned the specificity of the dot blot Ag85 immunoassay for orangutans,<sup>6</sup> and the results here seem to suggest a high background reactivity to this antigen that may make it poorly specific for detecting *M. tuberculosis* infections in this species.

In order to further evaluate the effectiveness of all of these tests at determining true *M. tuberculosis* infection status, they will need to be evaluated on orangutans found to be culture positive for *M. tuberculosis*.

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## HEALTH ASSESSMENT, MEDICAL AND DENTAL INTERVENTIONS FOR A GROUP OF 33 CHIMPANZEES (*Pan troglodytes*)

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### **Abstract**

Construction of a building designed to temporarily house chimpanzees (*Pan troglodytes*) facilitated health assessments, and medical and dental interventions in an established group of 33 chimpanzees. The chimpanzees lived in a completely outdoor South Florida habitat. Little medical information on these animals was previously available due to challenges involved in immobilizing, treating, and reintroducing chimpanzees in this environment. This report describes strategies to introduce and habituate the chimpanzees to the facility and will also describe procedures including physical exams and vasectomies. Viral survey results and histopathologic findings will be presented.

### **Introduction**

The chimpanzee colony at Lion Country Safari was established in the early 1970's when chimpanzees were brought to this zoological park from diverse sources including U.S. medical research laboratories. Breeding continued within the colony through 2002. This colony is one of the largest groups of chimpanzees among American Zoological Association accredited zoos. This group includes some of the oldest chimpanzees in the U.S., animals of African origin, and young offspring. Behavioral and scientific data from these animals has been and continues to be shared with a number of research groups exploring questions of chimpanzee aging, ethology, evolution, genetics, health, husbandry, and pathology.

### **Methods**

For several years, the chimpanzees have been divided into sub-groups within the outdoor exhibit to promote compatible social grouping. Established sub-grouping was maintained as much as possible before, during, and after anesthetic procedures. Planning the composition of any temporary social groups within the facility was important to encourage voluntary transfer of animals into the building and to help prevent intraspecific aggression between individuals when chimpanzees were reunited.

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Four months before any anesthetic procedures, chimpanzees were encouraged to transfer into the facility as a sub-group with food rewards. The sub-group would interact with keepers throughout the day within the facility, spend the night there, and then return to the outdoor exhibit.

Adult animals expected to become highly excitable during immobilization procedures were given crushed diazepam (Diazepam, Abbott Laboratories, North Chicago, Illinois 60064, USA; 0.2 mg/kg p.o.) in a small amount of fruit drink the previous afternoon and the morning of anesthesia. In general, this pre-anesthetic oral sedation was avoided to reduce the likelihood of vomiting and aspiration. Pre-darting stress was usually minimal when the animal was separated from other chimpanzees the day before and darted in the morning. Separated animals could still see and hear other chimpanzees in the facility and in the nearby exhibit. Darting was facilitated by having a staff member distract the chimpanzee from one end of the enclosure while the dart was fired from behind the animal.

A combination of ketamine (Ketaset, Fort Dodge Animal Health, Fort Dodge, Iowa 50501, USA; 10-15 mg/kg i.m.) and xylazine (Xylazine, Phoenix Scientific, Inc., St. Joseph, Missouri, 64506, USA; 0.1-0.2 mg/kg i.m.) proved most reliable for immobilization. Telazol (Telazol, Fort Dodge Animal Health, Fort Dodge, Iowa 50501, USA; 4.0 mg/kg and xylazine (0.6 mg/kg) failed to produce satisfactory sedation in one chimp. In assessing more than 25 immobilizations, it appeared that ketamine dosed at 10-15 mg/kg with xylazine at 0.2 mg/kg afforded the most efficient and complete immobilizations with less need for supplemental drugs. When additional supplementation was needed to reduce any movement, midazolam (Midazolam HCl, Bedford Laboratories, Bedford, Ohio 44146, USA; 0.2 mg/kg i.m) or ketamine (1-4 mg/kg i.m.) was effective. During induction, chimpanzees usually moved to a sitting position on the ground. Chimpanzees sometimes leaned forward, hyperflexing their necks. This was of concern as it potentiated upper airway obstruction; therefore, neck posture and tongue position were closely monitored. Once immobilized, chimps were strapped into a stretcher which assisted in control of any arm or hand movement.

Chimpanzees were placed in dorsal recumbency, intubated, and placed on isoflurane if procedures required more than 30 min of anesthetic time. Use of a long laryngoscope blade (Miller #4, Henry Schein, Inc. Melville, New York 11747, USA) facilitated efficient intubation.

Procedures performed on anesthetized animals included: physical examinations, blood collection, intradermal tuberculin testing, electrocardiographic studies, punch biopsies for genetic analysis, chest radiographs, and collection of feces for culture and parasitology. Blood was typically collected from the distal radial vein using a butterfly system that incorporates a vacutainer (Vacutainer Brand Safety-Lok Blood Collection Set, Becton Dickinson and Company, Franklin Lakes, New Jersey 07417, USA). The blood samples were then utilized to perform complete blood counts, serum biochemistry analyses and various types of serology.

## Vasectomy

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Six chimpanzees were vasectomized. Once anesthetized, these animals were maintained in dorsal recumbency. Towels were placed under the testicles to support their weight and to decrease tendency for dependent edema associated with surgery. Separate longitudinal prescrotal incisions were made on each side of the penis over each spermatic cord. Blunt dissection to the level of tunic was made, the tunic was sharply incised, and each vas deferens was clamped at two sites approximately 4 cm apart in its mid-section. A ligature of 2-0 polydioxanone (PDS, Ethicon, Inc., Somerville, New Jersey 08876, USA) was placed proximal to each clamp and the section of vas deferens between the ligatures was excised. The open tunic was flushed with chlorhexidine (Chlorhexidine Solution, The Butler Company, Columbus, Ohio 43228, USA) and a mixture of amikacin in 0.9% saline (Amiglyde-V, Fort Dodge Animal Health, Fort Dodge, Iowa, 50501, USA). The tunic and subcutaneous layers were sutured separately in simple interrupted fashion and a continuous subcuticular pattern was used to close the skin, with 2-0 PDS. A small amount of tissue adhesive (Nexaband Liquid, Abbott Laboratories, North Chicago, Illinois, USA 60064) was used over skin sutures in some cases. Antibiotic coverage included cefazolin (Cefazolin, Geneva Pharmaceuticals, Inc., Dayton, New Jersey 08810, USA; 25 mg/kg i.m. or s.c.) intraoperatively and chewable amoxicillin/clavulanate (Augmentin, GlaxoSmithKline, Research Triangle Park, North Carolina 27709, USA; 10 mg/kg p.o., b.i.d. for 5 days) post-operatively. Flunixin meglumine (Flunixinamine, Fort Dodge Animal Health, Fort Dodge, Iowa 50501, USA; 1 mg/kg i.m) was administered for analgesia prior to recovery.

#### Dental Procedures

Three, 30-55 yr-old, chimpanzees were transported to a veterinary dental clinic for complete dental radiography, evaluation, and treatment. Chronic fractures of the incisors and canines with periapical lesions were the most common abnormalities. A male that presented for a facial fistula was found to have an endodontically involved canine root fragment requiring lateral alveolar plate removal to access. Diseased incisors and canines were extracted and gingival flaps were performed. Osseointegrative synthetic bone graft material (Consil, Nutramax Laboratories, Inc., Edgewood, Maryland 21040, USA) was used to fill the extraction defects. Closure was achieved with simple interrupted sutures of 4-0 chromic gut (Ethicon, Inc., Somerville, New Jersey 08876, USA). Animals were maintained under gas anesthesia for 1-4 hr to perform the tooth extractions.

Chimpanzees undergoing invasive dental procedures received ceftiofur (Naxcel, Pharmacia & Upjohn Company, Kalamazoo, Michigan 49001, USA; 2.2 mg/kg i.m.) intraoperatively. Crushed chewable amoxicillin/clavulanic acid (9-11 mg/kg p.o., b.i.d. × 5 days) and acetaminophen suspension (Tylenol, McNeil Consumer and Specialty Pharmaceuticals, Fort Washington, Pennsylvania 19034, USA; 9 mg/kg p.o., b.i.d. × 3 days) were administered. The animals consumed the medications best when they were placed on soft fruits such as oranges or mixed with fruit drinks.

#### Respiratory Disease

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In July of 2003, a virulent respiratory disease swept through the entire colony, most severely affecting a subgroup of 18 individuals. This subgroup was brought into the facility for observation and treatment. The most severely affected individuals showed increased inspiratory effort, purulent nasal discharge, open-mouth breathing, and decreased appetite. Infant and juvenile animals and their dams were only minimally affected. Immobilizations were not performed on animals due to the increased anesthetic risk in animals with respiratory disease. Instead, severely affected animals were treated empirically by darting with ceftiofur (2-3 mg/kg i.m., s.i.d. × 5 days) followed by an oral regimen of amoxicillin/clavulanate (10 mg/kg p.o., b.i.d. × 7 days). All chimpanzees fully recovered in approximately 14 days.

## Results and Discussion

All chimpanzees, with the exception of an individual suffering from cerebrovascular insult, were clinically normal at the time of serologic sampling. A small number of seropositive tests for Hepatitis A (2 of 7) and Hepatitis B (1 of 9) occurred in some of the older animals; these chimpanzees were former laboratory animals believed to have been used in hepatitis research. Eight chimpanzees tested for Hepatitis C were negative. No hematologic or serum chemistry abnormalities compatible with hepatic illness were noted. All chimpanzees including those of wild origin were negative for human immunodeficiency virus, human T-lymphotrophic virus, simian immunodeficiency virus, and simian T-lymphotrophic virus.

For other viruses, the number of positive animals/number of animals tested were as follows: Influenza A (8/17), Influenza B (0/17), Parainfluenza I (2/7), Parainfluenza II (0/17), Parainfluenza III (11/17), Measles (0/17), Respiratory Syncytial Virus (0/17), Simian A-8 (0/17), Herpes simplex I (16/18), Herpes simplex II (0/17), Human Varicella (11/17), Chimp Cytomegalovirus (17/18), Epstein Barr Virus (18/18), Simian Foamy Virus (5/6). As most tested animals were seronegative for Parainfluenza III prior to the respiratory outbreak of July 2003, and several were seropositive after that time, it is postulated that this virus contributed to clinical disease expressed that summer. Several animals were seropositive for West Nile virus and/or St. Louis encephalitis virus.

Seventeen chimpanzees were given intradermal tuberculin tests (Tuberculin Mammalian, Human Isolates Intradermic, Synbiotics Corporation, San Diego, California USA) and were negative. Nine of these chimpanzees also had blood tuberculosis tests for *Mycobacterium tuberculosis* complex that were negative. (Primagam, BioCore Animal Health, Omaha, Nebraska, USA).

A 38-yr-old male chimpanzee had acute left-sided hemi-paresis and depression. A diagnosis of cerebral infarction was made via CAT scan. The animal had normal radiographic, electrocardiographic, and cardiac ultrasound findings during this illness. It further declined and expired 5 days after presentation. Severe myocardial fibrosis was seen on histopathology. An approximately 40-yr-old female chimpanzee also succumbed to cardiac disease characterized by myocardial fibrosis. Because fatal cardiac fibrosis has been associated with vitamin E deficiency in primates, analysis for liver concentration of alpha-tocopherol was performed.<sup>3,4</sup> Hepatic levels

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of vitamin E were approximately 24 µg/g in the male and 2.3 µg/g in the female. Expected liver vitamin E level in human adults is 20 µg/g (E. Dierenfeld, personal communication) It has been postulated that depressed vitamin E levels may lead to myocardial fibrosis that is not reversed by subsequent vitamin supplementation.<sup>4</sup> The lifetime dietary history of these two chimpanzees was not known. It has also been theorized that viral infections may contribute to myocardial fibrosis.<sup>1,2</sup> The viral survey performed in this colony lends insight into the identities of viruses that may contribute to cardiac disease.

No anesthetic-related mortalities occurred. One chimpanzee had brief, severe hypoxia from upper airway obstruction due to hyperflexion of the neck soon after being darted. Vomiting occurred in three individuals in the post-operative (vasectomy) period. These three chimpanzees were among the first immobilized and had received a small amount of diazepam in juice within 2 hr prior to darting, a practice which was discontinued as anesthetic dosages were refined. Vomiting could also have been caused by abnormal gastrointestinal motility as a result of gas anesthesia or the use of xylazine.

All vasectomized chimpanzees recovered from surgery without significant complications. Two animals had scrotal swelling that was most apparent several days post-operatively. This complication occurred despite intraoperative attempts to support the weight of the scrotum and to provide gentle tissue handling. These chimpanzees were monitored daily. Although use of anti-inflammatories was considered, the post-operative inflammation resolved on its own. One animal had a mild incision line infection that was successfully treated with minor debridement and oral antibiotics.

No complications from dental procedures were observed. All chimpanzees were awake and ambulatory within 4 hr post-extubation and all consumed juice and/or fruit in the immediate post-operative period. Outward manifestations of oral pain were not apparent in any chimp. The male that underwent removal of a retained canine fragment and a female that underwent incisor and canine extraction exhibited substantially brighter behavior following the procedure. Although these chimps had not been known to have difficulty chewing or drinking prior to or after the procedure, keepers noted them to be more energetic and vocal following the extractions. Keepers described the male as a “new chimp.”

#### **ACKNOWLEDGMENTS**

Special thanks to Drs. Pat Frost, Steve Bolin, Jim Oosterhuis, and David Fagan for several telephone consultations. Thanks also to Wildlife Director Terry Wolf, chimpanzee keepers Kelly Greer, Andrew Halloran, and Michele Schwartz for their tireless efforts with this unique group of animals. The support of Lion Country Safari veterinary technicians and curators is greatly appreciated.

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## PREVALENCE OF WEST NILE VIRUS IN MIGRATORY BIRDS DURING SPRING AND FALL MIGRATION

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### Abstract

Since the discovery of West Nile virus (WNV) in New York in 1999, this disease has spread throughout 44 of the lower 48 states and to Canada and the Caribbean and Mexico. Migrating birds are often thought of as the principle mechanism for the dissemination of this virus. We investigated the prevalence of WNV and antibodies to WNV in birds during the spring and fall migrations at 8-10 sites in the Atlantic flyway during 2001-2003 and 5 sites on the Mississippi flyway during 2002 and 2003. We obtained blood samples from 13,402 birds captured in mist-nets, representing 135 species. Seroprevalence each season was low (<5%) at most sites but was as high as 18.4% (Memphis, Tennessee; fall 2002). In the Atlantic flyway, gray catbirds (*Dumetella carolinensis*) and northern cardinals (*Cardinalis cardinalis*) were most commonly found with antibody to WNV, as well as the first and third most commonly sampled species. In the Mississippi flyway, antibody to WNV was most commonly detected in northern cardinals, the most commonly sampled species. Additionally, two birds in this flyway had detectable WNV viremias, an indigo bunting (*Passerina cyanea*) and downy woodpecker (*Picoides pubescens*). Both individuals were sampled in fall 2002 at Mark Twain National Wildlife Refuge, Illinois. No viremic birds were detected in the Atlantic flyway.

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## AVIAN WEST NILE VIRUS SURVEILLANCE AT THE NWHC: A 5-YEAR SUMMARY

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### **Abstract**

West Nile virus (WNV) emerged in the New York City region in 1999 and has rapidly spread across the North American continent in the short course of 5 yr. At this time, much remains unknown about the ecology of WNV in North America. There are two unusual characteristics of the North American epidemic: high avian mortality associated with the human and equine epidemic and the increasing number of species in which WNV has been detected. Mortality rates have been particularly high in corvids (crows, jays) and raptors, and 226 avian species have been reported by state and federal public health, veterinary and wildlife agencies. The USGS National Wildlife Health Center (NWHC) has been actively involved in testing dead birds submitted through state and federal WNV surveillance programs since 1999. Due to changing surveillance programs, variation in the data collected by each state, and the constantly evolving role of the NWHC in surveillance programs, the wild bird data are impossible to interpret epidemiologically. However, the surveillance testing does serve as an indicator of the avian mortality that has occurred since 1999. Although WNV has been a major cause of avian mortality during this time period, the data shows that other uninvestigated, and therefore unknown, causes of death have probably contributed to the avian mortality, even in those species found to be particularly susceptible to fatal WNV infection.

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## OUTBREAK OF WEST NILE VIRUS IN RAPTORS FROM VIRGINIA DURING 2003: CLINICAL, DIAGNOSTIC AND EPIDEMIOLOGIC FINDINGS

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### Abstract

Since its introduction to North America in 1999, West Nile virus (WNV) has impacted a broad range of animals and humans.<sup>6</sup> Reports of raptors infected with WNV are limited in the scientific literature; however, there is evidence of regional increases in raptor mortality.<sup>3</sup> Although impact on avian populations secondary to WNV infection is unknown, anecdotal reports suggest a likely negative effect in the forthcoming years.<sup>1,3</sup>

As part of avian surveillance for WNV in Virginia (2003), cloacal and/or oropharyngeal swabs collected from 61 live raptors admitted to the Wildlife Center of Virginia (WCV) were tested at Virginia Department of Consolidated Laboratory Services (DCLS) for WNV by real time reverse-transcriptase polymerase chain reaction (RT-PCR) using FAM- and TAMRA-labeled probes and primers that previously have been reported.<sup>2,5</sup> Forty raptors, including nine species, were positive for WNV by RT-PCR on oropharyngeal and/or cloacal swabs (Table 1) with red-tailed hawks (*Buteo jamaicensis*) (RTH) and great-horned owls (*Bubo virginianus*) (GHO) over-represented (15/40; 37.5% and 16/40; 40%, respectively). Seventeen of 32 birds (53%) tested only with oropharyngeal swabs were positive. In addition, 23 of 29 birds (75.8%) tested positive with combined oropharyngeal and cloacal swabs. Four birds (two GHOs and two RTHs) (4/29; 13.8%) were positive on oropharyngeal swabs but negative on cloacal swabs. Two RTHs (2/29; 6.9%) were positive on cloacal swabs but negative on oropharyngeal swabs.

Physical examination, hematology, serum chemistry profile, and radiographs were performed on WNV infected birds. Clinical presentation varied between species. The most common findings on physical examination in all species were non-specific signs of illness including depression, dehydration and emaciation. The main presenting signs in GHOs included head bobbling, head tremors, and ataxia. Hematology (n = 10) showed a moderate anemia, marked leukocytosis, heterophilia with left shift and a monocytosis. Chemistry results (n = 4) suggested dehydration. On radiography (n = 7), GHOs had a mild to moderate interstitial lung pattern (4/7). One GHO had mild splenomegaly and one had hepatomegaly. In contrast, RTHs presented with non-specific signs of illness with minimal neurologic signs. Hematology (n = 9) showed a moderate to marked anemia, moderate leukocytosis and heterophilia with left shift. No consistent

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chemistry (n = 3) findings were noted. On radiography, RTHs were emaciated (4/5) with decreased splenic mass (3/5). Two of five RTHs also had fractured long bones.

The mean monthly numbers of raptors admitted to WCV for the previous 10 yr were compared to the monthly number of raptor admissions for 2003. There was a decrease in the number of nestlings received during May and June 2003. The number of cases in 2003 showed a marked increase during August and September followed by a marked decrease in admissions for October to December compared with the previous 10 yr. Retrospective review of medical records from 2002 suggested a similar epidemiologic pattern and clinical presentation, although less marked; however, no WNV cases were confirmed.

Of great concern is the impact of West Nile virus infection on threatened species and those of ecological importance. For example, two bald eagles (*Haliaeetus leucocephalus*) were identified as WNV positive by RT-PCR, which were euthanatized due to poor prognosis for recovery. In addition, four juvenile peregrine falcons (*Falco peregrinus*) were positive for WNV detected by RT-PCR, including three juveniles that were submitted directly to DCLS by Virginia Department of Game and Inland Fisheries (VDGIF). These falcons were part of the VDGIF Peregrine Falcon Restoration Project: Falcon Trak (<http://www.dgif.state.va.us/wildlife/falcontrak/index.html>).

Early detection of clinical cases with accurate and rapid diagnosis will aid in monitoring the spread of WNV. This is the first clinical description of WNV in red-tailed hawks and will assist in recognition of this disease. The clinical presentation of great-horned owls is consistent with previous reports of WNV infection in owls.<sup>3</sup> Oropharyngeal and cloacal swabs for WNV RT-PCR provided a reliable ante-mortem diagnosis of current infection in field samples and is consistent with the findings of Komar, et al. (2002).<sup>4</sup> Due to the difference in RT-PCR results in 6 birds, testing of both oropharyngeal and cloacal swabs is recommended. RT-PCR of oropharyngeal and cloacal swabs correlated well with clinical presentation of WNV in great-horned owls and red-tailed hawks. The epidemiologic findings are consistent with outbreaks of WNV infection in raptors from Virginia during 2002 and 2003. In addition, the change in the monthly distribution of raptor admissions may indicate declines in local populations and provides evidence to support that WNV is having a negative impact on local raptor populations. The apparent increased number of WNV infected raptors during 2003 is consistent with the generalized spread of WNV in Virginia compared with previous years (D.N. Gaines, pers.communication). Studies are urgently needed to determine if the decline in the number of raptor admissions during spring and winter represents more widespread raptor population declines.

#### **ACKNOWLEDGMENTS**

We thank David N. Gaines, Ph.D., and Suzanne R. Jenkins, V.M.D., M.P.H., of the Virginia Department of Health, for their assistance. In addition, we thank the Virginia Department of Game and Inland Fisheries for their cooperation and the wildlife rehabilitation staff of the Wildlife Center of Virginia for their expertise and animal care.

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**Table 1.** Raptors from the Wildlife Center of Virginia positive for West Nile virus by RT-PCR using oropharyngeal and/or cloacal swabs during 2003.

Species	Scientific name	Number positive	Percent positive
Great-horned owl	<i>Bubo virginianus</i>	16	40.0
Red-tailed hawk	<i>Buteo jamaicensis</i>	15	37.5
Broad wing hawk	<i>Buteo platypterus</i>	2	5.0
Bald eagle	<i>Haliaeetus leucocephalus</i>	2	5.0
Sharp-shinned hawk	<i>Accipiter striatus</i>	1	2.5
Peregrine falcon	<i>Falco peregrinus</i>	1	2.5
American kestrel	<i>Falco sparverius</i>	1	2.5
Barn owl	<i>Tyto alba</i>	1	2.5
Black vulture	<i>Coragyps atratus</i>	1	2.5
Total		40	100

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**DETECTION OF WEST NILE VIRUS FROM ORAL SWABS OF NESTLING CLIFF SWALLOWS: POTENTIAL USE AS AN EARLY SURVEILLANCE METHOD**

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**Abstract**

We report early seasonal activity of West Nile virus (WNV) infection in cliff swallow nestlings from the Fort Collins, Colorado area. Using TaqMan reverse transcription-PCR we were able to detect WN virus in oral swab samples taken from nestling cliff swallows. The timing of virus activity in the nestling population predates the general human activity of WN in the Fort Collins area by 5 wk. West Nile virus activity in nestlings corresponded spatially to case reports of viral infection in humans. This surveillance method may prove useful in designing a sensitive, spatially-explicit, early-detection monitoring system that can predict risk to human populations and thus help guide mosquito control efforts.

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**SEROSURVEY FOR ANTIBODIES TO FLAVIVIRUSES IN WILD MAMMALS OF THE CENTRAL AND EASTERN UNITED STATES**

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**Abstract**

ELISA techniques were used to detect antibodies to flaviviruses and West Nile virus (WNV) in wild mammals. Two different monoclonal antibodies (6B6C-1 and 3.1112G) were used. More than 500 serum samples from over twenty mammal species captured in five states (CO, LA, NY, OH, PA) were screened for flaviviruses, and those which were flavivirus positive were screened for WNV. Antibodies to flaviviruses were detected in multiple species. This number was significantly reduced for WNV as was the overall prevalence of antibodies, indicating that multiple flaviviruses may have been present at some study sites. High prevalence rates were noted for select species in both assays. Future work will employ plaque reduction neutralization tests to detect neutralizing antibodies to WNV and other flaviviruses.

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**EVIDENCE OF INFECTIONS BY *Anaplasma phagocytophilum* AND *Borrelia burgdorferi* SI IN AMERICAN BLACK BEARS, WOODRATS, AND DOMESTIC DOGS FROM INLAND FOREST HABITATS OF NORTHERN CALIFORNIA**

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**Abstract**

Transmission cycles of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sl appear parallel with reservoirs in wild rodents and transmission by *Ixodes* spp. ticks. Dusky-footed woodrats are the putative reservoir hosts of both of these organisms in California. We report high seropositivity to both organisms in woodrats, American black bears, and domestic dogs sampled in inland forest habitats, and DNA of *A. Phagocytophilum* was PCR-amplified from 10% of woodrats and 3.8 % of bears, but none of the dogs, suggesting greater risk to humans than is appreciated locally. However, the sequence of DNA typed as *A. phagocytophilum* from woodrats appears slightly different from reported sequences. Moreover, current evidence suggests that dusky-footed woodrats are the reservoir for *Borrelia bissettii* (a genospecies closely related to *B. burgdorferi* within the *B. burgdorferi* sl complex) but they may not be the most important reservoir of *B. burgdorferi* ss, the primary pathogen associated with human Lyme disease in the western US. Thus, the reservoir(s) of both of these important zoonotic pathogens remains unclear, at least in northern California.

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## THE ROLE OF HOST BIODIVERSITY, DENSITY AND TRANSMISSION ROUTES IN GENERATING NON-LINEARITIES IN TICK BORNE INFECTIONS

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### Abstract

The dynamics of tick-borne infections incorporates a series of non-linear phenomena operating in the transmission processes between ticks, hosts and pathogens. Ticks feed on a diverse range of hosts that vary in their competence to transmit the pathogen and vary in the routes of transmission.

We explore the consequence of variations in the relative abundance of the two host species (deer and rodents) and the interaction with the transmission routes on the persistence and success of Lyme disease and Tick-borne encephalitis in Trentino (northern Italy). More generally we wish to explore the consequences of host abundance and non-linearities in the transmission processes on the likelihood of tick borne diseases emerging as significant threats to human and wildlife health.

We develop a general model on tick borne infections,<sup>1</sup> predict the relative conditions that would lead to disease persistence ( $R_0 > 1$ ) and then test the model against surveillance data we have collected from northern Italy.

### LITERATURE CITED

1. Rosà, R., Pugliese, A., Norman, R. & Hudson, P.J. 2003 Thresholds for disease persistence in models for tick-borne infections including non-viraemic transmission, extended feeding and tick aggregation. *Journal of Theoretical Biology* 224, 359-376.

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## ECOLOGY AND MANAGEMENT OF CHRONIC WASTING DISEASE IN NORTHERN COLORADO MULE DEER

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### **Abstract**

Chronic wasting disease (CWD) has been endemic in northern Colorado for over 2 decades. We analyzed prevalence data from mule deer (*Odocoileus hemionus*) populations in Larimer County to discern the likely influences of temporal, spatial, and demographic factors on patterns observed in naturally infected populations and to look for evidence that recent management actions have affected temporal trends. We observed spatial heterogeneity among wintering mule deer subpopulations, marked difference in CWD prevalence by sex and age groups, and clear local trends of increasing prevalence over a 7-yr period that largely preceded management intervention. For both sexes, prevalence peaked in the 4–6-yr old age class, with the largest increase occurring between the 2–3-yr-old and 4–6-yr-old age classes. This differential was larger for males (5.9% among 2–3-yr-olds vs. 19.4% among 4–6-yr-olds;  $P = 0.0002$ ). Demographic, spatial, and temporal factors all appear to contribute to the marked heterogeneity in CWD prevalence in endemic portions of northcentral Colorado. These factors likely combine in various ways to influence epidemic dynamics and responses to management on both local and broad geographic scales.

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## COMPARISON OF GENETIC VARIABILITY IN NORTH AMERICAN RUMINANT PRION PROTEIN (PrP) SEQUENCES

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### Abstract

We obtained genomic DNA from members of ten species and subspecies of wild North American ruminants and two Old World cervids and determined the DNA sequence of the prion protein (PrP) genes to investigate the degree of similarity between them. We also assessed genetic variability of this gene in the different species by ascertaining the number and location of any commonly recurring polymorphisms. The species sampled included the three natural hosts for chronic wasting disease (CWD), a transmissible spongiform encephalopathy seen in *Cervus elaphus*, *Odocoileus hemionus*, and *Odocoileus virginianus*. We also sampled *Alces alces shirasi*, *Rangifer tarandus*, *Odocoileus hemionus columbianus*, *Odocoileus hemionus sitkensis*, *Antilocapra americana*, *Bison bison*, *Ovis canadensis*, *Dama dama*, and *Capreolus capreolus*. We compared the locations of interspecific substitutions and of polymorphic loci relative to the other species and to the elements of secondary structure of the normal cellular protein.

Our findings are consistent with previous, large-scale comparisons of PrP protein sequences across a wide array of taxa that show conservation of amino acid sequence in the two beta-sheet and three alpha helix regions of mammalian prion proteins. The interspecific differences and twelve to fifteen intraspecific polymorphisms of the mature polypeptides in the ruminant species examined fell in the carboxy terminal two-thirds of the protein. Most substitutions occurred outside the secondary structure features of cellular PrP, although in addition to the well-known substitution in beta-1 of leucine for methionine at codon 132 which occurs at low frequency in *Cervus elaphus nelsoni*, we noted an isoleucine-for-methionine substitution at 209 in *Alces alces shirasi*; this falls in alpha helix 3. In our samples (n = 1 to >300) the number of non-rare, fixed polymorphic loci per species varies from 0 to 3, and none exhibits more than two alleles per locus. None of the polymorphisms are the same in any two species. About half of the 11 interspecific differences fall outside elements of secondary structure, while two each occur within the second and third alpha helices. In addition, bison, bovines and pronghorn carry a sixth copy of the eight-amino acid repeated region in the amino terminus of the protein. Overall, we found amino acid identities within these species to vary between 96% and 100% as compared to *Cervus elaphus* PrP.

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## INVESTIGATION OF CHRONIC WASTING DISEASE STRAIN VARIATION USING FERRETS (*Mustela putorius furo*) AS A MODEL

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### Abstract

We investigated evidence for strain variation in the chronic wasting disease (CWD)-associated prion (PrP<sup>CWD</sup>) of deer (*Odocoileus* spp.), as well as the utility of domestic ferrets (*Mustela putorius furo*) as a common host for such studies. Ferrets (n = 3 per group) were inoculated intracerebrally with brain material from single natural cases of CWD from northeastern Colorado that had occurred in a) a captive mule deer (*O. hemionus*) prior to 1985, b) a captive mule deer in 2000, c) a free-ranging mule deer, d) a captive white-tailed deer (*O. virginianus*) in 1999, and e) a free-ranging white-tailed deer; two additional groups of ferrets (uninoculated and inoculated with CWD-negative mule deer brain) were maintained as controls. Clinical signs and postmortem findings consistent with CWD in ferrets were observed in four of five groups inoculated with tissue from infected deer, but not in the free-ranging white-tailed deer or control groups. Incidence and incubation periods were consistent among affected groups. Moreover, Western blots (WB) revealed no apparent differences in glycosylation patterns among WB-positive ferrets. No strain variation in PrP<sup>CWD</sup> was evident among these representative cases of CWD in captive mule deer and white-tailed deer and free-ranging mule deer from northeastern Colorado; however, no variation was expected among the three groups inoculated with materials from the same captive facility. Based on our experiences, domestic ferrets have limited utility as a laboratory model for studying CWD. Despite our findings, further investigation of potential strain variation among more geographically and epidemiologically distant cases of CWD in deer still appears warranted.

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## CHRONIC WASTING DISEASE VACCINE RESEARCH

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### **Abstract**

Chronic wasting disease (CWD) in deer and elk is a transmissible spongiform encephalopathy (TSE) purportedly caused by prions. Studies by other research groups have elucidated key contact sites on normal cellular prion protein that are needed for duplication of the abnormal, disease-causing prion form. When these sites are blocked with antibodies, the course of the disease may be altered. We are investigating vaccines that include peptide sequences found within these sites that may be used to elicit a protective, active immune response. These vaccines tested in rabbits have elicited an antibody response. The next phase of research will investigate vaccine efficacy in the face of disease challenge using a mouse scrapie model.

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## INFECTIOUS DISEASE SURVEY OF SAGE-GROUSE IN NEVADA AND OREGON

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### Abstract

The U. S. Fish and Wildlife Service has received numerous petitions for listing sage-grouse (*Centrocercus urophasianus*) as a threatened or endangered species. The role of infectious diseases in reduced productivity and population declines in sage-grouse over their range is not known. Information on diseases of sage-grouse is limited. Therefore, to determine if there were a high prevalence of disease in sage-grouse in portions of their range, we surveyed sage-grouse (n = 40) from southeastern Oregon in April, 2003, for serologic evidence to selected disease agents including: *Salmonella typhimurium*, *S. pullorum*, *Mycoplasma gallisepticum*, *M. synoviae*, avian influenza, Newcastle disease, *Chlamydophila psittaci* (n = 36), and West Nile virus (n = 27). All were negative. We also surveyed the same sage-grouse from SE Oregon (n = 40) and additional (n = 37-38) sage-grouse from northwestern Nevada for serologic evidence of exposure to avian infectious bronchitis virus (AIBV; Arkansas 99, Massachusetts 41, and Connecticut types) using the hemagglutination-inhibition test. Avian infectious bronchitis virus causes early chick mortality in domestic poultry, and we had observed unexplained early sage-grouse chick mortality in southeastern Oregon. We found 46% (36/78) had positive titers (1:16) for AIBV Arkansas 99 type, 8% (6/77) for Massachusetts 41 type, and 53% (20/38) for Connecticut type. During October, 2003, attempts to isolate AIBV from Nevada sage-grouse tracheal and cloacal swabs (n = 21) by egg-culture and fluorescent antibody techniques were unsuccessful. This is the first known published report that sage-grouse have positive antibody titers to AIBV. The effects AIBV may have on sage-grouse populations are unknown. The importance of surveys for parasites and diseases in sage-grouse cannot be overemphasized. Only with such knowledge, can proper management of this dwindling species be accomplished.

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**AN EPIDEMIC OF AVIAN POX VIRUS IN LARKS (*Calandrella rufescens*) AND PIPITS (*Anthus bertelotti*) IN THE CANARY ISLANDS, SPAIN**

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**Abstract**

Over the past 2 yr on the islands of Fuerteventura and Lanzarote in the Canary Islands off the west coast of Africa, ongoing ecological studies of desert passerines have uncovered the occurrence of an apparent epidemic of health problems in two species, the short-toed lark, *Calandrella rufescens*, and Berthelot's pipit, *Anthus bertelotti*. Two other native passerine species which are found in the same steppe habitats associated with dairy goat farming, the Spanish sparrow, *Passer hispaniolensis*, and the trumpeter finch, *Bucanetes githagineus*, have been studied simultaneously. Over 800 birds from the four species have been trapped and ringed over the past 2 yr in April, July and November. Of 465 individuals from the two affected species studied over the various collection periods, 28% to 49% (mean 42.5%) of the birds had clinically obvious pox-like lesions involving the legs, feet and, occasionally face. Of a similar number of trumpeter finches and sparrows trapped at the same locations at the same time, none showed evidence of infection. Histopathology and electron microscopy have confirmed the presence of poxvirus in the lesions, whereas serology using standard, fowl and pigeon poxvirus-based diagnostic agar gel immunodiffusion techniques yielded negative results. Serology was not diagnostic in this case because of the limited (74.6%, pipit; 74.9% lark) similarity between the viruses in our species and fowlpox virus on which the serologic tests are based. Using a 575 base pair DNA fragment from the 4b core gene of a fowlpox virus strain, the virus isolated from dried lesions of *C. rufescens* has only 80.5% similarity with the virus isolated from *A. bertelotti*, and 91.3% similarity with canarypox, whereas *A. bertelotti* poxvirus has 80% similarity with canarypox, which indicates that these are two distinct, and possibly new avian poxviruses.

The conservation implications of this epidemic of avian pox among birds in the Canary Islands are considerable. We have discovered a high prevalence of disease similar to that described in well studied, native passerines in Hawaii that are known to be threatened, endangered, or even extinct, at least in part due to poxvirus infection. Of the species we have studied, all except the sparrow are designated threatened. Other globally endangered species exist in the same habitats in the Canary Islands of Fuerteventura and Lanzarote, such as the houbara bustard, *Chlamydotis undulata*, and the stone curlew, *Burhinus oedicephalus*. Subspecies of these birds on the mainland have been diagnosed with poxvirus infections. It will be essential to investigate environmental and biologic factors that are contributing to the increased disease susceptibility of these isolated

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populations of vulnerable bird species, in order to reverse this alarming trend in disease occurrence.

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## LONG-TERM NEUROLOGIC EFFECTS OF EXPOSURE TO DOMOIC ACID IN STRANDED CALIFORNIA SEA LIONS

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### Abstract

Domoic acid is an excitatory neurotoxin that is produced by a number of marine algae, including the diatom *Pseudonitzschia australis*. Acute domoic acid toxicity can result in a number of neurologic signs in affected California sea lions (*Zalophus californianus*) such as ataxia, disorientation, seizure, and possibly death. However, the long-term, sublethal effects of domoic acid toxicity have not been fully investigated. This study describes the neurologic lesions associated with the long-term effects of acute exposure to domoic acid in stranded sea lions, and investigates the survival of animals with these lesions using satellite-linked telemetry. Animals in the study were suspected of having long term effects of domoic acid toxicity if they exhibited neurologic signs typical of domoic acid toxicity yet stranded during a time of no known domoic acid producing algal blooms, restranded after initial treatment for domoic acid toxicity, or continued to exhibit neurologic signs after multiple courses of anti-convulsant therapy. The animals were then screened for other causes of neurologic disease by performing complete blood counts, serum biochemistry analysis, serology for *Toxoplasma* sp. and *Sarcocystis* sp., radiographs, cerebrospinal fluid evaluation, and magnetic resonance imaging (MRI). If the animals showed no clinical signs of neurologic disease for at least 10 days after the end of anti-convulsant therapy, they were fitted with a satellite-linked transmitter and released.

Ten animals (2 male, 8 female) were suspected of having long-term effects of domoic acid toxicity and entered into the study. Eosinophilia was noted in eight animals. Radiographs and cerebrospinal fluid evaluation did not reveal any significant abnormalities. Markedly elevated and rising *Toxoplasma* sp. titers were detected using an immunofluorescent antibody test in one animal, while weak positive titers were found in two additional animals. Evaluation of MRI studies in the animals revealed a variety of lesions. The most common MRI findings were varying degrees of unilateral and bilateral hippocampal atrophy found in all 10 animals.

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Additional lesions suggestive of cerebritis in two animals and cerebral hemorrhage in one animal that were seen with MRI and confirmed with histopathology were not considered to be typical of domoic acid toxicity. Histopathology from the other three animals that either died or were euthanatized revealed neuronal necrosis and gliosis in the limbic system considered typical of California sea lions naturally exposed to high doses of domoic acid. One animal was released without a satellite-linked transmitter. Five animals were released with satellite-linked transmitters and monitored for up to 106 days post-release. Of these five, two animals re-stranded and were euthanatized, one animal had questionable success after release due to premature failure of the telemetry system, one animal displayed normal behavior after release, and it is still too early to evaluate the success of the last animal. This study highlights the difficulties encountered with the diagnosis of long-term effects due to acute domoic acid toxicity in stranded California sea lions. The variable post-release success of animals diagnosed with long-term effects of domoic acid toxicity suggests that diagnosis of the severity of lesions must be improved in order to better evaluate the prognosis for affected California sea lions.

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**AN UNUSUAL GENOTYPE OF *Toxoplasma gondii* IS COMMON IN CALIFORNIA SEA OTTERS (*Enhydra lutris nereis*) AND IS ASSOCIATED WITH MORTALITY**

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**Abstract**

*Toxoplasma gondii*-associated meningoencephalitis is a significant disease of California sea otters (*Enhydra lutris nereis*), responsible for 16% of total mortality in fresh, beachcast carcasses. *Toxoplasma gondii* isolates were obtained from 35 California otters necropsied between 1998 and 2002. Based on multi-locus PCR-RFLP and DNA sequencing at conserved genes (*18s rDNA*, *ITS-1*) and polymorphic genes (*BI*, *SAG1*, *SAG3* and *GRA6*), two distinct genotypes were identified: type II and a novel genotype, here called type x, that possessed distinct alleles at three of the four polymorphic loci sequenced. The majority (60%) of sea otter *T. gondii* infections were of genotype x, with the remaining 40% being of genotype II. No type I or type III genotypes were identified. Epidemiologic methods were used to examine the relationship between isolated *T. gondii* genotype(s) and spatial and demographic risk factors, such as otter stranding location and gender, as well as specific outcomes related to pathogenicity, such as severity of brain inflammation on histopathology and *T. gondii*-associated mortality. Differences were identified with respect to *T. gondii* genotype and sea otter gender and stranding location along the California coast. Localized spatial clustering was detected for both type II (centered within Monterey Bay) and type x (centered near Morro Bay) -infected otters. The Morro Bay cluster of type x-infected otters overlaps previously reported high-risk areas for sea otter infection and mortality due to *T. gondii*. Nine of twelve otters that had *T. gondii*-associated meningoencephalitis as a primary cause of death were infected with type x parasites.

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# INTERNAL ANATOMY OF THE HORNBILL CASQUE BY RADIOGRAPHY, CONTRAST IMAGING, AND COMPUTED TOMOGRAPHY

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## **Abstract**

Techniques of standard radiography, computed tomography, and contrast radiography were systematically applied to post-mortem skull and casques of representative species of the hornbill family. Skeletal preparations were completed of these specimens for direct examination. Diagnostic images and photographs were evaluated to document internal casque anatomy.

## **Introduction**

The unique features of the Family Bucerotidae include a casque, or ornamentation on the dorsal maxillary beak. Each of the 54 extant hornbill species has a different shape, size, and coloration of casque that may be as simple as a ridge or as elaborate as a recurved horn. The casque is essentially an air-filled cavity enclosed by minimal bone, except in one species (greater helmeted hornbill, *Buceros vigil*).<sup>2-4</sup> A horny keratin overlies the casque and produces the bulk of its visible mass. It originates from a highly vascular ridge of the maxillary rhamphotheca in the juvenile. Progressively enlarging until sexual maturity, a different casque contour is produced by each gender with males generally having larger, more elaborate structures than the females. Detailed descriptions of the external appearance are available but little information is available about the internal anatomy.<sup>2,3</sup> The clinical challenge of a structure poorly described has become apparent with the hornbill casque (personal communications: T. McNamara, 2000; G. Pirie, 2003).<sup>6,7</sup> The casque is subject to self-induced trauma, conspecific injury, environmental damage, and neoplasia (personal communications: R. Aguilar, 2003; T. McNamara, 2000; K. Petrini, 2000; G. Pirie, 2003; R. Wagner, 2001).<sup>5,7,8</sup> Surgical exploration, resolution of disease, and reconstruction of this area would be improved by anatomic knowledge.

To date, evaluation of the casque has been essentially through standard radiography. Although multiple views have assisted with assessment of disease, they have not substantially advanced the anatomic knowledge of the structure. In domestic animal medicine, computed tomography (CT) have been utilized for imaging the nasal planum, nasal sinuses, and surrounding tissues.<sup>1,6</sup> Furthermore, traditional radiography has been expanded in domestic species by contrast techniques. These techniques were systematically applied to advance clinical understanding of the Bucerotid casque anatomy. Specifically, the interconnections of the casque space with the sinuses, the nares themselves, and the oropharynx may vary by species (K. Petrini, personal communication, 2000).<sup>3,4</sup>

## **Methods**

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By an addendum to the established necropsy protocol of the Coraciiformes Taxon Advisory Group (TAG), seven specimens were obtained for evaluation: silvery-cheeked (*Bycanistes brevis*) (female), trumpeter (*Bycanistes bucinator*) (female), rhino (*Buceros rhinoceros*) (male), wreathed (*Rhyticeros undulatus*) (female), greater Indian (*Buceros bicornis*) (female), wrinkled (*Aceros corrugatus*) (male and female), and Jackson's (*Tockus deckeni*) (male).

The intact skull and casque were maintained frozen until thawed for imaging. Standard radiography (right lateral, left lateral, ventrodorsal, dorsoventral, and skyline-rostrocaudal) was completed then the specimen shipped on ice for CT imaging (Texas A&M University College of Veterinary Medicine, College Station, TX 77843, USA) in standard positioning (lateral and ventral recumbency) for a clinical avian patient. Both sagittal and craniocaudal sectioning was performed at 2-5mm slice thickness depending on size of the specimen. The specimen was then returned on ice for the contrast radiography. The casque internal space was estimated by multiplication of linear measure (in mm) of the casque (height, length, width) from the plain films. This calculated volume (in ml) of radio-opaque contrast media (RenoCal 76, 37% organic bound iodine, Bracco Dx, Princeton, NJ 08540, USA) was instilled into the casque and the standard radiographic series repeated. Professional preparation (Skulls Unlimited, 10313 South Sunnyslane, Oklahoma City, OK 73160, USA) of the skull and casque was performed and specimens sectioned sagittally to allow access to the interior of the casque and skull.

## **Results and Discussion**

Diagnostic images were evaluated for internal casque anatomy (dimensions, contour, shape, and interconnections to sinuses within the skull and beak). The skeletal preparations were directly examined and photographed to document anatomic features. The images and skull preparations were compared directly then considered between the genera, the species, and genders.

This assembled data is interpreted for clinical applications. Utility of the casque interior as a depot for pharmaceuticals, including antibiotics and anti-neoplastics, may be considered. Surgical approaches and limits to the casque, skull, and sinus are available from the TAG veterinary advisor. Continued documentation to obtain similar image studies from the originally selected twelve hornbill species as representatives of the captive population is ongoing.

## **ACKNOWLEDGMENTS**

Funding for this endeavor was provided by a generous grant (in-kind) from the Schubot Center for Avian Medicine, Texas A&M University, College Station, Texas, and through annual research funding from the Association of Avian Veterinarians. I appreciate the submitted specimens from the following institutions: Audubon Institute, New Orleans, Louisiana; Chaffee Zoo, Fresno, California; Dallas Zoo and Aquarium at Fair Park, Dallas, Texas; San Antonio Zoological Gardens, San Antonio, Texas; and Wildlife Conservation Society, Bronx, New York. I thank Chris Sheppard, Ph.D., Coraciiformes TAG chair, and the entire Coraciiformes TAG for their steadfast support of the endeavors of their veterinary advisor.

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## RENAL CREATINE DISPOSITION IN THE PIGEON (*Columba livia*): INFERRING FROM $^{99m}\text{Tc}$ TRACER STUDIES

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### Abstract

Available indicators of renal dysfunction (blood urea nitrogen, uric acid) in avian species have been shown to be relatively nonspecific and insensitive because they are readily affected by diet and hydration status, and they are often elevated late in the disease course after the renal functional reserve has been depleted. One aspect of ongoing studies employing the pigeon (*Columba livia*) model (n = 26) compared disposition curves ( $V_d$ ) of  $^{99m}\text{Tc}$ -DTPA (diethylene pentacetic acid) and  $^{99m}\text{Tc}$ -MAG3 (mercapto-acetyl-triglycine), two commonly used renal function tracers, to that of the endogenous marker creatine. The calculated  $V_d$  for  $^{99m}\text{Tc}$ -DTPA ( $5.24 \pm 0.67$  %; n = 4) and  $^{99m}\text{Tc}$ -MAG3 ( $3.64 \pm 0.69$  %; n = 5) were smaller than for creatine ( $20.4 \pm 3.43$  %, n = 8). Our results in the pigeon are consistent with the view that  $^{99m}\text{Tc}$ -DTPA is filtered by the glomeruli, and  $^{99m}\text{Tc}$ -MAG3 is secreted in the renal tubules. The short-lived tracers  $^{99m}\text{Tc}$ -DTPA and  $^{99m}\text{Tc}$ -MAG3 show great promise for noninvasive real-time glomerular and tubular assessment of renal function in this representative avian species. Further work is required to clarify whether creatine may be reabsorbed in the renal tubules as well.

### Introduction

Renal disease remains a clinically challenging diagnosis in avian species. Available markers of renal dysfunction principally consist of those that accumulate in the blood due to reduced clearance or those released into the urine as a result of renal epithelial injury. Previous work in the pigeon and other avian species suggested the potential value of blood urea nitrogen (BUN) and uric acid for renal disease detection. However, both these markers have been shown to be relatively nonspecific for indication of disease because they are readily affected by diet, hydration status, and other factors. In addition, the elevation of these parameters in the blood occurs relatively late in the disease course, and only after the renal functional reserve is depleted. The development of improved methods for the assessment of renal disease would greatly aid the diagnostic capabilities of avian clinicians. In addition, the use of dynamic study methods may facilitate the early detection of renal function deficits whenever therapeutic efforts may be most effective.

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The present study in the pigeon included the following objectives: 1. characterizing the time course of the renal function tracers,  $^{99m}\text{Tc}$ -DTPA (diethylene pentacetic acid) and  $^{99m}\text{Tc}$ -MAG3 (mercapto-acetyl-triglycine); and 2. estimating the volume of distribution ( $V_d$ ) of these tracers as compared to the  $V_d$  for the endogenous marker creatine. These objectives were undertaken to allow meaningful comparisons of the disposition curves of these two commonly used renal function tracers in the pigeon, and to make a crucial step towards the characterization of creatine disposition in the avian kidney.

## Methods

Twenty-six healthy cull pigeons (*Columba livia*) were fed a standard laboratory pigeon diet and water *ad lib* for 1 wk prior to several renal function studies. For the present study, all animals were randomly selected by treatment and anesthetized with isoflurane (Aerrane, Anaquest, Madison, Wisconsin, 53713, USA) and oxygen. Once anesthetized, each pigeon had 22-24 ga heparinized catheters (Abbocath, Abbott labs, North Chicago, Illinois, 60064, USA) placed into both a medial tarsal vein (blood sampling), and a brachial vein (tracer/marker infusion). Each bird was recovered from anesthesia, administered 10 ml of 0.9% saline s.c. over the dorsum, and maintained in a quiet environment with low light 1.5 hr prior to study.

Pigeons were anesthetized just prior to study with a combination of ketamine (25 mg/kg i.m., Ketaset, Fort Dodge Animal Health, Fort Dodge, Iowa, 50501, USA) and xylazine (2 mg/kg i.m., TranquiVed, Vetco, St. Joseph's, Missouri, 64507, USA). Once anesthetized, each bird was placed directly on the detector surface of the Anger camera (Siemens Orbiter, Siemens AG, D-80333, Munich, Germany) in dorsal recumbency on a disposable absorbent plastic barrier-backed sheet. Tissue depth was kept constant by careful positioning. A baseline blood sample was drawn from the medial tarsal vein to measure baseline creatine levels. A combination of tracer ( $^{99m}\text{Tc}$ -DTPA {diethylene pentacetic acid} and  $^{99m}\text{Tc}$ -MAG3, {mercapto-acetyl-triglycine}, Mallinckrodt Medical, St. Louis, Missouri, 63134, USA) and marker (creatinine) was suspended in saline. This tracer/marker cocktail was injected i.v. into the brachial vein over a period of 30-45 sec for a total volume of 2.85 ml. Images were collected at 1/sec, and collapsed into twelve 1-min time series composite images using Image J (via download, National Institutes of Health, <http://rsb.info.nih.gov/ij/index.html>). During the scanning period, serial small volume (0.25-0.5 ml) blood samples were collected to assay for both the tracer and the marker.

Regions of interest (ROI) were delimited with the area drawing tool. The mean counts, the count area (of the ROI), and area normalized counts were calculated. ROIs for the "vascular phase", the "renal phase," and the "excretory phase" were selected and delimited from the heart, kidney, and cloaca regions respectively. Preliminary validations included correcting for background, defining the time course of the vascular, renal, and excretory phases, and surveying for intrarenal functional differences. The volume of distribution ( $V_d$ ) for each tracer and creatine were calculated by previously reported methods.<sup>1</sup> Briefly,  $V_d$ s of the tracers and creatine were estimated from log transformed y intercepts (time = 0), body weight, and the amount of tracer or creatine given. Creatine was assayed by methods described previously.<sup>3</sup>

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## Results

Initial analyses revealed a peak in the vascular phase approximately 2 min post-injection for both tracers. The excretory phase was derived from area normalized subtraction of the heart ROI curve from the cloacal ROI curve. For  $^{99m}\text{Tc}$ -DTPA, the excretory phase ( $n = 7$ ) started at  $4.36 \pm 0.18$  min (mean  $\pm$  s. e.;  $n = 7$ ), whereas, for  $^{99m}\text{Tc}$ -MAG3 it occurred at  $3.07 \pm 0.41$  min ( $n = 7$ ). The “renal excretion phase” was selected as the time period from 2-7 min post-injection for subsequent analyses.

The calculated  $V_d$  for  $^{99m}\text{Tc}$ -DTPA ( $5.24 \pm 0.67$  %;  $n = 4$ ) and  $^{99m}\text{Tc}$ -MAG3 ( $3.64 \pm 0.69$  %;  $n = 5$ ) were smaller than for creatine ( $20.4 \pm 3.43$  %,  $n = 8$ ), suggesting that the creatine disappearance curve was at least partially determined by a larger volume of distribution as compared to the tracers used.

Mean slopes of log transformed renal phase disposition curves for the two tracers were significantly different ( $P = 0.034$ ) between the two tracers. Likewise, the overall disposition curves for birds receiving each tracer indicated that  $^{99m}\text{Tc}$ -MAG3 ( $n = 5$ ) excretion appeared more rapid, and had a steeper negative slope than was observed for  $^{99m}\text{Tc}$ -DTPA ( $n = 5$ ).

## Discussion

The short-lived tracers  $^{99m}\text{Tc}$ -DTPA and  $^{99m}\text{Tc}$ -MAG3 show great promise for noninvasive real-time assessment of renal function in this representative avian species. Our results in the pigeon support similar findings in the human that  $^{99m}\text{Tc}$ -DTPA is filtered by the glomeruli, whereas,  $^{99m}\text{Tc}$ -MAG3 is secreted in the renal tubules.<sup>2</sup> Based on earlier work (Wimsatt and Steyn, unpublished, 1997), creatine appeared to have an even slower excretion rate than  $^{99m}\text{Tc}$ -DTPA in the pigeon. Some of this effect is explained by its large volume of distribution, however, further work is required to clarify whether creatine may be reabsorbed in the renal tubules as well.

## ACKNOWLEDGMENTS

We thank Dr. Phillip Steyn of Colorado State University for contributing to earlier studies upon which the present studies are based, and Dr. Klaus Beyenbach of Cornell University for helpful advice. Funding for this project was provided by a generous grant from the Morris Animal Foundation (DO2ZO-79 to JW).

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## PHARMACOKINETICS AND CLINICAL EFFICACY OF TERBINAFINE AGAINST ASPERGILLOSIS IN AVIAN SPECIES

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### Abstract

Current drug therapies used for treatment of aspergillosis in avian species have been largely ineffective, primarily because of their fungistatic mechanism of action. Terbinafine (an allylamine) has fungicidal activity and might provide a more efficacious therapeutic option against aspergillosis. Current research efforts are assessing the pharmacokinetics of terbinafine, formulating safe therapeutic dosing regimens, and determining clinical efficacy of this drug for treatment of aspergillosis in African penguins (*Spheniscus demersus*) and red-tailed hawks (*Buteo jamaicensis*). Preliminary results are inconclusive at this stage. Subsequent opportunistic field trials to determine clinical efficacy of terbinafine in the treatment of aspergillosis will utilize dosage regimens deemed therapeutic based on pharmacokinetic results in these species.

### Introduction

Aspergillosis is the most commonly occurring avian mycotic infection and typically causes chronic, debilitating disease and mortality in all representative species of psittacines, water birds and raptors.<sup>15</sup> A variety of drugs have been used in various combinations for treatment of aspergillosis; however, current treatment regimens are not often effective primarily because of their fungistatic nature.<sup>1,2,4,5,10</sup> Not uncommonly, a chronic, low-grade infection is maintained in individual birds or bird colonies, is exacerbated during times of stress, and then results in mortalities. Terbinafine (Lamisil,<sup>®</sup> Novartis, New York, New York 10020 USA) was released in 1996 for treatment of mycotic nail infections in human patients,<sup>9</sup> and pharmacokinetic studies have not been conducted to determine its value as a therapeutic agent in avian species. This drug has reported fungicidal effects *in vivo* and *in vitro* against a broad range of fungi, including *Aspergillus fumigatus*,<sup>11-13</sup> and can be administered orally or topically. Because of these characteristics, terbinafine has excellent therapeutic potential for the treatment of aspergillosis in avian species. Our ongoing research will assess the pharmacokinetics of terbinafine, formulate

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safe therapeutic dosing regimens, and determine clinical efficacy of this drug for treatment of aspergillosis, specifically in African penguins (*Spheniscus demersus*) and red-tailed hawks (*Buteo jamaicensis*).

## Methods

Several pharmacokinetic trials have been completed, and clinical field trials will be initiated based on preliminary results. Therapeutic doses for terbinafine (Phase I) are being determined by using three different dosages (15, 30 and 60 mg/kg in raptors and 3, 7 and 15 mg/kg in penguins; n = 10 birds/group) with a washout period between trials. Terbinafine was given orally to each bird and blood samples were collected at -5, 15, 30, 45 min, 1, 2, 4, 10, 12 and 24 hr post-administration. Optimal dosing frequencies for terbinafine (Phase II) are being determined by utilizing dosage requirements based on these results. A specific dosage will be given either once (s.i.d.) or twice (b.i.d.) daily for a total of four administrations to the same birds after an appropriate 1-2 wk washout period (n = 10 birds/group). Blood samples will be collected at 2, 4, and 8 hr after each administration, plus 1 hr prior to the next administration to detect peak and trough concentrations from birds (Phase II). Plasma is being stored at -4° C. until time of analysis by HPLC at the New Bolton Center.

## Results and Discussion

Because the MIC range of terbinafine against *A. fumigatus* in humans is reported to be at 0.02 to 5 µg/ml,<sup>3,6-8,13,14</sup> we *a priori* defined peak therapeutic serum concentrations in birds as between 2-4 µg/ml. Single dose pharmacokinetic parameters for raptors were calculated using non-compartmental analysis and demonstrated that terbinafine followed linear pharmacokinetics with peak concentrations of 0.5 ± 0.41 (mean + SD), 1.3 ± 0.39, and 2.7 ± 1.77 mg/L at 7 ± 4.7, 4 ± 1.6, and 7 ± 4.1 hr, respectively for 15, 30 and 60 mg/kg dosages. The half-life averaged between 20-21 hr for all dosages, and the area under the curve (AUC) was 12.2 ± 3.89, 23.2 ± 7.79, and 44.4 ± 27.0 hr mg/L, respectively for 15, 30 and 60 mg/kg dosages.

Because the 60 mg/kg terbinafine dosage only resulted in mean serum concentrations of 2.7 mg/L (low end of the range for therapeutic levels we were targeting), another Phase I trial using 120 mg/kg was conducted (n = 10 birds). Serum concentrations averaged 3.4 ± 0.37 mg/L (range: 1.5-7.4 mg/L) for this dose. However, birds started to regurgitate after multiple doses of 120 mg/kg/day were administered. Recently, 60 mg/kg/day dosages of terbinafine were given to four birds for four days with no apparent adverse responses. We would now like to conduct trials using 60 mg/kg s.i.d. for four days (n = 10 birds), following the original Phase II blood collection protocol. Three birds will then be sacrificed to collect lung, liver and muscle tissues and determine drug concentrations in these tissues during steady state. After a washout period, we will repeat this procedure using 60 mg/kg b.i.d. dosages for another 3 days (n = 7 birds). Blood samples will again be drawn and at least three birds will be sacrificed at the end to quantify tissue levels of terbinafine. We believe that terbinafine concentrations in these tissues might be

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adequate using 60 mg/kg dosages, even though serum concentrations during single-dose trials did not achieve therapeutic levels as currently defined, because the drug may be sequestered in these tissues. Additionally, it is possible that after a few days of dosing, serum concentrations may raise to the level desired.

Phase I penguin trials are currently being completed at the Seneca Park Zoo in New York. Single dose pharmacokinetic parameters for 3 mg/kg dosages were calculated using non-compartmental analysis. Peak concentrations reached  $0.082 \pm 0.06$  mg/L at  $2.7 \pm 0.9$  hr post-administration (n = 10). The half-life was  $24.8 \pm 11.4$  hr, AUC was  $0.909 \pm 0.34$ , and the clearance/fraction of dose absorbed was  $3.3 \pm 1.0$  L/hr (n = 4).

Subsequent field trials to determine clinical efficacy of terbinafine in the treatment of aspergillosis (Phase III) will utilize dosage regimens deemed therapeutic based on pharmacokinetic results. Opportunistic trials with African penguins will be conducted at SeaWorld facilities in Orlando, Florida, San Diego, California, San Antonio, Texas and Aurora, Ohio as well as at the Oregon Coast Aquarium. Field trials with red-tailed hawks will be conducted at the University of Minnesota Raptor Center. Treatment efficacy will be evaluated by evidence of remission based on evaluation of the same diagnostic parameters utilized (i.e., a combination of: 1) history and predisposing factors, 2) radiographs, 3) blood samples for complete blood chemistry and serum chemistry panels, 4) ELISA, and 5) aspergillus antigen and antibody levels). Efficacious treatment of aspergillosis cases in other avian species might require adjustments to dosing regimens and routes of administration because of differences in drug metabolism among avian species and individual variations in health status.

#### ACKNOWLEDGMENTS

We thank Dr. Steven Brown (Oregon Coast Aquarium) for initial project planning assistance, and the Morris Animal Foundation for funding this research.

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## AN UPDATE OF WEST NILE VIRUS VACCINE TRIALS IN A BIRD COLLECTION

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### **Abstract**

Since the arrival of West Nile virus (WNV) in North America in 1999, preventive measures have been taken to control the spread of the disease in bird collections in the zoo community. One approach is the use of a killed vaccine approved for equines. A currently accepted vaccine protocol consists of a three vaccine series administered 3-4 wk apart. Blood samples are collected each time the bird is inoculated. The Houston Zoo has vaccinated over 350 birds and tested over 550 blood samples for WNV.

### **Introduction**

West Nile virus (WNV) has been well studied, both by the human and veterinary medicine sector, since it first entered the USA in fall 1999.<sup>1</sup> One of the very first available WNV vaccines to be used was a killed vaccine (West Nile-Innovator, Ft Dodge Animal Health, Iowa 50501, USA) licensed for horses. This vaccine has been used in vaccine trials in selected birds. The first case of WNV confirmed in a bird in Houston occurred 17 June 2002.<sup>4</sup>

### **Materials and Methods**

In anticipation of the arrival of WNV, the Houston Zoo launched a vaccination program for the bird collection in August 2001, primarily those more susceptible species such as the corvids (crows, magpies and jays) and those housed outdoors.

The WNV vaccine being used in this project was a killed vaccine (West Nile-Innovator, Ft Dodge Animal Health, Iowa 50501, USA) licensed for equines. Initially, the birds were given between 0.25 ml to 1.0 ml i.m. in the pectoral muscles, depending upon bird size. However, this protocol was later changed to 1.0 ml, regardless of weight. Birds weighing less than 1.0 kg received the vaccine subcutaneously in the flank web. The vaccine was given at day 0, and then repeated at 3-4 wk intervals for a total of three inoculations. Blood samples were collected at pre-vaccination, then at each following vaccination, and finally at 4 wk after the third and final vaccination. Blood collections were performed mostly under manual restraint using a 25-ga needle with a 1.0 or 3.0 ml syringe. Venipuncture sites included the jugular vein (most frequent), ulnar (wing) vein and metatarsal (leg) vein. The blood collected was transferred into a heparinized collection tube and transported to the hospital laboratory. After centrifugation for 5-10 min, plasma was separated, transferred into a cryovial, and stored in an ultracold freezer at -70°C before shipment to the Animal Health Diagnostic Lab (New York State College of

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Veterinary Medicine, Cornell University, Ithaca, NY 14853) for processing using the Plaque Reduction Neutralization Test (PRNT). This project was part of the American Zoo and Aquarium Association (AZA) on-going study. Additionally, an initial batch of pre-vaccination samples was submitted to Zoonosis Control Division (Public Health Region 6, Texas Department of Health (TDH), Houston, TX 77023) for processing using the hemagglutination inhibition (HI) test.

The bird collection consisted of approximately 800 specimens representing 250 species. The vaccination program commenced in August 2001 and continues to the present time. To date, the zoo has vaccinated well over 350 birds and tested more than 550 samples for WNV (Table 1).

## Results and Discussion

The batch of pre-vaccination samples (primarily Attwater's prairie chicken *Tympanuchus cupido attwateri* and Chilean flamingo *Phoenicopterus chilensis*) that was sent to TDH tested negative for WNV. This result was expected since it was a naïve population prior to the arrival of WNV in Houston.

As a result of the lack of seroconversion seen in the Attwater's prairie chicken in the initial phase of the study,<sup>2,3</sup> all birds regardless of weight thereafter received 1.0 ml of the vaccine. It was believed that the recommendation of the manufacturer to use a dose of 1.0 ml for horses also applied to the birds tested, as this was the minimal volume required to invoke an immune response.

Of the 132 birds tested for WNV pre-vaccination, none were positive. On that same number of birds tested for WNV post-vaccination, only three species demonstrated seroconversion: the white-necked raven (*Corvus albicollis*), the green jay (*Cyanocorax yncas*) and the Chilean flamingos (*Phoenicopterus chilensis*). (Tables 1 and 2.)

At this point, it is uncertain whether vaccination with the WNV vaccine has conferred protective immunity. None of these birds were clinically challenged as many of them are rare and endangered species. However, we suggest that for several of the species, immunity might have been conferred based on the relatively few numbers of WNV-related cases seen after WNV arrived in Houston. These five cases included the Palawan peacock pheasant (*Polyplectron emphanum*), the Marianas/Guam crow (*Corvus kubaryi*), the Mauritius pink pigeon (*Columba mayeri*), and two Ne-nes (*Branta sandvicensis*). Only the crow received the three WNV vaccine series, while the others did not. Except for the Ne-nes, the remaining cases involved other medical complications.<sup>2</sup>

In all the vaccine trials, no adverse side effects have been observed. A possible exception existed in a corvid (plush-crested jay, *Cyanocorax chrysops*) wherein minor feather loss occurred at the injection site.

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In summary, until another vaccine is proven to be more efficacious, the Houston Zoo will continue to use this particular WNV vaccine to vaccinate susceptible bird species, and will administer boosters on an annual basis, since the vaccine does not appear to be harmful and may afford some immunity.

#### **ACKNOWLEDGMENTS**

I thank the Veterinary and Bird staff involved in this project; Lorna Schnase, zoo volunteer, for tabulation of data; Dr Amy Glaser at NY State Diagnostic Lab for processing plasma samples; and AZA for funding of the study.

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**Table 1.** West Nile virus (WNV) vaccination serology results for Houston Zoo, 2001-2003.<sup>a</sup>

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Common Name	Scientific Name	No.	Pre-Vax	Post-Vax
Attwater's prairie chicken	<i>Tympanuchus cupido wateri</i>	24	Neg <sup>b</sup>	7 N/A <sup>c</sup> 17 Neg
Lesser bird-of-paradise	<i>Paradisaea minor</i>	2	Neg	N/A
Red bird-of-paradise	<i>Paradisaea rubra</i>	1	Neg	N/A
Red-tailed hawk	<i>Buteo jamaicensis</i>	1	Neg	Neg
American kestrel/sparrowhawk	<i>Falco sparverius</i>	1	Neg	Neg
Barred owl	<i>Strix varia</i>	1	Neg	Neg
Eastern screech owl	<i>Otus asio</i>	1	Neg	Neg
Great horned owl	<i>Bubo virginianus</i>	2	Neg	Neg
Short-eared owl	<i>Asio flammeus</i>	1	Neg	Neg
Cinereous vulture	<i>Aegypius monachus</i>	2	Neg	N/A
Bald eagle	<i>Haliaeetus leucocephalus</i>	1	Neg	Neg
Chicken (domestic)	<i>Gallus domesticus</i>	17	Neg	N/A
Chilean flamingo	<i>Phoenicopterus chilensis</i>	34	Neg	15 Pos <sup>d</sup> 19 Neg
Common trumpeter	<i>Psophia crepitans</i>	1	Neg	N/A
Common crow	<i>Corvus brachyrhynchus</i>	1	Neg	Neg
White-necked raven	<i>Corvus albicollis</i>	2	Neg	1 Pos <sup>e</sup> 1 Neg
Crested screamer	<i>Chauna torquata</i>	2	Neg	N/A
Green jay	<i>Cyanocorax yncas</i>	3	Neg	1 Pos <sup>f</sup> 2 Neg
Plush-crested jay	<i>Cyanocorax Chrysops</i>	4	Neg	Neg
Purplish-backed jay	<i>Cissilopha beecheii</i>	3	Neg	Neg
Lady Ross' plantain-eater	<i>Musophaga rossae</i>	1	Neg	N/A
Collie's magpie jay	<i>Calocitta formosa colliei</i>	4	Neg	Neg
Green magpie	<i>Cissa chinensis</i>	2	Neg	Neg
Azure-winged magpie	<i>Cyanopica cyana</i>	2	Neg	Neg
Lesser Bornean crested fireback pheasant	<i>Lophura ignita ignita</i>	1	Neg	N/A
Kenya crested guinea fowl	<i>Guttera puchetani</i>	2	Neg	N/A
Red-crowned crane	<i>Grus japonensis</i>	3	Neg	Neg
Duck (various)	<i>Anas spp.</i>	8	Neg	1 Pos <sup>f</sup> 7 Neg
Ne-ne	<i>Branta sandvicensis</i>	1	Neg	N/A
Coscoroba swan	<i>Coscoroba coscoroba</i>	2	Neg	N/A
Yellow-knobbed curassow	<i>Crax daubentoni</i>	2	Neg	N/A

<sup>a</sup>Total number birds vaccinated; Pre-vax, serology prior to vaccination; Post-vax, serology after vaccination series with plaque reduction neutralization test (PRNT) seroconversion noted after inoculation #1, #2, or #3.

<sup>b</sup>Negative PRNT titer 1:20.

<sup>c</sup>No blood drawn post-vaccination.

<sup>d</sup>Positive titer seroconversion variable, refer to Table 2.

<sup>e</sup>Positive PRNT titer 1:20 3 wk after 2<sup>nd</sup> vaccination.

<sup>f</sup>Positive PRNT titer 1:20 4 wk after 3<sup>rd</sup> vaccination.

**Table 2.** Plaque reduction neutralization test (PRNT) serology results for West Nile virus (WNV) vaccination in Chilean flamingos (*Phoenicopterus chilensis*), for Houston Zoo, 2001. Interpretation of negative (Neg) or positive (Pos) and PRNT titer (1: “dilution level”) listed for samples collected 3-4 wk after each WNV vaccination.

Bird #	Day 0	Post-vax #1	Post-vax #2	Post-vax #3
1	Neg 1:20	Pos 1:40	Pos 1:80	Pos 1:80
2	Neg 1:20	Neg 1:20	Pos 1:40	Pos 1:40
3	Neg 1:20	Neg 1:20	Neg 1:20 <sup>a</sup>	Pos 1:20
4	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
5	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
6	Neg 1:20	Pos 1:80	Pos 1:80	Pos 1:40
7	Neg 1:20	Pos 1:160	Pos 1:160	Pos 1:80
8	Neg 1:20	Pos 1:80	Pos 1:40	Pos 1:20
9	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
10	Neg 1:20	Pos 1:80	Pos 1:20	Pos 1:20
11	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
12	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
13	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
14	Neg 1:20	Pos 1:40	Pos 1:20	Pos 1:20
15	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
16	Neg 1:20	Pos 1:80	Pos 1:160	Pos 1:80
17	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
18	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
19	Neg 1:20	Pos 1:160	Pos 1:80	Pos 1:40
20	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
21	Neg 1:20	Neg 1:20	Neg 1:20 <sup>a</sup>	Neg 1:20 <sup>a</sup>
22	Neg 1:20	Neg 1:20	Neg 1:20 <sup>a</sup>	Neg 1:20 <sup>a</sup>
23	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
24	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
25	Neg 1:20	Pos 1:40	Pos 1:40	Pos 1:40
26	Neg 1:20	Pos 1:160	Pos 1:160	Pos 1:80
27	Neg 1:20	Pos 1:20	Pos 1:20	Pos 1:20
28	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
29	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
30	Neg 1:20	Pos 1:80	Pos 1:40	Pos 1:20
31	Neg 1:20	Neg 1:20	Neg 1:20	Pos 1:20
32	Neg 1:20	Neg 1:20	Pos 1:20	Neg 1:20
33	Neg 1:20	Neg 1:20	Neg 1:20	Neg 1:20
34	Neg 1:20	Neg 1:20	Neg 1:20 <sup>b</sup>	Neg 1:20 <sup>b</sup>

<sup>a</sup>Sample showed 50% plaque reduction at negative 1:20.

<sup>b</sup>Sample showed 60% plaque reduction at negative 1:20.

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## TESTING OF A DNA-PLASMID VACCINE FOR PROTECTION AGAINST WEST NILE VIRUS CHALLENGE IN RED-TAILED HAWKS (*Buteo jamaicensis*)

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Originally prepared for Proceedings of the Association of Avian Veterinarians, 2004.

### Abstract

West Nile virus (WNV) has caused morbidity and mortality in more than 150 bird species in the United States since 1999. The threat to avian collections and conservation programs led to the investigation for an effective vaccine to protect birds. A recombinant *E. coli* DNA-plasmid preparation that contained WNV genes coding for specific antigenic proteins was mixed with proprietary aluminum hydroxide adjuvant to produce the experimental vaccine. The vaccine was administered i.m. to 20 permanently disabled red-tailed hawks (*Buteo jamaicensis*) twice 3 wk apart. Five hawks served as sham-vaccinated controls. Four wk after completion of the vaccine series, hawks were challenged with a Louisiana strain of WNV. Blood samples were collected throughout the study, initially to evaluate serologic status by plaque reduction neutralization test for WNV antibodies after vaccination, and later to evaluate the degree of viremia after WNV antigen challenge. A significant difference in level of viremia post-challenge between vaccinates and non-vaccinates was found.

### Introduction

West Nile virus (WNV) became one of the first arthropod-associated viral diseases ever described when it was isolated from the blood of a febrile woman in Uganda in 1937.<sup>5</sup> Historically, this member of the *Flavivirus* genus in the Flaviviridae family has caused occasional disease in humans and horses in Africa and Asia, with sporadic outbreaks in Europe.<sup>7</sup> West Nile virus is considered one of the most widely distributed of all flaviviruses.<sup>3</sup> In Africa, up to 70% of humans in endemic areas are seropositive suggesting that the majority of WNV infections are mild or subclinical.<sup>1</sup>

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West Nile virus was introduced into the United States in 1999 and became a significant cause of morbidity and mortality among wild birds (especially crows), horses, and humans. A member of the flavivirus group, WNV is a vector-borne disease transmitted primarily by *Culex* spp. mosquitoes.<sup>4</sup> By fall of 2003, it had become endemic in all states but those west of the Rocky Mountains with epidemics occurring at the front of westward movement in each of the years 2000, 2001, 2002, and 2003.

Owing to the more than 150 bird species in which WNV has been documented since its introduction and since many of these are components of collections and conservation programs, protection in the form of effective vaccination was deemed desirable.<sup>6</sup> Introduced in 2000, a killed vaccine licensed for use in horses received limited testing in birds<sup>8</sup> and became widely used by avian veterinarians. A recombinant DNA-plasmid vaccine was reported effective in protecting crows against challenge with WNV.<sup>9</sup> The DNA-plasmid vaccine was further tested in California condors (*Gymnogyps californianus*).<sup>6</sup> The *E. coli* DNA-plasmid contains WNV genes pCBWN that express the prM and E glycoproteins of WNV which elicit an immune response.<sup>2</sup>

## Methods

In mid-September 2003, we began a vaccine trial with this recombinant DNA-Plasmid West Nile Vaccine obtained from a manufacturer of stable DNA plasmids (Aldevron, Inc. Fargo, North Dakota 58105 USA) under a research agreement with the Centers for Disease Control and Prevention (CDC), Department of Health and Human Services, USA. The DNA concentration was 500ug/500ul in PBS and it was mixed with proprietary aluminum hydroxide adjuvant (Biomune Co., Lenexa, Kansas 66215 USA).

A group of 20 permanently disabled red-tailed hawks (*Buteo jamaicensis*) was obtained from The Raptor Center and various rehabilitators throughout the USA in accordance with provisions of permits issued by the United States Fish and Wildlife Service. All subsequent procedures were conducted in accordance with an approved IACUC protocol. Hawks were given physical examinations. Blood samples were collected from the hawks for CBC and serology. Blood samples were tested for antibodies to WNV by the plaque reduction neutralization test (PRNT) and only antibody negative birds were used for further study. Fifteen birds were selected for immunization at random and placed in a treatment group room; five birds were used as sham-vaccinated controls and placed in a separate room.

Hawks were housed indoors in controlled light and ventilation rooms constructed of cinder block walls and concrete floors all sealed with epoxy resin finishes. Temperature was maintained constant at 20°C and photoperiod was regulated at 12L:12D. Hawks were fed *ad lib* coturnix quail (*Coturnix japonica*) supplied at the rate of approximately ¾ of a quail per hawk per day. Water for drinking and bathing was provided in shallow floor pans. Rope-covered perches of suitable size and height for non-flying red-tailed hawks were placed in various locations on the floor. All rooms were cleaned once daily with the hawks left *in situ*.

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Hawks received two 1-ml doses of the vaccine with a 3-wk interval between doses. Inoculations were delivered by i.m. injection in the pectoral musculature with a 25-ga needle. Blood samples were drawn before the first vaccination, at the time of the second vaccination, and 3 wk after the second vaccination. Blood samples were spun by centrifuge and plasma was drawn off for antibody testing by PRNT.

Following the third phlebotomy, the hawks were loaded into fiberglass animal holding kennels and driven by van to Baton Rouge, Louisiana for challenge with live WNV at the School of Veterinary Medicine, Louisiana State University. Upon arrival, hawks were transferred to suitable animal holding space similar to that described previously and allowed 1 wk of acclimation prior to additional experimentation.

For challenge, birds were inoculated s.c. in the inguinal web with 0.1 ml of a suspension containing  $10^5$  PFU of the Louisiana Strain of WNV. This virus strain had been passaged once in Vero cells after isolation from the kidney of a blue jay (*Cyanocitta cristata*), which had died in Louisiana during the summer of 2001. Blood samples were collected on day 1, 2, 4, 6, 8, 10, 12, and 14 to assess the degree of viremia. Swabs were taken from the pharynx and cloaca during this time to assess viral shed patterns. Birds were humanely euthanatized upon completion of the study. Due to the development of neurologic signs, one bird was euthanatized on day 10 post-challenge and was subjected to full necropsy. One bird that exhibited no viremia post-inoculation was re-inoculated 6 wk after the first inoculation, and was sampled for viremia as previously.

Geometric mean values for viremia were compared using a 1-tailed t-test. A P-value  $<0.05$  was taken as an indication of significant difference between vaccinates and non-vaccinates.

## Results and Discussion

A significant difference in level of viremia post-challenge between vaccinates and non-vaccinates was found. In the vaccinated hawk that was challenged twice with live WNV, viremia results were negative at all samplings. These findings render the possibility of developing an avian specific vaccine for WNV more probable in the near future. Details of differences between groups along with other findings from this experiment will be presented and ultimately published.

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## PAROXETINE THERAPY FOR FEATHER PICKING AND SELF-MUTILATION IN THE WALDRAPP IBIS (*Geronticus eremita*)

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### Abstract

Paroxetine (Paxil®, SmithKline Beecham Pharmaceuticals, Philadelphia, Pennsylvania 19101, USA) is a selective serotonin reuptake inhibitor (SSRI) used to treat human patients diagnosed with psychiatric conditions such as depression, anxiety, obsessive-compulsive disorder, and post-traumatic stress disorder.<sup>1</sup> Its advantages over tricyclic antidepressants include an absence of cardiovascular side-effects, and a wide margin of safety. Of the SSRIs used for disorders involving self-mutilation, paroxetine appears to be the one of the few with a fairly low incidence of side effects. In human patients, the main side effect commonly seen with paroxetine is lethargy. (Gregory McFadden, MD, PhD, personal communication, 2002)

While the causes of feather picking or self-mutilation in avian patients may at times be difficult to determine, it is widely held that a large number of these cases have a strong behavioral component and/or may become exacerbated by stress. It could be postulated that some of these veterinary cases may be analogous to severe anxiety or obsessive-compulsive disorders in humans.

At the San Diego Zoo, two Waldrapp ibis (*Geronticus eremita*) presented with histories of chronic feather picking and self-mutilation of the dorsal thorax. The wounds were debrided and bandages changed for several weeks. Paroxetine therapy was initiated gradually, starting at 0.3 mg/kg once daily (s.i.d.) p.o. for the first week, followed by 0.6 mg/kg s.i.d. p.o. for the next 2 wk, and finally 1.0 mg/kg s.i.d. p.o. The birds began to show a decreased frequency of picking and mutilation 4 days after therapy was initiated. One month later, these behaviors appeared to have stopped. After 15 mo of therapy, the dosage was increased to 2.0 mg/kg s.i.d. p.o. whenever it appeared that the birds were picking again. The dosage was increased gradually in this situation due to a lack of published information on appropriate dosages for avian species. Since treatment of these ibises was initiated, the use of paroxetine in laboratory pigeons (*Columba livia*) has been reported. The dosage used in the pigeon was 3 mg/kg s.i.d. p.o.<sup>2</sup> Long-term oral paroxetine, administered once daily in a mouse, allowed the wounds to heal to the point of small scabs. During this time, the wounds required debridement and bandage changes for several months. Complete blood counts and serum chemistries were performed monthly for the first 6 mo, and then q 90 day for the following 12 mo. Results of these tests suggested no evidence of adverse effects from the medication. Lethargy was not observed in these birds. Periodic serum paroxetine levels were performed. Although the actual numeric quantity of paroxetine in the

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blood does not correlate with degree of clinical effect in human patients, this testing did at least allow us to verify that the drug was being absorbed from the gastrointestinal tract in our ibises. At the San Diego Zoo, treatment with paroxetine has also been used successfully in some individuals of other species: a feather-picking hyacinth macaw (*Anodorhynchus hyacinthinus*); and a self-mutilating Allen's swamp guenon (*Allenopithecus nigroviridis*.)

Although no side effects were observed while any of these animals were on paroxetine, care should be taken to taper the dosage over at least 3 wk if treatment is to be discontinued. Stopping paroxetine treatment abruptly in human patients may cause withdrawal symptoms such as nausea, hypomania, and dizziness.<sup>1</sup>

#### **ACKNOWLEDGMENTS**

I would like to thank the San Diego Zoo veterinarians, registered veterinary technicians, and hospital keepers for their long-term, dedicated care of these birds.

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## CORRECTION OF ANGULAR LIMB DEFORMITY IN TWO SPECIES OF FLAMINGO UTILIZING A TRANSPHYSEAL BRIDGING TECHNIQUE

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### Abstract

Three hand-raised American flamingo (*Phoenicopterus ruber ruber*) chicks and one hand-raised Chilean flamingo (*Phoenicopterus ruber chilensis*) developed valgus angular limb deformities of the proximal tarsometatarsus. All flamingos underwent surgical correction to unequally retard the growth plate using transphyseal bridging. Positive profile pins were placed in the proximal epiphysis and distal to the growth plate in the metaphysis on the convex side of the affected tarsometatarsus. Various banding techniques were used in each flamingo to create tension. Three of the four flamingos responded in 7-14 days with correction or slight over-correction of the valgus limb deformity. The fourth flamingo's leg deformity did not improve for reasons thought to be involved in improper implant placement. Growth plate retardation by transphyseal bridging proved successful in correcting valgus limb deformity of the proximal tarsometatarsus. This technique may be considered as an option for correction of angular limb deformities of the proximal tarsometatarsus in flamingos less than 90-120 days of age.

### Introduction

Angular limb deformities appear to be common in hand-raised long-legged avian species.<sup>8</sup> Etiologies in mammals include trauma, nutrition, genetics, and lack of or excessive exercise.<sup>1,4,5</sup> Similar etiologies are thought to be involved in angular limb deformities in avian species.<sup>2,6-8</sup> Treatments for mammalian species may be extrapolated for use in affected birds. This report presents a series of four flamingo chicks in which a procedure described in foals, physeal retardation by transphyseal bridging, was used.<sup>5</sup>

### Case Report

Four flamingo chicks developed tarsometatarsal deformities: three American flamingos (*Phoenicopterus ruber ruber*) and one Chilean flamingo (*Phoenicopterus ruber chilensis*). Initial medical therapy for tarsometatarsus valgus limb deformity in the three American flamingo chicks included tension taping of the medial aspect of the tibiotarsal/tarsometatarsal joint, and implemented exercise (walking) with hydrotherapy (swimming). In addition measured daily weight gains were decreased.

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### Case 1

An American flamingo developed valgus deformity of the left proximal tarsometatarsal bone at 34 days of age. Medical therapy as previously described was implemented for 14 days without clinical improvement. Radiographs of both tarsometatarsal bones revealed a  $14^\circ$  from normal valgus deformity of the proximal left tarsometatarsal bone. Minimally invasive surgical intervention was elected. The chick was anesthetized with Isoflurane (Isoflo, Abbott Laboratory, North Chicago, Illinois 60064, USA) via face mask and then intubated with a 4-mm Cole tube. The skin on the medial aspect of the left tarsometatarsus was aseptically prepared. Butorphanol (Torbugesic, Fort Dodge Animal Health, Fort Dodge, Iowa 50501, USA; 2 mg/kg i.m.) was administered for anesthetic sparing effects and pain management. Transphyseal bridging was performed using a sterile cordless hand held drill to place a 0.03-mm positive profile pin (IMEX Veterinary Inc., Longview, Texas 75603, USA) in the proximal epiphysis engaging the medial cortex of the left tarsometatarsal bone. A second pin was placed approximately 4 cm distal to the other pin in the metaphysis. The distal ends of each pin were bent  $90^\circ$  away from each other. The orthopedic pins were connected with 23-ga cerclage wire in a figure-eight pattern. A soft padded bandage was placed over the leg. Anesthetic recovery was uneventful and the bird was using the leg the same day. Exercise therapy was reinstated. Radiographs at ten days post-apparatus placement revealed a reduced valgus deformity of the tarsometatarsal bone and distraction of the pins due to bone growth. Orthodontic rubber bands were added to the pins and figure-eight wire to create constant dynamic tension. Radiographs at fifteen days post-operative indicated slight overcorrection with a  $1^\circ$  from normal varus deformity of the left tarsometarsus. The pins were removed from the left tarsometatarsus. The bird was immediately encouraged to walk and hydrotherapy was reinstated 8 days post implant removal when the skin over the pin holes had closed. Six weeks post-operative, the left leg was clinically straight.

### Case 2

A 75-day-old American flamingo chick presented with a mild valgus deformity of the proximal left tarsometatarsus. Radiographs verified a  $15^\circ$  from normal valgus deformity. Transphyseal bridging was performed as described in Case 1, and orthodontic rubber bands were applied instead of cerclage wire for constant dynamic tension. The orthodontic rubber bands had to be changed twice weekly to maintain continuous tension. At 14 days post-operative, rubber bands were changed and two wire twist-ties were placed around both pins and twisted to create additional tension. Radiographs at this stage revealed a reduction from  $14^\circ$  to  $4^\circ$  valgus deformity from normal. At 25 days post operative, the leg was visually straight and radiographs revealed a mild overcorrection with a  $2.5^\circ$  varus deformity. The pins were removed and the left leg appeared clinically normal.

### Case 3

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A 34-day-old American flamingo chick presented with a varus deformity of the proximal right tarsometatarsus. Medical therapy was implemented as described for Case 1. At 71 days of age, the proximal aspect and alignment of the right leg had straightened, however the varus deformity remained and had grown distally on the tarsometatarsus. At this stage surgical intervention was not elected.

At 79 days of age, a valgus deformity of the left leg had developed in the proximal tarsometatarsus. Radiographs revealed a 21° from normal valgus deformity and mild twisting of the mid-shaft right tarsometatarsus. Medical therapy as previously described, was unsuccessful, and transphyseal bridging was performed at 90 days of age. The proximal pin was placed in the more distal aspect of the epiphysis, at the epiphyseal/physeal junction. Orthodontic rubber bands and 23 ga cerclage wire were both applied in this procedure. Three days post-operative, the cerclage wire was replaced with a twist-tie and the rubber bands were changed. At this time the proximal implant pin appeared to be loosening. At 7 days post-operative, the bird was limping significantly on the left leg. Radiographs revealed that the proximal pin was migrating out of the proximal epiphysis. The valgus deformity was still at 21° from normal. At 14 days post-operative, the bird was still limping on the left leg with no visually apparent correction of the valgus deformity. The implants were removed and the limp improved within hours of recovery. Eventually the left tarsometatarsal valgus deformity improved visually although currently mild valgus deviation appears present.

#### Case 4

A Chilean flamingo presented at 62 days of age with a valgus limb deformity of the left proximal tarsometatarsus. Radiographs revealed a 17° from normal valgus deformity of the proximal tarsometatarsus. Transphyseal bridging was performed using a Jacobs hand chuck to place the two 0.03-mm positive profile pins, and 28-ga cerclage wire was placed in a figure-8 pattern for tension. Three days post-operative, two orthodontic rubber bands were placed around the pins. Radiographs at 7 days post-operative revealed a decrease to 4° from normal valgus deformity. At this point, the chick was slightly lame and some swelling was noted in the foot. Radiographs at 9 days post-operative revealed an over-correction to a 4° from normal varus deformity. The apparatus was removed, the lameness resolved, and the leg remained clinically straight.

#### Discussion

Angular limb deformities commonly occur in hand-raised long legged birds.<sup>8</sup> The etiology of the deformity is rarely elucidated. In ratites angular limb deformities have been associated with high planes of nutrition, lack of exercise, trauma, and genetics.<sup>6</sup> In cranes leg problems may result from the same etiologies, as well as management practices including incubation temperatures, hatching and rearing substrates, nutrition, ambient temperature, illumination, external stimuli and exercise.<sup>3</sup> Angular limb deformities of clinical significance left untreated may produce irreparable limb misalignment that may lead to joint abnormalities, muscle contracture, and ulcerative pododermatitis.<sup>7</sup>

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Medical management of angular limb deformities of long-legged avian species includes increasing exercise, slowing weight gain, correcting nutritional deficiencies, swimming or hydrotherapy, and bandaging or hobbling the legs into a more normal orientation.<sup>2,7,8</sup> Surgical procedures reported to address angular limb deformities include hemi-circumferential periosteal stripping and dome osteotomy techniques.<sup>6-8</sup> Periosteal stripping procedure was not elected due to the minimal amount of soft tissue present for closure over the tarsometatarsal bone. Dome osteotomy was not elected due the technique requirement of fracturing the affected bone for realignment and the immature age of the affected flamingos in this report.<sup>7</sup> The use of transphyseal bridging has been reported in foals and calves, but not to date in avian species.

Transphyseal bridging is a tension band technique that retards bone growth at the physis of the convex side allowing lengthening of the bone on the concave side to straighten the leg.<sup>5</sup> In foals, retardation of growth on the convex aspect of the deformity is consistently effective in correction of angular limb deformities and can be performed using several different systems of implants.<sup>5</sup> In this instance, due to the size of the four flamingos and their very small proximal tarsometatarsal epiphyses (radiographic measurements ranged from 3-4 mm), small diameter positive profile pins were used to engage soft epiphyseal bone and cortical bone of the metaphysis with minimal disruption of the physeal growth plate.

Methods to create tension were different in each case. Initially 23-ga orthopedic wire in a figure-8 pattern was used. The small gauge orthopedic wire was difficult to manipulate to create the desired amount of tension. Orthodontic rubber bands created constant dynamic tension as long as they were changed every 3-4 days to ensure limited elasticity. Clinical results were not as rapid with rubber bands alone, therefore commercial grade twist-ties were added in a figure-8 pattern. This media tended to be much more pliable than cerclage wire. Based on the results from these limited cases and length of time for improvement in each case, small gauge ( $\leq 25$ ) orthopedic cerclage wire, or alternatively a commercial grade twist-tie, are recommended to create the initial non-elastic tension on the 0.03 mm positive profile pins during the growth phase of the bone. At 3-4 days post-operative, the addition of orthodontic rubber bands appears warranted to maintain constant dynamic tension on the pins. These bands should be changed once to twice weekly.

The age of chicks when a tension band apparatus is placed may also be an important consideration. In a study of Greater Flamingo (*Phoenicopterus ruber roseus*) chicks at the Basle Zoo, females reached about 90% of their adult tarsus length, and male fledglings 80% of their adult tarsus length, at 90-120 days of age.<sup>9</sup> Therefore, in these young rapidly growing chicks early correction would be necessary to prevent further abnormal growth and the resulting chronic orthopedic problems described in adults. It is doubtful that transphyseal bridging would have been successful after 90-120 days of age when their tarsometatarsus growth is nearly complete.

Case 3 was considered to be unsuccessful, although there was some improvement in the valgus deformity over time once the implant was removed. On postoperative radiographs the proximal pin was not centered in the small ephyseal bone. Due to decreased bone and increased cartilage

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engaged, the pin was unstable and migration was evident on serial radiographs over 14 days. This was also appeared to be causing pain, manifested as the clinical lameness observed. Improper pin placement appears to be at least part of the cause for failure, and illustrates the importance of exact pin placement in the small epiphysis of this avian species.

Very few surgical options for angular limb deformities have been described in the literature for long-legged avian species. To the authors' knowledge, this is the first reported use of transphyseal bridging in avian species. The procedure appears to be safe, minimally invasive, and was reasonably well tolerated. The success of transphyseal bridging induced growth plate retardation appears encouraging based on the outcome in three of the four cases reported. Transphyseal bridging appears to be a viable surgical option to accelerate or affect the correction of proximal tarsometatarsal valgus angular limb deformities in young flamingo chicks.

#### **ACKNOWLEDGMENTS**

We thank Dr. Gary West for contribution of one of the cases and to Dr. John Hoover for manuscript review. We thank the bird departments of the Tulsa and Oklahoma City Zoos for their help with these cases.

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## INTESTINAL ENTRAPMENT IN THE RIGHT PULMONARY OSTIUM FOLLOWING CASTRATION IN A JUVENILE OSTRICH (*Struthio camelus*)

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### **Abstract**

A juvenile ostrich (*Struthio camelus*) was castrated in two procedures: right and left hemicastrations at 3 and 4 mo of age, respectively. The right hemicastration was performed with the bird in left lateral recumbency through a right lateral celiotomy with an incision that extended caudally from the third last rib to a point midway between the inguinal skin fold (between the stifle and body wall) and the vent. In locating the right testicle, the air sac walls between the abdominal and thoracic regions of the coelom were incised. Ligating clips (Hemoclip, Weck Closure Systems, Research Triangle Park, NC 27709, USA) were placed dorsal to the testicle prior to its removal. The left testicle was unable to be visualized. The surgical wound was closed with polyglactin 910 (Vicryl, Ethicon Inc., Johnson and Johnson Medical Pty. Ltd., North Ryde, NSW 2113, Australia) suture in two layers: a simple continuous suture pattern in the abdominal muscle and a Ford interlocking suture pattern in the skin. The left hemicastration was performed with the bird in right lateral recumbency through a left lateral celiotomy with an incision that extended caudally from the inguinal skin fold approximately 12 cm towards the vent. The left testicle removal was performed in a similar manner to the right testicle removal. Wound closure was similar to the right lateral celiotomy closure. The bird had three episodes of depression, inappetance, and head-shaking with apparent dysphagia of 1-3 days duration during the 4 mo following the first surgical procedure. The bird was found dead at 7 mo of age with no clinical signs in the days preceding the death. At necropsy, the intestine was found entrapped in the right pulmonary ostium. Death likely resulted from compression of the air sacs and heart by the dilated bowel. Care should be taken to avoid disruption of the air sac wall integrity between the thoracic and abdominal regions of the coelom during the castration of juvenile ostriches. Bilateral celiotomies may be required to access both testicles. Incisions should be made caudal to the last rib, beginning from the inguinal skin fold (between the stifle and body wall) and extending caudally towards the vent.

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## USE OF PHOTODYNAMIC THERAPY AGAINST SQUAMOUS CELL CARCINOMA IN A ROSE-RINGED PARAKEET (*Psittacula krameri*)

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### Abstract

A 5 yr-old female African rose-ringed parakeet (*Psittacula krameri*) presented with an ulcerated mass in the medial post-patagial area of the right wing. Biopsy specimens of the mass demonstrated a well-differentiated squamous cell carcinoma. Photodynamic therapy (PDT) resulted in tumor cell necrosis and initial reduction in tumor burden but complete remission was not achieved.

PDT utilizes an intravenous photosensitizing agent, in this case Hexylether pyropheophobide-a (Photochlor, Roswell Park Cancer Institute, Buffalo, New York 14263 USA), that is subsequently activated by a diode laser. Upon activation with the light source, cytotoxic oxygen radicals are generated photochemically and destroy neoplastic cells. The particular wavelengths of light used in PDT depend upon the photosensitizing agent used and the reported response with various neoplasms.<sup>1</sup>

One injection of Photochlor (0.3 mg/kg i.v.) was administered into the basilic vein 18 hr prior to activation. A diode laser fitted with a 400 µm microlens filter was used to deliver 665 nm of light to the affected tissue. Although tumor cell necrosis and reduction in tumor burden was documented, complete remission was not achieved after five courses of PDT.

Based on this and other avian cases,<sup>2,3</sup> it appears that photodynamic therapy in avian species against squamous cell carcinoma using protocols modeled after canine, feline and human PDT protocols may not be useful. We hypothesize that differences in light penetration, photosensitizing agent pharmacokinetics, and wound healing properties in avian species necessitate alteration of PDT protocols if this treatment modality is to be effective in avian oncology. We suggest further investigation into either shortening the interval between injection and activation, altering the wavelength of light administered, or altering the treatment time during PDT.

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## UNILATERAL PECTORAL MUSCLE INFARCTION IN BIRDS ASSOCIATED WITH FUNGAL ARTERITIS

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### Abstract

A unique syndrome of unilateral pectoral muscle infarction associated with fungal arteritis of the great vessels or their branches has been recognized in birds at the Zoological Society of San Diego. Of the nine cases currently identified, all were passerine species. Reported clinical signs included unilateral wing droop (n = 6), inability to fly (1), and unilateral pectoral muscle swelling (1). One of the birds was simply found dead. One bird, after developing a unilateral wing droop progressively developed pelvic limb paresis.

At necropsy, the affected muscle tissue was pale tan compared to the contralateral pectoral muscle. In six cases the entire pectoral muscle was affected. The three remaining cases involved at least one-half of the pectoral muscle. Fungal infiltration of a major vessel was suspected on gross examination in six cases. Findings included tan masses surrounding the great vessels at the base of the heart (2), extreme thickening of the brachiocephalic trunk (1), thickening and occlusion of the left pulmonary artery (1), thickening and thrombosis of the right axillary artery (1) and thrombosis of the proximal aorta (1). Acute coagulation necrosis of muscle fibers was found in the affected pectoral muscle in all cases. The amount of accompanying inflammation varied and likely reflected the age of the muscle infarct. Histologic examination confirmed fungal arteritis in the six cases wherein it was suspected grossly. Fungal arteritis was detected in the three remaining cases and involved the brachiocephalic trunk (1), pulmonary artery (1), and a deep pectoral artery (1). In all cases, morphology of the fungus on H & E sections was typical of *Aspergillus* species.<sup>1</sup> *Aspergillus fumigatus* was cultured from one case; fungal cultures were not submitted from seven birds. In one case, there was no fungal growth from the sample. Additional significant findings included respiratory aspergillosis (3), cerebral infarcts associated with fungal vasculitis (2), and hepatitis associated with fungal vasculitis (1).

In addition to the nine cases discussed above, eight additional cases of unilateral pectoral muscle infarction were also identified. Six cases were in passerine species; one case each was in a galliform species and in a columbiform species. Histologic examination of the affected pectoral muscle revealed similar findings. Respiratory infections consistent with *Aspergillus* sp. were found in seven of these cases. In the remaining case, pectoral muscle infarction was the only finding. The brachiocephalic arch was thickened in this case, but histology failed to confirm fungal arteritis. In light of the previous nine cases, fungal infiltration and subsequent thrombosis

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was suspected in the seven cases with concurrent *Aspergillus* infections. Vascular lesions could not be confirmed.

Our findings suggest that unilateral pectoral muscle infarction due to fungal arteritis may be a differential for unilateral wing droop in passerine birds. Care should be taken to examine the great vessels, brachiocephalic arch and axillary arteries in cases where unilateral pectoral muscle changes are detected at necropsy and possibly in cases of suspected respiratory aspergillosis.

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## ADENOVIRUS IN FALCONS: DIAGNOSTIC ASSAYS AND OUTBREAK PREVENTION

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### Abstract

Adenoviruses are morphologically, genetically and biologically diverse agents that have been identified by ultrastructural or molecular methods in fish, birds, reptiles, mammals, and amphibia.<sup>1,2,5</sup> Most natural infections are subclinical and require co-infection with viral or bacterial pathogens, toxin exposure, or immune compromise to the host for significant disease to occur.<sup>6,7</sup> Occasionally, however, emergent viral strains, cross-species transmission, or high-dose infection of young, naive animals result in severe disease without the presence of co-factors.<sup>4</sup>

In birds, detailed molecular and cellular analyses been done on the adenovirus type species of group I (fowl adenovirus 1 of chickens), group II (hemorrhagic enteritis virus of turkeys), and group III (egg drop syndrome virus of chickens) viruses of poultry.<sup>6</sup> In the genus *Falco*, adenovirus has been described in a merlin (*Falco columbarius*), seven American kestrels (*Falco sparverius*) and thirteen Maritius kestrels (*Falco punctatus*).<sup>3,8,9</sup> These studies brought attention to the danger of adenoviruses in captive falcons but were limited to morphologic descriptions of viral particles and lesions in affected birds. The source, genotype, involvement of co-pathogens, and interspecies communicability of falconid adenoviruses have not been reported.

In 1996 at a captive breeding facility in Idaho, anorexia, dehydration, and diarrhea or sudden death occurred in Northern aplomado falcons (*Falco femoralis septentrionalis*) from 9 to 35 days of age and peregrine falcons (*Falco peregrinus*) from 14 to 25 days of age. Sixty-two Northern aplomado and six peregrine falcons died. Epidemiologic analyses indicated a point source epizootic, horizontal transmission and increased relative risk associated with cross-species brooding of eggs. Affected birds had inclusion body hepatitis, splenomegaly and enteritis. The etiology in all mortalities was determined by molecular analyses to be a new species of adenovirus distantly related to the group I avian viruses, serotypes 1 and 4, genus *Aviadenovirus*. *In situ* hybridization and polymerase chain reaction (PCR) demonstrated that the virus was epitheliotropic and lymphotropic and that infection was systemic in the majority of animals.

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Adeno-associated virus was also detected by PCR in most affected falcons, but no other infectious agents or predisposing factors were found in any birds. Subsequent to the 1996 epizootic, similar disease caused by the same adenovirus was found in three other falcon species: two orange-breasted falcons (*Falco deiroleucus*), two Teita falcons (*Falco fasciinucha*) and a merlin (*Falco columbarius*), a peregrine falcon subspecies (*Falco peregrinus nesiotes*), and two gyrfalcon X peregrine falcon hybrids (*Falco rusticolus/peregrinus*) from Wyoming, Minnesota, Oklahoma and California, respectively. The adenovirus appeared to be the primary cause of disease and death in all birds. A specific and sensitive PCR for detection of this adenovirus in feces or tissues is now available at the on-site Molecular Diagnostics Laboratory (Zoological Society of San Diego, San Diego, CA, 92112) and is a valuable method for screening animals and preventing future outbreaks.

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## OVIDUCT PROLAPSES IN KING PENGUINS (*Aptenodytes patagonicus*)

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### **Abstract**

Edinburgh Zoo has lost several breeding female king penguins (*Aptenodytes patagonicus*) over the past few years due to oviduct prolapses. When cases were detected early, prolapses were reduced and secured with a purse string, and penguins were treated with an anti-inflammatory drug and an antibiotic. Pathologic findings from four post mortem examinations did not identify a definitive or common cause, but did suggest that these cases of oviduct prolapse could be seen as a sequel to egg laying.

### **Introduction**

Oviduct prolapse may occur secondary to egg laying, physiologic hyperplasia of the oviduct, or as a sequel to dystocia. Underlying causes include deformed eggs, malnutrition, or obesity. Underlying causes may be ruled out through submission of samples for bacterial culture and sensitivity and through radiography.

Treatment varies and depends upon the underlying cause and the amount of protruding tissue present. Part of the oviduct may be protruding with vaginal and cloacal tissue. Treatment includes removal of an egg if present, reduction of the swelling in exposed tissue and maintenance of oviduct-vaginal patency. The prolapsed tissue should be replaced after cleansing and a purse string applied or cloacopexy performed to prevent reoccurrence.<sup>3</sup> Medical treatment includes broad spectrum antibiotics, non-steroidal anti-inflammatory drugs, nutritional support, and medication to decrease hormonal levels (e.g., leuprolide acetate, medroxyprogesterone or human chorionic gonadotropin).<sup>2</sup> Surgical treatment is an alternative with salpingohysterectomy.<sup>1</sup> Prognosis is generally good after treatment of most prolapses, and return to normal breeding function may be accomplished in most birds as long as underlying predisposing factors are identified and eliminated.<sup>5,6</sup>

### **Methods**

Edinburgh Zoo houses four species of penguins in its zoological collection: Gentoo penguins (*Pygoscelis papua papua*), rockhopper penguins (*Eudyptes crestatus moseleyi*), macaroni penguins (*Eudyptes chrysolophus*), and king penguins (*Aptenodytes patagonica patagonica*).

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King penguins are fed 8-10 herrings per day and supplemented from February through September with two multivitamin tablets (Mazuri™ fish eater tablet, Witham, Essex, CM8 3AD, United Kingdom), one vitamin B1 tablet (Benerva, Roche, Welwyn Garden City, AL7 3AY, United Kingdom, 100 mg) two cod liver oil tablets (Isoactive, Edinburgh, EH89PP, United Kingdom, 400 mg), and one vitamin E tablet (Millpledge pharmaceuticals, Retford, Notts, DN22 9NA, United Kingdom, 1000 mg). Vitamin B1 and vitamin E are not supplemented during the remainder of the year (a practice currently under review).

Breeding success has been variable within the king penguin colony. Last year, one male chick was successfully hand-reared. The breeding success has been partially hampered by the death of females due to oviduct prolapses. Prolapses have occurred infrequently in the past and have been noted in other zoological collections (personal communication, E. Flach, 2004). This problem did not occur with the other penguin species held within the collection at Edinburgh Zoo. In the majority of cases, the king penguin females were found dead, however minor prolapses were detected in a few females at an earlier stage.

A portion of the oviduct was protruding with vaginal and cloacal tissue in a few cases in king penguins. When identified, the prolapse was reduced and a purse string suture was placed. The penguins were treated with an anti-inflammatory drug (carprofen, Rimadyl™, Pfizer Ltd., Sandwich, Kent, CT13 9NJ, United Kingdom, 4 mg/kg i.m. b.i.d.) and an antibiotic (enrofloxacin, Baytril™, Bayer PLC, Strawberry Hill, Newbury, RG14 1JA, United Kingdom, 10 mg/kg, p.o. b.i.d.). Itraconazole (Sporanox™, 20 mg/kg, p.o s.i.d.) was given prophylactically to prevent against aspergillosis. In the majority of incidences, the oviduct was presumed to have prolapsed during the night as the animal was found collapsed or dead in the morning. Penguins that showed a mild prolapse the previous season had a lethal prolapse the following 1-2 yr.

Full post mortems of the four cases seen since 2000 were performed at the Lasswade Veterinary Laboratory Agency, (Pentlands Science Park, Penicuik, E26 0PZ, United Kingdom) and have been summarized in Table 1. No routine bacterial cultures were performed on reproductive tissue of post mortem specimens. Salmonella screening was performed routinely on liver and intestine and was negative in all cases.

## **Results and Discussion**

No common denominator could be identified as to the primary or underlying cause for the oviduct prolapses. Various parameters such as age, diet, husbandry, and disease were reviewed in each case. None of the birds were related. Keymer (1980), in his overview of the disorders of the avian female reproductive tract, states an unusually high percentage of affected penguins (close to 25%), probably due to the high number of older birds and evidence of hypovitaminosis A.<sup>4</sup>

Of the four birds, only one (24 yr) falls into the former older age category. Longevity in captivity reaches approximately 30 yr. Obesity, another contributing factor to reproductive problems, was

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only noted in one bird (19 kg). The average recorded weight for king penguins at Edinburgh Zoo is 12-13 kg.

Pathologic findings suggest that these cases of oviduct prolapse could be seen as a sequel to egg laying. Breeding cycle in the wild is every 2-3 yr as parents take 15-18 mo to raise their chicks. Breeding is more intense in captive breeding programs with cycles every 1-2 yr.

#### **ACKNOWLEDGMENTS**

I thank the pathologists at VLA Lasswade, the penguin keepers at Edinburgh Zoo, and colleagues at the exotic animal service.

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**Table 1.** Summary of post mortem evaluation for king penguins (*Aptenodytes patagonicus*) with oviduct prolapse at the Edinburgh Zoo, 2000.

Animal no.	Age	Clinical signs	Postmortem findings	Breeding record
92BA06	10 yr	None reported	Excess fat (19 kg), inactive ovaries.	Proven
770702	24 yr	None reported	Longitude tear in oviduct wall. Fully formed egg free in the pelvic cavity. <sup>a</sup>	Proven
90CA02	16 yr	Prolapsed and died after laying an egg. History of cloacal prolapse treated 2 yr prior to death. <sup>b</sup>	No significant findings.	Proven
92AA32	11 yr	Died 2 wk after treatment for proctodeum prolapse. History of egg passage with some blood and mild cloacal prolapse that self-reduced 15 mo prior to presentation.	Visceral gout consistent with kidney failure. Damaged ureters	Proven

<sup>a</sup>A longitude tear was found in the oviduct wall and may account for the egg in the pelvic cavity.

<sup>b</sup>Hematology was normal at that time. Bacterial culture at that time isolated a heavy mixed growth of *Escherichia coli* and *Enterobactercloacae* and a scant moderate growth of *Candida albicans*.

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## TAURINE DEFICIENCY IN MANED WOLVES (*Chrysocyon brachyurus*) MAINTAINED ON TWO DIETS MANUFACTURED FOR PREVENTION OF CYSTINE UROLITHIASIS

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### Abstract

The captive population of the maned wolf (*Chrysocyon brachyurus*) in the United States has an alarmingly high prevalence of cystinuria, a metabolic disorder that has proven to have an autosomal recessive mode of inheritance in both humans and domestic dogs.<sup>3-5,7</sup> Previous research on the nutritional management of cystinuria in maned wolves<sup>2</sup> led to the development of a commercially manufactured maintenance maned wolf diet that had been fed to almost all of the maned wolves in the United States since 1998. In an effort to make further improvements on this diet, an experimental diet was developed which was demonstrated to significantly raise the urine pH in the maned wolves tested.<sup>6</sup> This study was performed in an effort to assess the long-term effects of this experimental diet vs. the commercially available manufactured diet on the overall nutritional and health status of the captive maned wolf.

Six adult maned wolves (three males, three females), maintained at the National Zoological Park's Conservation and Research Center were used in the study. For 14 wk, two pairs of maned wolves were maintained on the commercially available maintenance diet, while two individually housed wolves were maintained on the experimental diet. All six wolves, both at the beginning and at the end of the diet trial, had severely decreased plasma levels of taurine (as compared to the normal canine reference range of 60-120 nmol/ml)<sup>1</sup> with average taurine levels of 16 nmol/ml at the beginning of the study and 3 nmol/ml at the end of the study. There was no statistically significant difference in the taurine levels between animals on the maintenance vs. experimental diets. After receiving the taurine results following the trial, both diets were immediately supplemented with taurine at a level of 0.3%. All study animals were eventually switched to the taurine-supplemented version of the commercially manufactured, maintenance diet and subsequent samplings were performed to monitor plasma taurine levels. A final sampling, performed approximately 5 mo following the initiation of taurine supplementation, revealed an average taurine level within the target canine reference range (90.25 nmol/ml), although two individuals continued to exhibit levels below the reference range. There are numerous physiologic (e.g., possible unique metabolism and requirements for taurine in this species as compared to other canids) and dietary factors (e.g., effects of the types and levels of both fiber and protein on nutrient availability, taurine metabolism, and enterohepatic circulation of taurine-conjugated bile salts; impaired taurine synthesis secondary to low cysteine

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availability) that could be potential contributors to the development of taurine deficiency in the maned wolves in this study.

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## ABNORMAL TOOTH LOSS IN A CAPTIVE CROCODYLIAN COLLECTION

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### Abstract

A large captive crocodylian collection was evaluated because of abnormal tooth loss. This problem was most severe in *Crocodylus* species. It had been noticed in a few animals several years previously and the number of affected animals was increasing. Bone and tooth biopsies were collected for histologic evaluation and mineral analysis. Plasma and whole blood samples were submitted for mineral analysis. Plasma samples were also analyzed for vitamin A, E and C levels. Significant findings included low plasma vitamin A (retinol <0.2 µg/ml) and E (alpha-tocopherol <1.0 µg/ml) as well as elevated blood, tooth and bone lead concentrations in many of the affected crocodylians. Histologic evaluation of the tooth biopsy samples from affected animals revealed dentine resorption, as well as gingival hyperkeratosis. The affected animals were fed skinned nutria without abdominal contents. Nutrient analysis of samples from three randomly selected nutria revealed low vitamin A and E content, and elevated lead content in one animal. The affected animals were treated with an injectable combination of a vitamin A and E preparation (Vital E + A, Schering-Plough Animal Health, Union, NJ 07083 USA; retinyl palmitate 200,000 IU/ml, D-alpha-tocopherol 300 IU/ml, 3-6 ml i.m. once per month) for 3 mo, and the plasma vitamin concentrations rechecked. Additionally, the diet was changed to a 50:50 mixture of a commercial carnivore diet (Dallas Crown, Inc., West Fair, Kaufman, TX 75142 USA) and alligator pellets (Burriss Mill & Feed, Inc, Franklinton, LA 70438 USA) combined into a sausage.

### ACKNOWLEDGMENTS

The authors thank the general curator, John Brueggen, and reptile curator, David Kledzik, at the Saint Augustine Alligator Farm for their assistance in this clinical study.

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## METABOLIC BONE DISEASE IN CAPTIVE HUMBOLDT PENGUINS (*Spheniscus humboldti*) AND NORMAL SERUM IONIZED CALCIUM, PARATHYROID, AND VITAMIN D VALUES

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### Abstract

Three cases of metabolic bone disease (MBD) were identified in young Humboldt penguin (*Spheniscus humboldti*) chicks. Diagnosis, monitoring, and treatment of these cases were very challenging, in part because radiographs and traditional serum biochemistries could not provide adequate information for appropriate clinical management. Evaluation of ionized calcium (iCa), 25-hydroxyvitamin D<sub>3</sub> (25-[OH]D<sub>3</sub>), and parathyroid hormone (PTH) levels may have provided valuable diagnostic information about these chicks, but access to these tests was not available at the time of clinical presentation. Additionally, normal values for these parameters have not been reported for any sphenisciformes species. This study aimed to establish normal reference ranges for the tests to provide an important method for assessing clinical cases of MBD and other diseases.

Blood samples were collected from birds considered to be in normal health in conjunction with standard annual physical examinations. Ionized calcium was measured immediately after sample collection using an i-STAT portable chemical analyzer with an EG7+ cartridge (i-STAT Corporation, East Windsor, New Jersey 08520 USA) and also by shipment to a commercial laboratory (Michigan State University, Diagnostic Center for Population and Animal Health, Lansing, Michigan 48909 USA). The same commercial laboratory was used for analysis of PTH and 25-[OH]D<sub>3</sub>. Not all measurements were obtained from every animal when collected quantities of blood were insufficient. Ionized calcium results were consistent with other avian species and this test should provide a useful clinical parameter for diagnosing and monitoring MBD cases, as well as other diseases. Obtained values for PTH were clustered in the low end of the detectable assay, raising uncertainties about the validity of the assays in this species. Results of the 25-[OH]D<sub>3</sub> test were also near the limit of detection for the assay (4-5 nmol/L), which questions the validity and clinical usefulness of the assay in this species.

No additional cases of MBD have occurred in the penguin collection and three additional chicks have been successfully hand reared. The ability to closely monitor future cases with these tests, especially iCa, allows for close monitoring of neonates and provides rapid results to immediately adjust therapy with only a very small sample of blood. The tests are also a valuable alternative

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to radiography, as newly hatched chicks are difficult to evaluate due to minimal bone calcification.

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## SERUM CONCENTRATIONS OF INTACT PARATHORMONE AND IONIZED CALCIUM IN VITAMIN D DEFICIENT EMPEROR TAMARINS (*Saguinus imperator*): RESPONSE TO ENVIRONMENTAL MODIFICATIONS

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### Abstract

Clinical and biochemical signs of vitamin D deficiency and secondary hyperparathyroidism were identified in a colony of emperor tamarins (*Saguinus imperator*) housed at St. Paul's Como Zoo. Suggestive clinical signs occurring over a 2-yr period, including the death of three juveniles with identification of skeletal abnormalities upon necropsy in two of the juveniles and the clinical presentation of a mature male with limb bowing, thoracolumbar scoliosis, and altered gait (stiffness and difficulty climbing), prompted clinical, radiographic, and biochemical evaluation of all colony members.

At the time of initial evaluation, the tamarin colony consisted of seven adults (4.3) and one juvenile (0.1). Animals were housed indoors without access to direct sunlight or an artificial UVB light source. They were fed a morning meal of mush (Zupreem Marmoset Diet (Premium Nutritional Products, Mission, Kansas 66202 USA), bananas, Mazuri<sup>®</sup> Leaf Eater Primate Diet (PMI Nutrition International, Brentwood, Missouri 63144 USA, wheat germ, calcium, vitamin C, honey, water) and an afternoon meal of fruits, vegetables, a mealworm or cricket, and Mazuri<sup>®</sup> Marmoset Jelly (PMI Nutrition International, Brentwood, Missouri 63144 USA).

Animals were anesthetized with injectable ketamine HCl (8-12 mg/kg i.m.) for physical examination, whole body radiography, and collection of blood for serum 25-hydroxyvitamin D<sub>3</sub> (25[OH]D<sub>3</sub>), ionized calcium (iCa), and intact parathormone (iPTH) measurements. All animals, except an adult male who was euthanatized shortly after evaluation, were in good body condition and exhibited no abnormal clinical signs. However, radiographic lesions, including variable degrees of limb bowing (varus deviation distal to the elbow with separation of the radius and ulna), physeal abnormalities, radiolucency, and apparent pathologic fractures, were identified in six of eight animals. Initial serum concentrations of 25[OH]D<sub>3</sub>, iPTH, and iCa revealed seven of eight animals with low 25[OH]D<sub>3</sub>, compared to levels reported in healthy wild-caught callitrichids,<sup>1,2</sup> and seven of eight with apparent high iPTH and normal iCa levels based on human "normals" used by the reporting laboratory (Minnesota State Diagnostic Laboratory, Animal Health Diagnostic Laboratory, Lansing, MI 48909 USA). Changes were the most severe in older animals (>11 yr of age).

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Based on these results, a series of environmental and dietary manipulations were initiated. During the first 3-mo period all males were moved into a screened, outdoor enclosure for the summer, while the females remained indoors. At the end of this period, 25[OH]D<sub>3</sub> concentrations changed little in either group. However, iPTH concentrations decreased in the males with little change in females. During the second 3-mo period, all animals were provided access to an artificial UVB light source (Reptisun 5.0, Zoo Med Laboratories, San Luis Obispo, California 93401 USA) and are scheduled for repeat serum evaluation. Regular evaluation and management modification will continue over the next 6 mo in this colony. This study provides baseline values for 25-[OH]D<sub>3</sub>, intact parathormone, and ionized calcium levels in a colony of emperor tamarins. It also provides information about the clinical and biochemical impact of specific sequential dietary and environmental modifications that are practical and feasible in a zoo setting.

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**DIETARY INFLUENCES ON BONE METABOLISM IN THE COMMON MARMOSET (*Callithrix jacchus*): ASSESSMENT OF BONE STATUS BY BIOCHEMICAL BONE TURNOVER MARKERS AND DENSITOMETRY**

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**Abstract**

This study was designed to optimize the diet composition and nutrient uptake of common marmosets (*Callithrix jacchus*) at the animal husbandry unit, F. Hoffmann-La Roche in Basel, Switzerland, with a particular emphasis on bone health protection. The additional influence of UVB-irradiation on vitamin D-status was also examined.

Four groups of animals (n = 12/group) each consisting of evenly distributed male and female marmosets between 1 and 4 yr old, were subjected to either a standard diet (SD), a SD and UVB-irradiation, a trial diet (TD), or a TD and UVB-irradiation. The SD consisted of marmoset pellets (Kliba 3450, Provimi Kliba AG, Kaiseraugst, Switzerland) and Hills Prescription Diet Canine I/d™ (Hill's Pet Nutrition Inc., Topeka, Kansas 66601, USA). The TD contained ssniff® Mar meal for marmosets (ssniff Spezialdiäten GmbH, Soest, Germany) supplemented with 17.5% gum arabic (GA) (10% in the basal feed, the rest given in fluid form as a separate meal). Both diets were supplemented with eggs, carrots, raisins, bananas, or apples. Food items were provided in three portions throughout the day. The diets contained comparable nutrient amounts in accordance with the National Research Council<sup>3</sup> and vitamin D<sub>3</sub> concentrations of 2500 IU/kg (SD) and 2800 IU/kg (TD) respectively. Weight gain, food intake, biochemical markers of bone turnover (bone formation: serum osteocalcin [sOC], serum N-terminal propeptide of human pro-collagen type I [sP1NP], bone-specific alkaline phosphatase [bALP]; bone resorption: urinary pyridinoline [uPYD], urinary deoxypyridinolone [uDPD], serum C-terminal crosslinks of human collagen type I (C-telopeptide) [sCTX]), vitamin D<sub>3</sub> metabolites (25(OH)D<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub>), intact serum parathyroid hormone (iPTH), and routine blood biochemistry for general health monitoring were determined at the beginning of the study and then in monthly intervals for a duration of 6 mo. Bone mineral density (BMD) was determined by peripheral

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quantitative computed tomography (pQCT) and dual-energy X-ray absorptiometry (DEXA) at the beginning and the end of the study. Groups receiving UVB irradiation were exposed to a light source (Ultra-Vitalux®UV-Strahler, Osram AG, Winterthur, Switzerland), claiming to produce UVB wavelengths, for 30 min each day.

When compared with the SD-groups, the TD-groups had significant weight gains and reduced food intakes, sufficient to maintain their significantly higher body weights. Possible nutrient deficiencies due to the reduced food intake of the TD-group could be excluded based on the measured serum parameters. All groups showed a decrease in bone turnover rate (with a lower rate for the TD- than the SD-groups) and decreasing iPTH values. The significant weight gain and lower bone turnover of the TD-groups, may have been due to better digestion, absorption, and utilization of the diet as a result of the GA supplement. The addition of GA to the diet has been shown to lengthen transit time (allowing for better digestion and absorption of dietary nutrients) in marmosets<sup>4</sup> and to increase absorption of volatile fatty acids, increase mineral absorption, and have a trophic effect on large intestinal mucosa in rats<sup>2,5</sup>. The lack of significant differences in bone turnover between the SD- and TD-groups can be explained with the two diets evidently fulfilling the nutrient requirements of marmosets. The food fractionation over the course of the day is an important contributing factor as well, underlining the importance of the feeding regime for bone metabolism. Serum 25(OH)D<sub>3</sub> levels showed a rise in all groups and like the 1,25(OH)<sub>2</sub>D<sub>3</sub> values, a decrease in variation in the course of the study. A vitamin D<sub>3</sub> intake of 87 – 55 IU/kg body weight appears sufficient to maintain serum 25(OH)D<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> values in the physiologic range. The role of artificial UV-irradiation seemed to be of secondary importance as no significant differences between groups could be detected. This can be the result of either a too short exposure to and/or insufficient irradiation in the desired UVB wavelengths. The DEXA and pQCT measurements indicated the TD had a positive influence on BMD. An overlap with growth effects in single animals could not be completely excluded, however, as peak bone density in marmosets is reached at approximately 2 yr of age.<sup>1</sup>

The TD was considered well suited to replace the SD and had a positive influence on bone metabolism and health. Gum arabic appears to be an essential dietary fiber for marmosets and should constitute at least 10 % of the provided diet. Particular attention should be paid to food fractionation with a minimum of three meals per day and the basal feed always given as the first food in the morning to secure sufficient nutrient uptake.

#### **ACKNOWLEDGMENTS**

The authors wish to thank Mr. W. Stamm, Mr. H. Brunner, and Mrs. U. Gerwert for technical assistance and data processing. This study was conducted as part of the thesis of A. Angeliewa currently in progress, toward a doctorate in Veterinary Medicine at the Free University of Berlin, Germany.

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## COULD A GLUTEN-FREE DIET FOR MARMOSETS BE THE SOLUTION FOR WASTING MARMOSET SYNDROME?

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### Abstract

Wasting marmoset syndrome (WMS) is a major cause of morbidity and mortality in marmosets and tamarins kept in captivity. In a prior study we demonstrated that WMS is an enteric malabsorption process with morphology similar to human celiac disease. The main objective of this research is to describe the clinical and pathologic features of marmosets on a gluten-free diet compared to animals fed a gluten diet.

The research was conducted at the Criatório Mucky de Proteção aos Pequenos Primatas, a small-primate conservationist breeding and rehabilitation center located in Jundiaí, state of São Paulo. The species included in the study were: *Callithrix jacchus* (common marmoset, n = 14), *Callithrix penicillata* (ear-tufted black marmoset, n = 30), *Callithrix geoffroyi* (Geoffroy's marmoset, n = 3) and hybrid marmosets (n = 31). The animals were born at this facility or had been there for at least 6 mo. Animals were kept in outdoor enclosures in pairs or in kin groups. Group 1 (three males, five females) was the control group. It consisted of animals that did not exhibit physical signs of WMS. Group 2 (18 males, 22 females) consisted of marmosets diagnosed with WMS. Groups 1 and 2 received a diet consisting of grain-based products (such as cereal and bread), fruits, cooked vegetables, cooked chicken, eggs and lactose-free milk through October of 2001. At this time a diet change was initiated for all animals. The new diet was similar to the original diet, but did not contain cereal products. As a result of the diet change, animals that were still alive and previously classified as Group 2, were classified as Group 3. The study animals underwent physical examinations, fecal observation, and periodic weight measurements.

Group 1 animals died as a result of other diseases not related to enteropathies. At necropsy their jejunum was used for histologic examination. Group 2 animals had progressive weight loss and diarrhea, but no other identifiable diseases that would elicit these symptoms. These animals received oral vitamin and amino acid supplementation, antibiotics, antiparasitics, and subcutaneous fluid therapy. Twenty five marmosets from this group died and were necropsied before October 2001. Group 3 animals were diagnosed with WMS and received subcutaneous vitamin and mineral treatments and oral pancreatic enzymes. Twenty five marmosets from this group died and were necropsied before August 2003.

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The main clinical changes exhibited by animals in Group 3 compared to those in Group 2 included stabilized weight loss, decreased incidences and intensities of diarrhea bouts, total or partial recovery of hind limb paralysis, increased survival rates, and decreased incidences of abdominal distention at necropsy.

The histopathologic evaluation of included villus:crypt ratio, villus height, crypt hyperplasia, the intensity of the inflammatory process, the intensity of the mononuclear infiltrate, and the density of intraepithelial lymphocytes. Group 1 animals were within standard normal ranges. Group 2 animals had severe, atrophic enteritis with partial to complete villi loss, severe crypt hyperplasia and lymphocytic-plasmacytic infiltrate in the lamina propria. Group 3 animals had chronic enteritis with mild and partial reduction of intestinal villi height, crypt hyperplasia, and lymphocytic-plasmacytic infiltrate in the lamina propria. The villus:crypt ratio, the villus height, and crypt hyperplasia were significantly different ( $P < 0.001$ ) among all groups. The intensity of the inflammatory process, the intensity of the mononuclear infiltrate, and the density of the intraepithelial lymphocytes were significantly different ( $P < 0.001$ ) between the control group (Group 1) and the WMS groups (Groups 2 & 3), but not significantly different between Groups 2 & 3. The presence of ulcerations and neutrophilic or eosinophilic infiltrate was not statistically significant for any of the groups. Intestinal parasites were not found in either group of animals diagnosed with WMS.

Removing gluten from the diet of animals with WMS resulted in symptomatic and histologic improvements. Feeding captive marmosets diets with gluten may have profound negative effects on their intestinal morphology and consequently on their health. Removing the prolamines of wheat, rye, barley, and oats from the diet of marmosets kept in captivity may help control and possibly eradicate WMS.

#### ACKNOWLEDGMENTS

We would like to thank Livia Bótar and the technical staff from Criatório Mucky for facilitating and encouraging this research. This work was supported financially by FAPESP with grants 00/04412-1; this study is part of the PhD project of L.R.M. de Sá.

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## USE OF FRESH PLANT MATERIALS IN FEEDING AND MANAGEMENT OF CAPTIVE ANIMALS SHOULD BE SCIENCE-BASED

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### **Abstract**

Over the past 25 yr, there has been an increased use of fresh plant materials in captive animal management. These plant materials, including leaves, branches, flowers, and fruits in various stages of development, may serve as primary nutrient sources or as supplements to the diet, or they may be used as sources of environmental enrichment, providing perching sites, manipulable objects, or enclosure plantings that increase the natural appearance of exhibits.<sup>8,11</sup>

Most institutions employ the use of indigenous plant species collected from various sources in the facility's immediate surroundings. Other institutions have combined these sources with plants identified in a target animal species' home range, or documented the natural diet, and cultivated those plants for routine harvesting and use.

Many animal species (e.g., koalas) cannot be maintained in captivity without adequate quantities of appropriate (i.e., species, stage of development) browse plants<sup>18</sup>. Other animals demonstrate positive objective responses to inclusion of browse plants as a significant component of the daily ration (e.g., giant eland, giant pandas).<sup>4,12</sup>

Captive-born animals do not have the same experiences as wild animals in food selection and avoidance of potentially hazardous material.<sup>11</sup> The presumption that naïve animals are innately capable of recognizing nutrient concentrations or toxicants within a food source (nutritional wisdom) is not supported by evidence.<sup>10,17</sup>

As a consequence, with the increased use of plants in animal diets, our industry has reported an increased incidence of morbidity and mortality associated with inappropriate or incompatible pairings of animals with plants.<sup>1-3,6,7,9,13,15,16,19</sup>

The use of fresh plant materials in animal diets brings with it inherent risks that are not present with (or are different than) commercially available foods. The perceived benefits of using fresh plant materials should be evaluated against those risks. Personnel making the decisions to include fresh plant materials in an animal care program must fully understand and assume those risks and bare the burden of those decisions. These staff members must have an intimate understanding of the interactions between both the plants and the animals consuming them. They would not seek out that expertise from producers of commercial produce or harvested

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forages (e.g., hays), nor should they look for those decisions to be made by the “producers” of these browse materials.

Animal-care personnel, preferably those with strong backgrounds in the sciences of animal nutrition and plant physiology, should scrutinize these browse plants with all the safeguards (and more) used to evaluate any food item used for feeding captive wildlife.<sup>5,14</sup> Plant species, stage of growth, growing conditions, exposure of this plant or plants in the vicinity to pesticides, herbicides, or environmental pollutants, and handling of the materials post-harvest are just a few of the issues that should be evaluated before plant materials are approved for use with the targeted animal species.

As with any other aspect of captive animal care, diets, including the use of plant materials for any purpose, require continued scrutiny and daily management. Appropriate diets are the foundation of proper animal care, and it is unethical to acquire and exhibit wild animals without the supervision of scientifically trained and qualified personnel making critical management decisions that ultimately affect animal health and welfare.

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## JAGUAR (*Panthera onca*) SPECIES SURVIVAL PLAN

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### **Abstract**

Jaguars (*Panthera onca*) are the largest felid species in the New World and the only member of the genus *Panthera*, the roaring cats, that occurs in the Americas. It is estimated that 10,000 jaguars are left in the wild with an unknown number in captivity throughout Latin America. There are currently 81 jaguars held at 38 AZA institutions.<sup>5</sup> Jaguars housed at AZA institutions can serve as ambassadors to instill concern for the conservation of this species and the diverse ecosystems in which jaguars live.

In 2003, the Jaguar Species Survival Plan (SSP) Guidelines for Captive Management of Jaguars was published.<sup>2</sup> These guidelines provide information from natural history to health care. Additionally, the Jaguar SSP website ([www.jaguarssp.org](http://www.jaguarssp.org)) was recently updated and provides a great deal of information on the biology and care of jaguars.

Data from a study conducted in 2003 on the morbidity and mortality of captive jaguars at AZA institutions can be used for making recommendations on captive husbandry and veterinary care.<sup>1</sup> This study confirmed that cage mate aggression is a frequently encountered problem and can lead to significant morbidity and mortality. Dental disease, often consisting of canine fractures, is also very common. Both these problems can be minimized with proper housing. In this study, gastrointestinal disease associated with parasites was common among all age groups; thus the need for routine fecal evaluations and preventive anti-helminthic measures is warranted. Diseases associated with advanced age included arthritis, spondylosis, intervertebral disk disease, and renal and urinary bladder diseases. The study also found that epistaxis was surprisingly common within all age groups. It is recommended that jaguars that present with epistaxis receive complete diagnostic work ups to determine etiology. Female jaguars are known to have a high prevalence of reproductive tract neoplasia and disease.<sup>3,4</sup> This was confirmed in the 2003 morbidity and mortality study. It is interesting to note that the presence of a MGA implant increased morbidity associated with the female reproductive tract but not mortality.<sup>1</sup> These data support the recommendation that the safest contraceptive measure is ovariectomy.

There are a number of *in situ* and *ex situ* projects of which the SSP is currently aware. A sample of these projects will be presented in this talk. Dr. Linda Munson continues her studies on female reproductive tracts. The SSP requests that all tracts be sent to Dr. Munson. Dr. Rebecca Spindler, the Jaguar SSP reproduction advisor, and her colleagues are studying male and female reproductive health both in North and Latin American captive jaguars and free-living jaguars in

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Latin America. Dr. Kay Backues and her colleagues at the Tulsa Zoo are working in Honduras and Guatemala on captive jaguar health issues. In Bolivia, the SSP is working with the Santa Cruz Zoo to import confiscated jaguars currently housed in captivity in Bolivia. Imports of healthy jaguars from home range countries are important due to the low genetic diversity of the captive population in North America. Jaguars to be imported should only be those that have been confiscated from the illegal animal trade or considered problem animals and that are known to be healthy and free of transmissible diseases.

The Jaguar SSP veterinary advisor welcomes submissions of published, anecdotal, and experimental findings related to jaguar health. Information gathered by health professionals working with jaguars is the key to expanding our knowledge on jaguar health and diseases. The SSP veterinary advisor has a working bibliography on jaguar health issues. This bibliography can be sent to all interested persons.

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## OLD WORLD NONHUMAN PRIMATE RETROVIRAL SURVEY-RESULTS

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### **Abstract**

A survey was sent via the American Association of Zoo Veterinarians (AAZV) list serve (AAZV-L) to American Zoo and Aquarium Association (AZA) institutions housing old world nonhuman primates (OWP), asking what retroviral testing was done on these animals, and if testing was done, would the institutions like to share results with the survey author and old world monkey Taxonomic Advisory Group (TAG). Forty-nine percent of zoos housing OWP responded to the initial survey asking if they tested for retroviruses and 27% said they do no testing, while 73% tested for at least one retrovirus. A follow-up survey was sent to responders and via the AAZV-L asking which species were tested for simian immunodeficiency virus (SIV), simian foamy virus (SFV), simian retrovirus (SRV), and simian T-cell lymphotropic virus (STLV) and what the results were. Thirty-three institutions responded to the follow-up survey (37% of zoos housing OWP) and some of these institutions also were testing prosimians, new world primates, and apes for the specified retroviruses. A total of 621 individuals representing 43 taxa (genus, species/subspecies) were tested for SIV with a positive percentage of 3.1% (19 individuals). Four hundred and forty-two individuals representing 44 taxa (genus, species/subspecies) were tested for SFV with a positive percentage of 50.9% (225 individuals). Three hundred and fifty-seven individuals representing 35 taxa (genus, species/subspecies) were tested for SRV (types 1-3) and six individuals were positive (1.6%). Four hundred and fifty animals representing 35 taxa (genus, species/subspecies) were tested for STLV with 31 individuals (6.8%) testing positive.

### **Results**

Results ranked by suborder/family are as follows.

#### Prosimians

19 individuals tested for SIV, 0 positives  
43 individuals tested for SFV, 0 positives  
6 individuals tested for SRV, 0 positives  
4 individuals tested for STLV, 0 positives

New World primates:

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1 individual tested for SIV, 0 positive  
3 individuals tested for SFV, 3 positives (100%)  
1 individual tested for SRV, 0 positives  
0 individuals tested for STLV

#### Old World monkeys

432 individuals tested for SIV, 18 positive (4.2%)  
277 individuals tested for SFV, 166 positive (60%)  
249 individuals tested for SRV, 2 positives (0.8%)  
305 Individuals tested for STLV, 26 positive (8.5%).

#### Apes

143 individuals tested for SIV, 0 positive  
102 individuals tested for SFV, 45 positive (44%)  
78 individuals tested for SRV, 0 positives  
123 individuals tested for STLV, 5 positive (4%)

### **Discussion**

Results included new world primates, prosimians, old world monkeys and apes. Several confounders to this data are: small sample size compared to overall population in AZA accredited institutions, individuals were only counted once even if they had been tested numerous times, and if they ever tested positive, that was recorded; indeterminate results were not counted unless confirmed on a later date. There was no standardization of laboratories used. Some animals were listed by common names, with no species/subspecies listed. Those individuals were counted under genus only. Even though the survey was originally intended to just look at retroviral status in old world monkeys, the inclusion by some zoos of results for prosimians, new world primates and apes lent some interesting data to the survey.

### **Conclusions**

In recent years, concern over the prevalence and zoonotic risk potential of retroviruses in captive nonhuman primate collections at zoos has grown.<sup>1-4</sup> In addition to the zoonotic risk potential, there has also been concern over the potential impact these viruses may make on TAG recommendations for breeding, moving animals, etc. While the Infectious Disease Committee (IDC) of the AAZV and Animal Health Committee (AHC) of the AZA has been working on Occupational Primate Disease Safety Guidelines for Zoological Institutions to protect zoo workers, as well as zoo nonhuman primates, from zoonotic risks, sound advice about species management has been difficult because the retroviral prevalence in the captive monkey populations is largely unknown. There have been no coordinated efforts to pull all of this data

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together and develop standardized testing and reporting. These preliminary results point out the need for such research to be done. Once a complete analysis of morbidity, mortality and viral prevalence is accomplished, sound recommendations on both health screening, zoonotic risks, as well as species management, can be made.

#### **ACKNOWLEDGMENTS**

The authors would like to thank all responding institutions, who shall remain anonymous.

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## CORACIIFORMES TAG VETERINARY ADVISORS' REPORT

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### Abstract

Two additional giant hornbills (*Buceros bicornis*) succumbed in the past year to invasive squamous cell carcinoma of the casque for a total of nine (6.3) birds. This has been documented in the literature previously in three cases. To date, this condition has been uniformly fatal, but advances are made with each case that may facilitate future resolution. Early detection of the neoplasm is essential to have a potential chance of recovery. It is strongly encouraged that this species be evaluated annually with a full radiographic series of the skull and casque. A project coordinated by the Veterinary Advisor is seeking an underlying cause for this condition though carotenoid pigmentation of the casque.

The project of creating a uniform bibliography (natural history and veterinary) for the Order continued this year. The first installment of over 250 citations were placed on the website ([www.coraciiformestag.com](http://www.coraciiformestag.com)) for free access. These citations are available through the Veterinary Advisors. Further installments will be available by the end of August 2004.

This year, a substantial effort was undertaken to categorize the pathology reports for the Order, 1990-2003. At the time of this abstract, 37 entries had been made from 59 institutions into a database, with 5 institutions reporting no deaths within the time period. Once completed, the database will be available for searches for comparison to future cases on request to the Veterinary Advisors. It is also a reminder to provide current pathology (gross and histopathology) reports to the Advisors for inclusion in the annual updates routinely planned.

Trends identified in the database accumulated to date:

Hemochromatosis often suggested for the Asian hornbill species has not been confirmed.

*Tetrameres* sp. have been identified at necropsy in several species of the Order and, in at least one hornbill case, was associated with proventricular pathology.

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## PARESIS AND DEATH IN ELK (*Cervus elaphus*) DUE TO PRESUMPTIVE LICHEN TOXICOSIS IN THE RED RIM HABITAT AREA OF SOUTH CENTRAL WYOMING

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### Abstract

In February and March 2004, 304 elk (*Cervus elaphus*) developed paresis in the Red Rim habitat area southwest of Rawlins, Wyoming. Elk were found in sternal recumbency, alert and responsive, but unable to rise. Bright red discolored urine was adjacent to many affected elk. Many elk progressed to lateral recumbency followed by dehydration, obtundation, and death. Several elk provided feed and water remained alive and responsive but never became ambulatory. The majority of elk were euthanatized due to the poor prognosis for survival and return to normal function. Postmortem examinations were performed on 12 elk from the field, and at necropsy these animals were in fair to good body condition. Most of the elk that were recumbent for a day or more demonstrated gross evidence of myopathy, with pallor and streaking in skeletal muscles, particularly the semimembranosus, semitendinosus, and gastrocnemius muscles. Microscopic examination of tissues from the majority of elk was unremarkable, with significant lesions most consistently observed in skeletal muscles. In affected muscles, there were degenerative lesions of varying duration, severity, and distribution, some with early mineralization and attempts at regeneration and some associated with degenerating protozoal cysts (*Sarcocystis* sp.). Sporadic lesions were observed in other tissues from a small number of elk, including mild tubular degeneration/necrosis in kidneys, mild fibrinoid degeneration/change in small blood vessels of adrenal glands and a few other organs, and mild hepatocellular degeneration or less frequently apoptosis/necrosis.

Common infectious, inflammatory, toxic, and traumatic causes of weakness, paresis, and recumbency were ruled out via histopathology, virus isolation and associated tests, bacterial culture, parasitology analyses, and toxicology analyses. During field investigations, large quantities of ground lichen (*Xanthoparmelia chlorochroa*) were observed in the area where affected elk were found. This lichen was found in the rumen contents of several elk. Approximately 50 kg of lichen was collected and fed to captive research elk. Three elk initially were offered a diet of 100% lichen for 7 days. After 7 days, elk were offered free choice alfalfa hay and lichen. After 7 days on this diet, one elk became sternally recumbent and was unable to rise. After 10 days on the diet, a second elk went down in a similar manner. Both elk were

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ethanatized and necropsied. Gross and microscopic lesions were consistent with lesions from the affected elk in the field and red discolored urine was noted in the pens where the elk had been housed.

Our preliminary conclusion is that this lichen was responsible for recumbency and death. Interestingly, cattle, horses, mule deer, and pronghorn also were observed on Red Rim habitat area during the elk mortality event, with access to the lichen, but were unaffected. The toxic compound of the lichen has not yet been identified. We plan to analyze the lichen for toxic compounds and analyze the diets of the other herbivores in the area to determine if they ate the lichen.

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**AN INTERAGENCY INVESTIGATION INTO CAUSES OF BALD EAGLE (*Haliaeetus leucocephalus*) AND GOLDEN EAGLE (*Aquila chrysaetos*) MORTALITY IN MARYLAND 1988-2004**

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**Abstract**

Eagles are protected under the Endangered Species Act (ESA), Bald and Golden Eagle Protection Act, the Lacey Act, as well as the Migratory Bird Treaty Act. States also protect eagles through state threatened and endangered species laws. National efforts to protect eagles have focused on habitat conservation, minimizing contaminant effects, monitoring wild bird nests, and coordinating captive propagation and release programs. Bald eagle (*Haliaeetus leucocephalus*) and golden eagle (*Aquila chrysaetos*) populations are growing in the U.S. The Chesapeake Bay is home to hundreds of nesting pairs of eagles. Currently, eagles are listed as threatened in Maryland.

Four organizations work collaboratively on eagle morbidity and mortality in our region. The Maryland Department of Natural Resources (MD DNR) and U.S. Fish & Wildlife Service (FWS) serve as regulating and permitting agencies. Tri-State Bird Rescue and Research and the Baltimore Zoo are the two facilities permitted to conduct treatment and rehabilitation for eagles. Carcasses of eagles that die or are found dead in Maryland are transferred to FWS and frozen for eventual shipment either to the National Eagle Repository or the FWS Forensics Laboratory.

To examine eagle mortality in Maryland, databases from the four organizations were reviewed. Minimal information existed on cause of death for Maryland eagles prior to 1988, so the period examined begins with January 1988 and extends through March 2004. A total of 220 eagles were found dead or died in Maryland during the study period with an average of approximately 14 deaths per year (range 2-29). For all eagles, causes of death included 63 traumatic injuries: collision (34), other physical trauma (29). Other causes of death included electrocution (32), poisoning (23), drowning (9), disease (5), entanglement (3), gunshot (5), and unknown causes (80). Little is known about eagles in the unknown category other than the date and location of the incident. Legal cases are still open, and no information is available until the cases are settled.

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However, the majority of eagles in the unknown category had no cause of death information listed.

The databases were reviewed for temporal and spatial significance. Three separate years (1988/ 28, 1997/ 22, 2003/ 29) accounted for nearly one-third of the total number of eagle mortalities. No other clusters of mortalities were found in the period examined; however, an overall increasing trend in mortalities was noted. From a geographic perspective, eagles were reported dead in 21 of 23 counties in Maryland. The majority came from three counties: Charles (34), Harford (34) and Dorchester (31) accounting for nearly half of all mortalities reported (99/220). Since these counties are located in different regions of the state, the mortalities did not represent a geographic concentration. Additionally, 124 eagles were identified as adults, 72 as immature and 24 were not identified by age.

The primary objective in this study was to examine the causes of eagle mortality in Maryland. Results indicated that eagles died throughout most counties of the state over the entire study period for a variety of reasons. However, the majority died from unknown causes. Through our cooperative efforts, we hope to better identify causes of eagle mortality that will lead to identification of threats and impacts to Maryland populations. This collaboration will serve as a model for future wildlife investigations and conservation efforts in our region.

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## DISEASE MONITORING IN CAPTIVELY PROPAGATED AND REINTRODUCED RIPARIAN BRUSH RABBITS (*Sylvilagus bachmani riparius*) IN CALIFORNIA

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### Abstract

The riparian brush rabbit (*Sylvilagus bachmani riparius*) is a state- and federally-listed endangered species. It is native to riparian communities in the northern San Joaquin Valley of California. Riparian habitat in the San Joaquin Valley has been reduced to less than 1% of its historic extent, primarily due to clearing of natural vegetation, irrigated agriculture, livestock grazing, impoundment of rivers, and stream channelization. At the time of state and federal listing of the species, there were only two known remnant populations of riparian brush rabbits in California, one in Caswell State Park along the Stanislaus River, and another along a overflow channel of the San Joaquin River (Paradise Cut). The size of both populations was too low to provide sufficient captures to estimate population sizes with capture-recapture population estimator models. To recover riparian brush rabbits, the US Fish and Wildlife Service set a goal of establishing three or more self-sustaining populations outside of Caswell Memorial State Park within the historic range of the species. Because the extant populations at Caswell State Park and Paradise Cut were isolated from other suitable sites that are currently uninhabited, it was determined that reintroduction of individuals derived from existing populations would be required to achieve this goal. The USFWS contracted with California State University Stanislaus' Endangered Species Recovery Program to design and implement a controlled propagation and reintroduction program (plan available at [www.esrp.csustan.edu](http://www.esrp.csustan.edu)).

The UC Davis Wildlife Health Center (WHC) drafted guidelines for monitoring and maintaining the health of the captive and reintroduced riparian brush rabbit populations, and has provided veterinary input on all aspects of the program since its inception. Veterinary oversight has generally been in the form of:

- Health screening of all founding adult breeders captured at Paradise Cut before translocation into the controlled propagation pens at the start of the breeding season
- Health screening of all progeny born in the controlled propagation pens before reintroduction into San Joaquin National Wildlife Refuge

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- Disease monitoring in the captive and reintroduced populations via complete necropsies and histopathologic evaluations of all dead rabbits for which sufficient remains are recovered and by serologic surveys for select pathogens
  - Disease screening of other lagomorph species at the reintroduction site prior to reintroduction of riparian brush rabbits
  - Opportunistic disease screening in sympatric mammals
  - Individual animal treatment and care as needed

Health screens typically consist of a physical examination under gas anesthesia, and blood collection for complete blood count, serum chemistry, and serum banking. Ectoparasites are collected opportunistically. Fecal analysis for gastrointestinal parasites in live rabbits is not performed routinely, except when disease due to parasite infections is suspected in clinically ill rabbits. Additional diagnostics performed on several rabbits have included radiography, ultrasonography, ophthalmologic examinations, and cytology and biopsies of superficial masses

As of the end of 2003, 26 rabbits have been brought into captivity to serve as founding breeders; 340 offspring have been produced; and 243 rabbits have been reintroduced to the wild at San Joaquin National Wildlife Refuge. As of March 15, 2004, 63 necropsies have been performed on rabbits for which sufficient remains were available. Major causes of mortality have included: predation; parasitic encephalitis (presumed *Baylisascaris*), necrotizing typhlitis, trap-related trauma (including conspecific aggression), bacterial sepsis, inanition/starvation (in neonates) and lymphoproliferative disease. Principle causes of morbidity which have required therapeutic intervention have included: ocular disease (keratitis, uveitis, conjunctivitis), wounds related to radiocollars, and miscellaneous traumatic injuries, wounds and abscesses.

Thirty rabbits from the 2003 breeding season were screened for antibodies to *Encephalitozoon cuniculi* and *Treponema cuniculi*. All 30 rabbits were seronegative for *Treponema*, and one rabbit was weakly seropositive for *Encephalitozoon*. This rabbit was trapped in the wild at Paradise Cut for screening as a founding breeder, treated for a subcutaneous mass, and returned to the wild prior to testing. We have not seen clinical or pathologic evidence of disease due to either pathogen in the captive or reintroduced populations.

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**ANTIGEN RECOGNITION BY SERUM ANTIBODIES IN WHITE-TAILED DEER (*Odocoileus virginianus*) EXPERIMENTALLY INFECTED WITH *Mycobacterium bovis***

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**Abstract**

White-tailed deer (*Odocoileus virginianus*) have emerged as reservoirs of bovine tuberculosis (TB) in Northern America. For TB surveillance of deer, antibody-based assays are particularly attractive because deer are handled only once, and immediate processing of the sample is not required. Sera collected sequentially from 25 *Mycobacterium bovis*-infected and 7 non-infected deer were evaluated by ELISA, immunoblotting, and Multi-Antigen Print Immunoassay (MAPIA) for immunoglobulin specific to *M. bovis* antigens. Various routes of experimental *M. bovis* infection, such as intratonsilar inoculation (n = 11), aerosol (n = 6), and exposure to infected deer (in contact, n = 8) were studied. Upon infection, specific bands of reactivity at ~24-26 kDa, ~33 kDa, ~42 kDa and ~75 kDa to *M. bovis* whole cell sonicate were detected by immunoblot. Lipoarabinomannan-specific immunoglobulin was detected as early as 36 days postchallenge, and responses were detected for 94% of intratonsilar and in contact infected deer. In MAPIA, sera were tested with 12 native and recombinant antigens coated on nitrocellulose. All "in contact" infected (8/8) and 10/11 intratonsilarly-infected deer produced antibody reactive with one or more of the recombinant/native antigens. Responses were boosted by injection of tuberculin for intradermal tuberculin skin testing. Additionally, 3/6 deer receiving a very low dose of *M. bovis* via aerosol exposure produced antibody specific to one or more recombinant proteins. *Mycobacterium bovis* was isolated from 1/3 non-responding aerosol-challenged deer. Of the 12 antigens tested, the most immunodominant protein was MPB83; however, a highly sensitive serodiagnostic test will likely require use of multiple antigens.

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**RATES AND CHARACTERIZATION OF SAMPLES FROM *Salmonella* spp. ISOLATED FROM WILD AND CAPTIVE BROAD-SNOURED CAIMAN (*Caiman latirostris*) IN SÃO PAULO STATE, BRAZIL**

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**Abstract**

*Salmonella* sp. is an important worldwide zoonotic agent, frequently isolated among reptile microbiota. This genus has ever-growing importance to public and animal health, as well as food hygiene. In the present study, cloacal swabs were collected from *Caiman latirostris* individuals; 103 samples from animals captive in two different colonies and 12 from animals in the wild. All animals were sexed, and total length was measured at sampling. Swabs were inoculated in Tetrathionate broth and were cultured in MacConkey and XLT4 agars, and incubated at 37°C, for the isolation of *Salmonella* spp. Samples isolated were characterized according to their biochemical profile using API 20E (BioMérieux). Serotyping was performed according to the Kauffman-White method, and the pattern of susceptibility to antibiotics was verified using the disk diffusion method. Presence of four virulence genes (*invA*, *spvC*, *sefC* e *pef*) was assessed using Multiplex-PCR. Correlations between the presence of *Salmonella* spp., gender and total length were also analyzed. Frequency of animals positive for *Salmonella* spp. among captive animals was 30% and 48.38%, respectively. The agent was detected in 50% of the animals in the wild. There were significantly more positive males than females in one of the captivity sites and in the other, a significant correlation between the presence of the bacteria, and total length was observed. There were no significant differences in incidence of *Salmonella* spp. between captive and wild *Caiman latirostris*. A total of 45 *Salmonella* spp. samples from 15 different serotypes were isolated: *S. infantis*, *S. typhimurium*, *S. grumpensis*, *S. cerro*, *S. anatum*, *S. agona*, *S. enterica* subsp. *enterica*. (O: 13, 23), *S. coeln*, *S. enterica* subsp. *enterica* (rough strain), *S. enteritidis*, *S. newport*, *S. minnesota*, *S. enterica* subsp. *enterica* (O: 6, 8: ch: -), *S. enterica* subsp. *enterica* (O: 4, 12: ch: -), *S. schwarzengrund*. All of them were in the subspecies *Salmonella enterica* subsp. *enterica* (Group I). In general, strains were sensitive to all antibiotics tested, but resistances were observed for cotrimoxazole, chloranfenicol, neomycin, gentamicin and tetracycline in five samples of *Salmonella* spp. isolated from captivity. There were also three multiresistant strains in captivity (*S. infantis*, *S. typhimurium* and *S. grumpensis*). In relation to virulence genes, all samples presented the *invA* gene, *S. enteritidis* presented all genes studied and in *S. enterica* subsp. *enterica* (rough strain) was detected the genes *invA* and *sefC*.

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## NONPLAGUE YERSINIAE IN A ZOO

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### Abstract

Following the death of a hooded pitta (*Pitta sordida*) from the zoonotic bacterium, *Yersinia pseudotuberculosis*, at the Sequoia Park Zoo, Eureka, California, USA, in December 2001, we surveyed cloacal swabs and feces of 44 birds and 31 mammals, January through May 2002; four soil samples also were evaluated. Cold storage (4°C) incubation for 11-12 mo in trypticase soy broth was followed by isolation on Yersinia Selective Agar/Antimicrobial Supplement CN. Suspect strains were identified with an API 20E (bioMerieux) system and biotyping confirmed by the methods of Wauters, et al.<sup>2-3</sup> Eight strains were evaluated by pulsed-field gel electrophoresis.<sup>1</sup> Serotyping of one strain was attempted by slide agglutination.

No *Yersinia pseudotuberculosis* was found, including in eight samples of rat feces, supporting the notion that no enzootic focus for this bacterium is present at the zoo. *Yersinia enterocolitica* biotype 1A was isolated from a sacred ibis (*Threskiornis aethiopicus*), a cedar waxwing (*Bombycilla cedrorum*), an unidentified bird in the aviary, a nyala (*Tragelaphus angasii*), a black bear (*Ursus americanus*), and one soil sample; this was a 7.6% prevalence for all samples taken. One biotype 1A strain (ibis) tested by slide agglutination was too rough for serotyping; other strains were not serotyped. Based on a pulsed-field gel electrophoresis of eight biotype 1A strains, we observed 5 distinct profiles of *Y. enterocolitica* – evidence for several sources of the bacteria rather than a clone stemming from a single introduction. In addition, *Yersinia frederiksenii* was isolated from a nyala and *Y. mollaretii* was collected from an Argus pheasant (*Arguslanus argus*). There were no apparent patterns in *Yersinia* spp. isolations related to host class or geographic location within the zoo. *Serratia* spp. (10% of all samples) and *Providencia* spp. (5%) also were isolated from both birds and mammals.

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## A REVIEW OF PSEUDOTUBERCULOSIS AT A EUROPEAN ZOO: EPIDEMIOLOGY AND APPROACHES TO CONTROL

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### Abstract

Epidemiologic trends of pseudotuberculosis outbreaks in callitrichids and Rodrigues' fruit bats (*Pteropus rodricensis*) due to *Yersinia pseudotuberculosis* (*Y. pstb*) at Jersey Zoo are analysed from 1981-2000. The organism appears persistent in the population with peaks of disease in winter months. Sub-adult bats (2-3 yr) appear especially susceptible as do young (< 2 yr) and old (> 9 yr) callitrichids. Control of yersiniosis through vaccination and through monitoring techniques have not been effective at Jersey; however, improved husbandry methods are employed with apparent success to date.

### Introduction

*Yersinia pseudotuberculosis* (*Y. pstb*) is a globally distributed facultatively anaerobic gram-negative coccobacillus. Disease may cause sudden death or chronic illness; diagnosis is most reliable at necropsy where it is characterized by necrotic and caseous lesions in liver, spleen, lung and intestine. Almost all susceptible animals may become carriers. Disease is persistent and recurrent within populations and has zoonotic significance. Outbreaks have been reported in a wide range of species with varying susceptibility; more vulnerable species include New World primates, Indian Ocean fruit bats and certain bird species.<sup>2</sup>

Transmission is via faecally contaminated water and food sources and ingestion of infected prey. Wild-living mammals and birds are thought to act as carriers<sup>6,7</sup> but the relationship between the prevalence of *Y. pstb* in wild-living animals and infection in others is poorly understood. The bacterium may be part of the normal flora of small wild animals.<sup>5</sup> *Y. pstb* is reported to be widely spread in the environment in substrates such as soil, water, faeces and vegetation.<sup>4</sup> The organism is able to survive and replicate outside of a host for years due to minimal nutritional requirements and tolerance of temperature extremes.<sup>3</sup>

Outbreaks may be precipitated by stressors such as cold and wet weather, decreases or changes in food availability, overcrowding or capture. There is a well-established seasonal occurrence, with increased incidence associated with the colder temperatures of late autumn, winter and early spring. The nutritional state of animals may influence their susceptibility to yersiniosis; a low-calcium environment leads to increased secretion of antiphagocytic proteins, key in the virulence process.<sup>8</sup>

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*Yersinia pseudotuberculosis* presents a threat to captive breeding programmes. The often peracute nature of the disease presents little opportunity for therapeutic intervention; it brings a potentially high mortality rate and a degree of unpredictability with likelihood of recurrence in a broad host range. Management of risk factors to limit disease is important in every collection of captive animals involved in species conservation. Methods of control available are monitoring levels of the organism, husbandry changes and vaccination.

## Methods

Raw data was obtained from post mortem reports, ARKS and MEDARKS records from 1981-2000. Deaths were attributed to *Y. pstb* only where the organism had been isolated and identified post mortem. The analysis was limited to previously identified susceptible groups;<sup>2</sup> Rodrigues' fruit bats (*Pteropus rodricensis*) and callitrichid species: silvery marmoset (*Callithrix argentata*), Geoffroy's marmoset (*Callithrix geoffroyi*), cotton-top tamarin (*Saguinus oedipus*), golden-headed lion tamarin (*Leontopithecus chrysomelas*), pied tamarin (*Saguinus bicolor*) and Goeldi's monkey (*Calimico goeldii*).

## Results

From 1981-2000, a total of 28 fruit bats and 36 callitrichids were lost to yersiniosis. The pattern of losses over the years is different for these two groups, as illustrated in Figure 1. There are two main epizootics resulting in deaths in bats in 1986 (12 dead) and 1992 (seven dead), whereas the pattern of callitrichid deaths appears to be more random.

There is a distinct seasonal pattern in both bats and callitrichids. Two peaks, in January and March, together represent 82% of bat deaths due to yersiniosis; two peaks in March and May represent 50% of callitrichid deaths due to yersiniosis as shown in Figure 2. The summer months saw no deaths in the bats and three in the callitrichids.

Callitrichid species vary in susceptibility to yersiniosis, with the majority of losses in Geoffroy's marmosets and Goeldi's monkeys (each representing 28% of total callitrichid deaths due to yersiniosis) followed by silvery marmosets (23%). All other species each account for less than 10% of callitrichid deaths due to *Y. pstb*. There is no significant difference between the sexes in callitrichids lost to yersiniosis ( $\chi^2 = 2.314$ ,  $df = 1$ ) and no apparent relationship between the enclosures in which animals are housed or the number of conspecifics in the enclosure and death due to *Y. pstb*. Deaths occurred in free-ranging callitrichids as well as caged. Young ( $\leq 2$ yr) and old ( $\geq 9$ yr) animals appear more susceptible than others, with a higher percentage of deaths occurring in these age groups. These two ends of the age spectrum account for 43% and 20% deaths respectively.

There is no significant difference between the sexes in bats lost to yersiniosis ( $\chi^2 = 0.333$ ,  $df = 1$ ) and no apparent relationship between population size and deaths due to *Y. pstb*. The timing of

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outbreaks only coincides with the maximum holding once, in 1992. The distribution of ages that succumbed to yersiniosis shown in bats is different from that in callitrichids. In bats, 60% of deaths are in those aged 2-3 yr. This result does not simply reflect the age distribution within the population, as the proportion of the population of this age has not exceeded 21% since 1991. There are no deaths due to *Y. pstb* in bats older than 5 yr.

## Discussion

The total number of animals lost at Jersey is significant for a single infectious cause and could represent a serious obstacle to the success of conservation breeding programmes. The pattern of incidence reflects the recurrent nature of the disease and suggests that it never completely leaves the populations, resurfacing after varying intervals; this may be due to carrier animals. Outbreaks are mostly confined to winter months; a trend particularly evident in the bats, which are kept in a hot, humid environment on a constant light cycle and should be relatively unaffected by seasonal conditions. This lends support to the idea of a wildlife reservoir as rodents may move inside in cold weather, leading to increased food contamination. Affected bats were usually in good condition; this, coupled with the age distribution, may provide an explanation in that sub-adults, although not short of food, are having to consume fallen food which is more likely to be contaminated, whether by rodents or bats, in which the disease mainly causes a necrotizing enteritis resulting in sudden death. Older bats have established perches and feeding priority. Competition for space and food could set up a chronic stress to which males and females appear equally susceptible. The lack of correlation between population size and disease in the bats is surprising, but there may be a more specific relationship between the sub-adult population and disease depending on the relative roles of competition and contamination.

The callitrichids have outside access but show a less pronounced seasonal disease pattern with a significant number of cases in late spring as well as a peak in March. This may suggest increased susceptibility among lactating or parturient females. Stress is also implicated as young and old members of the population are affected more often than others, possibly reflecting association with immune challenge. It could also reflect external stress such as lack of priority access to food and shelter due to their status in the population and subsequent increased exposure to disease risk factors. The poor condition of affected callitrichids and signs such as lethargy, heat-seeking behaviour and weakness may reflect chronic disease prior to septicaemia with typical lesions on liver and spleen.

Work at Jersey has failed to demonstrate a reservoir in resident wildlife or in the soil.<sup>2</sup> It has become established wisdom that rodents and birds act as reservoirs for *Y. pstb* but details of their epidemiologic role are still unclear. They are often proposed as the principal source of contamination but convincing evidence is lacking. Might there need to be a certain level of infection within the population before the organism is found in local wildlife? If so, this would imply disease is not simply passing from wildlife to zoo animals, rather that transmission occurs as a result of interplay between numerous population and environmental factors.

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The multifactorial nature of yersiniosis means that it is a difficult disease to control. Use of faecal samples for monitoring is minimally invasive but the organism is not always shed into the intestinal lumen so isolation can be problematic. It requires cold-enrichment techniques and may be sufficiently sensitive to detect only clinical cases rather than carriers. The sensitivity of polymerase chain reaction tests, currently under development, may allow more effective population screening. Serologic diagnosis is complicated by the number of serotypes and limited by cross-reactions with other organisms.<sup>4</sup> Vaccination is widely used in Europe, mainly in zoos with recurrent outbreaks of yersiniosis, and has been advocated as a primary control method.<sup>1</sup> No challenge trials have been performed, however, and due to the sporadic nature of the disease it is difficult to assess efficacy in field trials. A study conducted at Jersey Zoo to measure antibody titers in callitrichids following vaccination showed scant response (unpublished data). Vaccination was used for a few years at Jersey Zoo but following the study, along with deaths due to *Y. pstb* in six vaccinated animals and two vaccine reactions it was discontinued. Other zoos consider the vaccine effective but disadvantages include the stress involved in capturing and handling the animals for injection; repeat injections are required every 6 mo, along with the time and cost involved. The development of oral vaccination may eliminate some of these problems.<sup>9</sup>

Husbandry changes at Jersey aimed at tackling stress factors in yersiniosis seem to have been effective in reducing the incidence of disease. Many changes have been made to the callitrichids' accommodation to provide as natural an environment as possible (e.g., increased foraging opportunities, densely planted larger outside areas to provide private areas, increased distance between visitors and animals).<sup>10</sup> In addition, efforts have been made to improve cage-cleaning routines which appeared to cause unusual stress to these animals; wood shavings provide a dry indoor environment and allow spot-cleaning which is less disruptive to the occupants. In the bats' enclosure, more extensive branching has been provided with increased availability of feeding and roost sites. Attention has also been given to nutrition; the callitrichids regularly receive probiotics. Calcium supplements are given to lactating and older callitrichids and prophylactic broad-spectrum antibiotics to those exhibiting concerning clinical signs typically associated with yersiniosis. These changes have been in place for the last 6 yr; there have been no deaths due to yersiniosis in callitrichids or bats at Jersey since 2000.

Much of the complex epidemiology of *Y. pstb* remains unclear; consequently control is difficult, but by committing to actions to improve the welfare and well-being of vulnerable species in captivity the likelihood of disease can be reduced. Improved monitoring of captive populations and environments would enable zoos to better protect animals against this insidious disease.

#### **ACKNOWLEDGMENTS**

Thank you to all the staff at Jersey Zoo, where this study was undertaken as a student elective project. Special thanks to Tony Allchurch, Ann Thomasson and Anna Feistner.

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Figure 1. Deaths due to *Yersinia pseudotuberculosis* in bats and callitrichids at Jersey Zoo

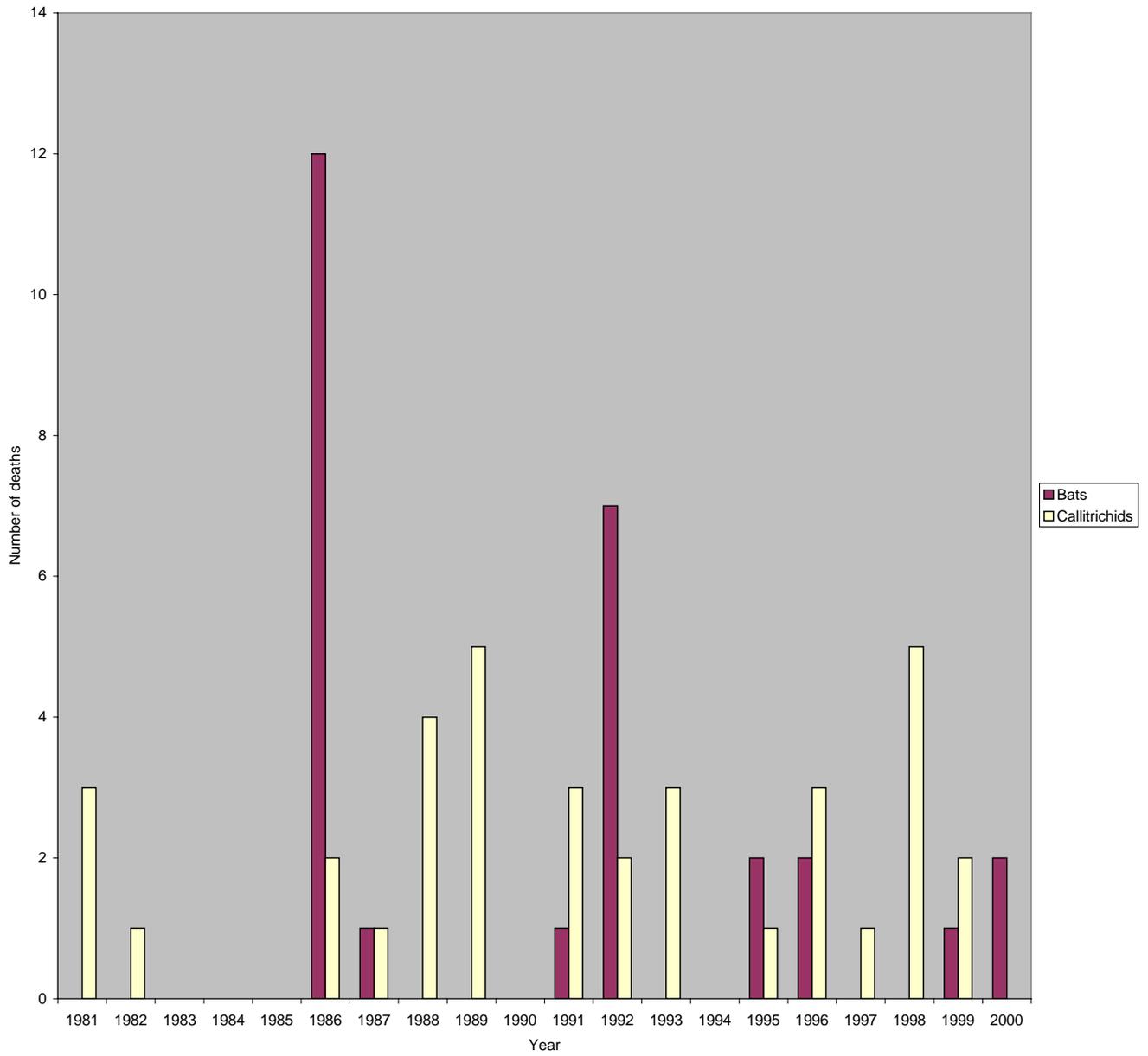
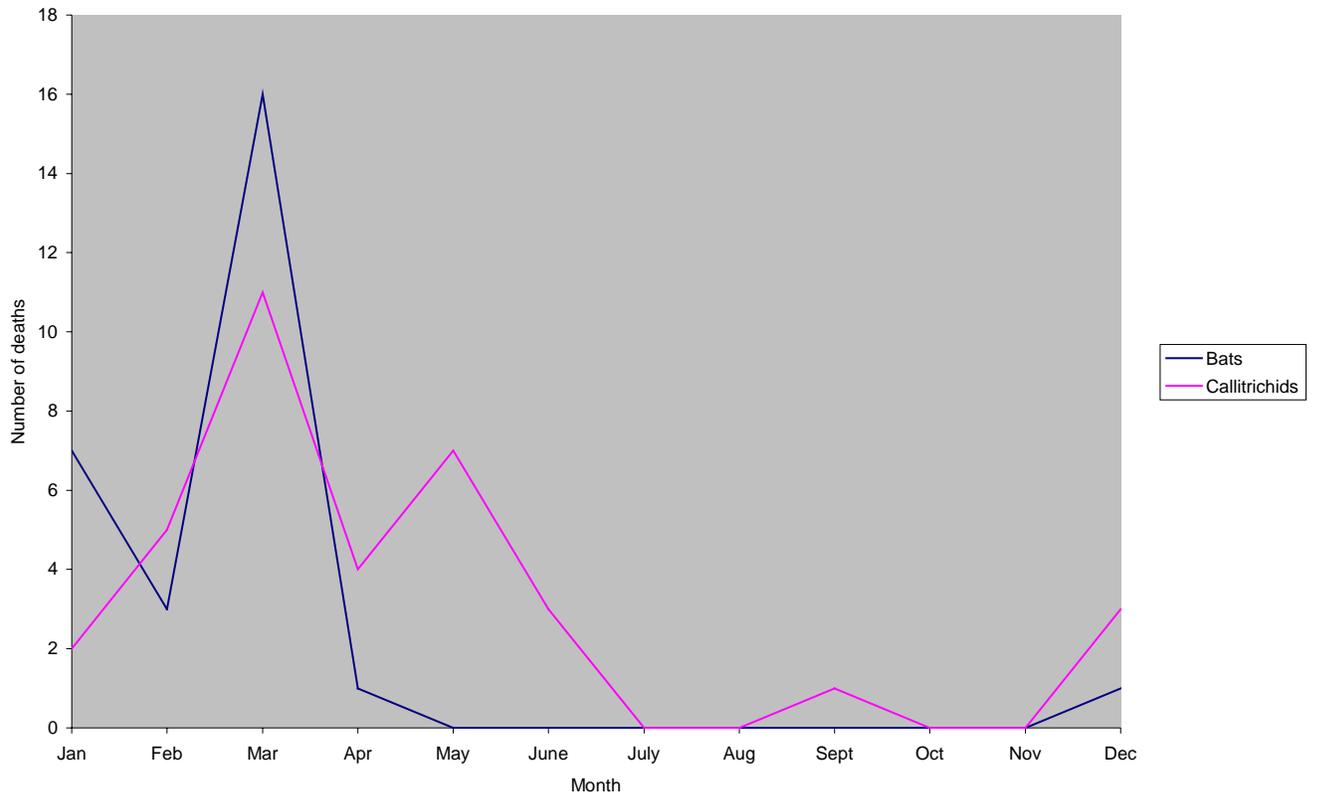


Figure 2. *Yersinia pseudotuberculosis* deaths with season (1981-2000)



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## PLAGUE INFECTION IN CANADIAN LYNX REINTRODUCED TO COLORADO: OCCURRENCE AND RESULTS OF A PILOT PLAGUE VACCINE TRIAL

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### Abstract

Plague appears to be a significant obstacle to successful reestablishment of lynx (*Lynx canadensis*) in Colorado. *Yersinia pestis* infections have been confirmed in six Canadian lynx reintroduced into Colorado as part of an ongoing species recovery program. Since 1999, plague was the primary cause in four of fifteen natural deaths, possibly contributed to one of six hit-by-vehicle deaths, and killed at least one kitten. In an attempt to minimize these impacts in future restoration efforts, we evaluated a recombinant capsular F1-V fusion protein vaccine that is safe and effective in black-footed ferrets. During January–April 2004, we vaccinated and serially bled 10 captive female lynx held in southwestern Colorado prior to their release in April 2004; 10 unvaccinated lynx served as controls. We observed no adverse effects of either the primary vaccine or booster doses on captive lynx. As of 15 March 2004, our study is still underway and serology results are pending. Based on observations to date, F1-V vaccine appears to be a safe vaccine in lynx; serologic responses and efficacy in reducing plague-related mortality remain to be determined.

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## ASSESSING CORAL REEF HEALTH IN AMERICAN SAMOA

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### **Abstract**

We surveyed corals in American Samoa for presence of lesions. We did 19 SCUBA and additional snorkel dives on six and seven sites on Tuituila and Ofu-Olosega, American Samoa. We photographed and took 70 samples from 49 corals comprising 29 species. Corals were fixed, decalcified, and sectioned on microscope slide to examine cellular architecture. Grossly, the most common lesions in corals were bleaching, growth anomalies, and tissue necrosis. On histology, depletion of zooxanthellae from coral tissue was most often seen followed by tissue necrosis associated with algae or fungi, hyperplasia of gastrovascular canals, or uncomplicated tissue necrosis. Two grossly bleached corals had evidence of pathologic lesions associated with invasion by ciliates (protozoa). One coral had evidence of primary infection with a fungus that manifested grossly as growth anomaly. One coral had evidence of skeletal enlargement associated with polychaete infestation. Incidental lesions included presence of bacterial aggregates or crustacea in normal tissues of several coral species. A gross diagnosis (e.g., bleaching) could have several different causes. This phenomenon underlines the importance of conducting microscopic exams on coral lesions to better define what the underlying causes of grossly visible changes. This study also provided the first baseline survey of corals in this region for pathogens and the first evidence that ciliates may, in some instances, be responsible for bleaching of selected coral colonies. This study also extended the documented range of growth anomalies in Acroporid corals. Future surveys should concentrate on systematically evaluating the spatial distribution of major lesions to allow for better comparisons among sites.

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## HOOKWORM ENTERITIS/BACTEREMIA COMPLEX IN CALIFORNIA SEA LIONS AND NORTHERN FUR SEALS, SAN MIGUEL ISLAND: A POPULATION DENSITY DISEASE

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### Abstract

During an investigation of high mortality of California sea lion (*Zalophus californianus*) and Northern fur seal (*Callorhinus ursinus*) pups on San Miguel Island in southern California, hookworm (*Uncinaria* spp.) enteritis with secondary bacteremia was found in 65% of the 225 pups examined. Ages ranged from 2 wk to 9 mo. Lesions found in these pups included parasitic enteritis, peritonitis, myocarditis, hepatitis, encephalitis, nephritis, pneumonia, and arthritis. Adult parasites including eggs were even found within the peritoneal cavity causing peritonitis. This severe epizootic hookworm infection is having an effect on the population of these two species of marine mammals. Over the last 30 yr or so the populations of California sea lions and fur seals have steadily increased causing the rookery to become fairly crowded, thus this recent problem with hookworms is considered to be a density dependent disease.

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## DEMOGRAPHICS, ECOLOGY, AND SEROSURVEY OF DOMESTIC DOGS IN THE ISOSO OF BOLIVIA

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### **Abstract**

Disease is increasingly recognized as a threat to the conservation of wildlife, and in many cases the source of disease outbreaks in wild carnivores is the domestic dog. For disease to spill over from a domestic to a wild population, three conditions must be satisfied: susceptibility of the wild species, presence of the disease agent in the domestic population, and contact between the two populations of interest. This study investigated the potential for disease spillover from the domestic dog population to the wild carnivore population in the Isoso of Bolivia, an area of tropical dry forest contiguous with a national park. Using questionnaires, data were gathered on the demographics of dogs, including adult and neonatal mortality, litter size, and hunting frequency. A large (6475 hunts) dataset containing self-recorded hunting information from 1996 to 2002 was analyzed to determine the extent of dog participation in hunting and duration, success, and frequency of hunting trips. Blood samples were taken from 98 Isoceno dogs for a serosurvey of canine pathogens of conservation concern, including canine distemper virus, canine parvovirus, canine herpesvirus, canine coronavirus, canine adenovirus, leptospirosis, toxoplasmosis, canine brucellosis, heartworm disease, and the sarcoptic mange mite.

Results from the demographic portion of the study indicate that the number of dogs present in the Isoso is remarkably high. The ratio of people to dogs is approximately 1.5:1, and each household has an average of 3.8 dogs. This is equivalent to more than 500 dogs in a village with a human population of 760. The average age of dogs is relatively low (3-4 yr), and the average litter size is 4.1 pups. These values, along with high neonatal (80%) and adult (38%) mortality rates, indicate that the population turnover among dogs is quite high, suggesting that the population is large enough to support diseases endemically by providing a constant source of susceptible hosts. Most (86%) dogs participate in hunting, and of these dogs, 82% hunt weekly or more often. The vast majority (97%) of hunts include dogs. The average hunt is 10 hr long and involves 2.8 dogs. Based on the average number of dogs participating in hunts, and the frequency and duration of hunts, the forest surrounding the Ioseño communities is subjected to an average of 30,000 dog-hr each week. Results of the serosurvey demonstrate a high seroprevalence of canine distemper virus (95%), parvovirus (95%), herpesvirus (68%), and sarcoptic mange (63%). These findings, as well as the high population turnover of dogs and

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frequent opportunities for contact between domestic and wild carnivores, indicate that domestic dogs represent a disease risk for wildlife in the Bolivian Isoso.

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## EXPERIMENTAL LEPTOSPIROSIS IN CAPYBARAS (*Hydrochaeris hydrochaeris*)

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### Abstract

The objective of the present trial was to characterize the periods of seroconversion, leptospiremia and leptospiruria in capybaras (*Hydrochaeris hydrochaeris*). In order to achieve this aim, six animals were infected intravenously, using *Leptospira interrogans* serovar pomona. The capybaras were anesthetized using intramuscular injections of ketamine (Vetaset, Fort Dodge; 1.5 mg/kg) and xylazine (Rompum, Bayer; 0.5 mg/kg). After the experimental infection, blood and urine collections were performed for culture of *Leptospira* sp., as well as serologic testing and polymerase chain reaction (PCR). The animal sera were tested by microscopic agglutination test using a collection of 24 serovars. The samples for culture were inoculated in semi-solid modified EMJH medium with 5 fluorouracil (300 mg/l) and nalidixic acid (20 mg/l) and after 24 hr, transferred to both Fletcher and modified semi-solid EMJH media without antibiotics. These media were incubated at 28°C and examined weekly for 8 wk. The capybaras were euthanatized after the experiment, and kidney and liver were collected for culture and PCR. Anti-*Leptospira* agglutinins started to be detected between day 2 and 10, and peak was reached between the 9th and the 27th day, coinciding with the 83rd day after infection (AI). Leptospiremia was detected until the period between days 12 and 14 AI. Leptospiruria was first detected between days 6 and 10 AI and was detected until the 43rd day AI. The culture of the tissues was negative. The control animal was negative to all diagnostics tests. Results of this study indicate that capybaras may have a role as *Leptospira* reservoirs and may contribute to the maintenance of this infection in rural and wild environments. This is the first description of experimental infection in capybaras with *Leptospira*.

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## WINTER MORTALITY OF BALD EAGLES ALONG THE LOWER WISCONSIN RIVER

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### Abstract

Unusual morbidity and mortality of bald eagles (*Haliaeetus leucocephalus*) wintering in two counties along the lower Wisconsin River, Wisconsin, began in 1994-1995 with the deaths of at least 14 eagles. Nine eagles were found dead, five were collected alive but died within 2 days, and two additional birds were found sick, then rehabilitated and released after 2.5 mo. Bald eagles at roosts from 10-65 km upriver and 10-150 km downriver from the affected region and elsewhere in the state were not found sick or dead. Beginning in 2000-2001, after a hiatus of 4 yr, during which eagle populations in the region were carefully monitored, similar bald eagle morbidity and mortality has recurred each winter. The area of concern has expanded to eight counties, with mortality events occurring primarily in January and February, and infrequent cases found from late November to early April. Of 85 bald eagles that have died in the target area within the appropriate time frame, 63 have been necropsied; some evaluations are still in progress. Sick eagles present in good body condition, with weakness, incoordination, tremors, vomiting and seizures. Snow or litter around dead eagles is often disturbed, consistent with observations of terminal seizures. Eagles brought into veterinary and rehabilitation facilities frequently have repetitive seizures, refractory to medication, over hours or days before death or euthanasia. No other avian or mammalian species have been involved. At gross necropsy, no consistent abnormalities have been found. By light microscopy, a minimum of 40 affected eagles had mild to severe multifocal to diffuse hepatocellular cytoplasmic vacuolation. Special stains revealed the presence of lipid in the vacuoles of a subset of affected livers. Vasculitis and microhemorrhages in the brain have been noted; it is unclear if the hemorrhages are a consequence of the seizures. This suite of lesions has not been seen in more than 4000 bald eagles from throughout the United States. The characteristic lesions of avian vacuolar myelinopathy, initially noted in 1994-1995 in Arkansas, have not been seen in Wisconsin bald eagles.

Extensive laboratory investigations on dead and sick birds have been inconclusive. Investigations have focused on agrochemical and veterinary drug use, contaminants associated with the Badger Munitions Plant, livestock mortality events, fish kills, and forage fish species available to eagles. Toxicologic tests have ruled out heavy metals, organophosphorus and carbamate pesticides, organochlorines, 4-aminopyridine, white phosphorus, strychnine,

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anticoagulants, and barbiturates as causative agents. Additional compounds, including sodium fluoroacetate and cyanide, have not been detected in a limited number of samples tested. Aerobic and anaerobic cultures, fungal cultures, viral cultures, assays for exposure to viruses and protozoal parasites, and tests for biotoxins have not found an etiology. Agricultural fields, where eagles feed on pig and duck carcasses, often with crows and turkey vultures, have been examined, farmers interviewed, and samples of pigs and ducks collected for evaluation. In 1995, a mortality event in the same area involving rock doves was investigated; the birds had severe, nonsuppurative encephalitis caused by pigeon paramyxovirus 1. There is no correlation with fish kills, and heavy metals and organochlorines in fish were below toxic concentrations. Forage fish have been tested for levels of thiaminase, with one species, gizzard shad, testing very high. The hypothesis that the syndrome is caused by a severe thiamine deficiency as a result of feeding largely on gizzard shad remains to be adequately tested. Evaluation of test results continues, and repeated multi-agency workshops allow for generation of new hypotheses.

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## EXPOSURE OF DESERT BIGHORN SHEEP TO SELECTED DISEASE AGENTS IN CENTRAL ARIZONA

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### Abstract

Twenty adult desert bighorn sheep (*Ovis canadensis mexicana*) were sampled from a population in the Mazatzal Mountains of central Arizona during multiple captures between June 2000 and October 2002. Serologic, nasal, pharyngeal, and cervical swab, fecal, and ectoparasite samples were examined for evidence of pathogen exposures during drought conditions prior to and following removal and exclusion of domestic livestock from the study area. Evidence of bacterial and viral activity persisted throughout the study, and was unremarkable in comparison to seroprevalence and antibody titers against disease agents reported for other desert bighorn populations. Results indicated lower than normal rainfall and removal of domestic livestock had no influence on exposure to leptospiral or viral diseases, and disease was not a factor limiting bighorn population growth and production. However, seroprevalence of antibodies against *Chlamydia* sp. increased during a year of exceptionally low rainfall, and incidence of pneumophilic bacteria in nasal swabs declined after livestock removal. We suggest our findings are consistent with enzootic stability and levels of immunity corresponding with absence of clinical disease in the Mazatzal Mountains desert bighorn sheep population. Continued monitoring of disease exposure and population trends in relation to variables such as presence or absence of domestic livestock, densities of bighorn and sympatric ungulates, rainfall levels, and translocations are key to understanding effects and etiology of desert bighorn sheep diseases.

### ACKNOWLEDGMENTS

Federal Aid in Wildlife Restoration Project W-78-R of Arizona Game and Fish Department and the Arizona Desert Bighorn Sheep Society provided financial support. We thank T. Smith, D. Conrad, L. Phoenix, B. Anthony, and J. Hanna for assistance in capture and sampling.

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**OVER THE FENCE AND THROUGH THE WEEDS: THE SPREAD OF *Brucella abortus* STRAIN 2308 FROM ELK (*Cervus elaphus*) TO ELK AND BISON IN A CAPTIVE FACILITY**

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**Abstract**

Brucellosis in Greater Yellowstone Area (GYA) bison and elk has been a source of controversy and wildlife management for many years. Research on brucellosis has been conducted in numerous facilities that house captive wildlife to generate data on the disease in elk, bison, and reindeer.

From 1999 to 2002, approximately 100 elk were held captive at the Idaho Department of Fish and Game Wildlife Health Laboratory in Caldwell, Idaho to evaluate the efficacy of single-dose, calfhood vaccination using *Brucella abortus* strain 19 (S19). These elk were challenged with  $1 \times 10^7$  CFU of pathogenic *Brucella abortus* strain 2308 (S2308) by bilateral intraconjunctival sac instillation on February 28, 2002. Abortions occurred between March and June 2002, and live births occurred in May and June 2002. All elk in the vaccine study were euthanatized by late June 2002.

All resident animals at the Wildlife Health Laboratory undergo annual health checks including serologic testing for brucellosis. None have been found to be seropositive to brucellosis until summer and fall of 2002. In July 2002, a 2-yr-old bull bison was found to be serologically positive to brucellosis at slaughter. On follow-up testing, two additional bison cows were found to be seropositive. In August 2002, three adult bull elk were found to be seropositive to brucellosis. One bull was culture positive for S2308 on a semen sample collected in December 2002 and again at slaughter in February 2003. In late summer and fall 2002, three adult elk cows were found to be seropositive to brucellosis. In addition, a group of six seronegative adult female elk were joined with a seropositive bull elk in fall of 2002. To date, three of these elk have seroconverted.

An extensive epidemiologic investigation was undertaken to try to determine the source and strain of the brucellosis that appears to have crossed at least two fencelines uphill from the elk challenged with S19. No common use area, random contamination or biosecurity break was

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identified that could explain the movement of the S2308 among these animals and through the various pens.

As of March 2004, all seropositive animals on site have been euthanatized and tissue samples submitted for culture. Summaries of serial serologic and culture results and epidemiologic data will be presented. The conclusions reached may have implications for future work with brucellosis in captive wildlife facilities.

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## SIBERIAN TIGER (*Panthera tigris altaica*) SPECIES SURVIVAL PLAN UPDATE

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### Abstract

Pathology Review: A review of Siberian tiger (*Panthera tigris altaica*) pathology records from 1915- 2000 has been completed. The mean age at death was 12.1 (+/- 5.9) yr, with females living longer than males (12.8 vs.11.3 yrs). Neoplasm was the most common diagnosis on necropsy, occurring in 25.5% of the reports. Degenerative musculoskeletal problems were noted in over 20% of the cats and renal disease was seen in over 25%. A complete review of the data is in preparation for presentation at the American Association of Zoo Veterinarians annual meeting in 2005.<sup>10</sup> Records are incomplete and any zoos that have not submitted information should contact Albert Lewandowski, DVM.

Vaccination: The veterinary advisor for the Tiger SSP recommends the vaccination of normal healthy tigers for Feline Rhinotracheitis, Calici Virus, Panleukopenia, Feline Leukemia, Rabies and Canine Distemper with killed vaccine. Only killed vaccines should be used in tigers, not modified live vaccines due to the potential risk of inducing disease. The following vaccination protocol is suggested for use in all Tiger SSP managed animals:

- 1 ml given intramuscularly of Purevax Ferret Distemper Vaccine, Merial, Inc., Athens, GA, USA. (Commercial killed vaccines for canine distemper are not available, this recombinant canarypox vectored vaccine has been used extensively in tigers with no observed problems.)
- 1 ml given intramuscularly of Fel-O-Vax LV-K, Fort Dodge Laboratories Inc., Fort Dodge, IA, USA.
- 1 ml given intramuscularly of Purevax Feline Rabies Vaccine, Merial, Inc., Athens, GA, USA, or Imrab 3 from Merial or other killed rabies vaccines.

Some institutions use 2 ml doses due to the greater body weight of tigers. Although there is no direct evidence that this is more effective, it also does not do any harm. Animals never before vaccinated should receive at least two and preferably three booster vaccinations approximately 3

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wk apart after 6 wk of age. Previously vaccinated animals should receive an annual booster. There are no vaccines, including those listed above, that are legally approved for use in non-domestic felids. This is particularly relevant with rabies vaccines where human exposure through bites may occur, especially in privately owned animals. We do not know how protective these vaccines actually are or statistically how effective they might be.

It is known that most species of large felids are susceptible to canine distemper virus and the virus should be regarded as a significant potential threat to zoo populations.<sup>1,2,7,9,11,13</sup>

Training programs: During the past 3 yr professionals from the Wildlife Conservation Society (WCS) Siberian Tiger Project, the Henry Doorly Zoo, and WCS zoo and field program professional staff as well as other groups have been active in building capacity in the Russian Far East (RFE) by training professional Russian tiger protection personnel who are required to respond to problem tiger situations. This is a group of trained Russian professionals that has the responsibility and capacity to respond with a variety of tools to problem tiger situations involving tiger conflict with humans. Through a series of workshops with their North American counterparts, Russian personnel responsible for dealing with problem tigers received intensive training in techniques dealing with this problem including wildlife health and disease management (including necropsy technique), immobilization and safe animal handling practices, and methods of animal – human conflict resolution. The two groups worked together sharing ideas and procedures to develop methods for coping with various aspects of the problem tiger issue. This training was made up of four workshops, carried out in Russia and the United States.<sup>12</sup> An advanced program is planned for the fall of 2004. This project has been supported by the Trust for Mutual Understanding.

### **Contraception (This section is taken from the 2002 tiger SSP report.)**

#### Tiger Contraception Recommendations

*Genevieve Dumonceaux (Busch Gardens, Tampa Bay) and Douglas Armstrong (Henry Doorly Zoo and Tiger SSP Veterinarian Advisor)*

While there are several methods of contraception in felids being investigated currently, none are currently useable. The recommended methods for contraception of large cats including tigers is as follows:

Physical separation: Typically the safest, most effective, reversible method with least risk of side effects. Available space, resources and facilities tend to be the limiting factors with this management method.

Male vasectomy: This involves the surgical removal of a section of each vas deferens to prevent transmission of semen to the female. Testicles are left intact. This method is very effective

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when performed properly. It does not inhibit testosterone-related behavior. It is not reversible and incurs an anesthesia and surgical risk.

Female tubal ligation: This is tying the fallopian tubes to prevent transmission of the sperm to oocytes at ovulation. The entire reproductive tract is left intact. This may or may not be reversible depending on technique, animal involved and the skill of the surgeon. This technique does not inhibit female estrogen-related behavior. This method incurs anesthetic risk and laparoscopic or surgical risk depending on the method used.

Neutering: This involves castration of the male or spaying the female for complete and permanent sterilization. This method is recommended primarily for animals of little or no future genetic value. It is 100% effective for contraception. There is anesthetic and surgical risk with this procedure.

Melengesterol acetate implants: Currently still the method of choice for temporary, reversible, minimally invasive contraception. This has proven to be successful as a primary means of contraception in felids and primates. This method is recommended for use for a maximum of 2 consecutive years at a time to minimize the risk of development of reproductive pathology. Repeated, continuous use of implants significantly increases the pathology risk. Pregnancy following or between implants may reduce the risk. This method does incur brief anesthetic and surgical risk.

Methods under investigation: Leuprolide acetate injections - An option for cat contraception but still very expensive at about \$600 per injection per Dr. Asa. Details on the use of this agent are still pending discussion with Dr. Briggs who has used it in various carnivores.

Deslorelin implants - As GnRH analogs this drug has potential for contraceptive action in the big cats. However, currently it is no longer available due to changes with companies manufacturing the product and FDA issues. Undetermined if it will be available in the future. Investigators including Dr. Cheri Asa of St. Louis Zoo are looking into other GnRH analogs but no available agents yet.

Zona Pellucida vaccine - Has been investigated in domestic cats and shows no immunocontraception in them per one study from University of Georgia. More information forthcoming.

## **Summary**

There are still no 100% effective, safe and reversible methods for contraception in large cats. Still the method of choice seems to be MGA implants as a temporary means of contraception but with significant side effects if used beyond 2 yr. Investigations continue in better, longer term safer means of contraception.

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Assisted Reproduction: In 1990 and 1991 single successes were achieved using fresh semen with each of the assisted reproduction techniques of *in-vitro* fertilization<sup>4</sup> and by laparoscopic artificial insemination<sup>5,6</sup> at the Henry Doorly Zoo in a cooperative project with the National Zoo and the Minnesota Zoo. The birth of Siberian tiger cubs following transvaginal artificial insemination with fresh semen was reported in 2000 by a team in Portugal.<sup>2</sup> There were no subsequent successes with any of these techniques in this species, in spite of multiple attempts with each until 2003 when a single cub was born at the Henry Doorly Zoo. The pregnancy had been produced by intrauterine insemination with fresh semen introduced to the uterus by laparoscopy. The cub was born after a 107-day gestation and had to be hand raised due to maternal neglect. It subsequently died at approximately 3 wk of age due to pneumonia.

At the present time, we believe that the primary problems preventing repeatable success with these techniques are attributable to the use in tigers of hormones derived from other species (human, equine, bovine) to manipulate the reproductive cycles. In essence the hormones used historically in these projects seemed to decline in effectiveness after initial use in any individual animal, possibly due to immunologic responses although this is not confirmed. In order to resolve this issue, we collected the pituitary gland from both an Amur tiger (*Panthera tigris altaica*) and from a snow leopard (*Panthera uncia*), extracted follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitaries and then determined the amino acid sequence for the FSH and LH for each of these species. These sequences were then compared to the known FSH and LH sequences for other species including canine, bovine, porcine, equine, ovine, and human. The protein structures for pig most closely matched the sequence for tiger hormones of all of those that were commercially available. Porcine FSH and LH were evaluated for efficacy in tigers during trials at the Henry Doorly Zoo. Although improved over hormone regimens utilized previously, the regimen of porcine hormones still proved less than ideal. The cats were inconsistent in the formation of corpora lutea and abnormalities in ultrastructure were found during electron microscopic evaluation of the oocytes.<sup>8</sup>

Concurrently during the above trial, a second project was undertaken to produce tiger hormones via transfection of cell cultures with the DNA sequence to produce tiger FSH and tiger LH. Plasmids containing the DNA sequences for the FSH and LH hormone subunits of tigers were produced and were used to transfect Chinese hamster ovary cell lines and cat kidney cell lines with these sequences. These cells were cultured, lines were selected for maximum hormone production by Western Blot analysis and these were propagated. Currently the effluent from these selected cell lines is in the process of being assessed for biologic activity in vitro in rat Leydig and rat granulosa cell bioassays. Large-scale production is being investigated by the National Cell Culture Center in Minnesota. Additional work remains, particularly determining the steps needed to produce purified hormone for injection. Initial hormone purification was done using a FLAG Tag system. It is not clear at this time whether additional purification steps will be needed or not. This project will have wide application to improve the success of assisted reproduction techniques in a variety of endangered cat species besides tiger. Initial support for this hormone production project came from the Morris Animal Foundation.

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## AN UPDATE ON JOHNE'S DISEASE

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### Abstract

Johne's disease or paratuberculosis is a transmissible, fatal disease of hoofstock. The primary route of transmission of the etiologic agent (*Mycobacterium avium* subsp *paratuberculosis*, *Map*) is thought to be the fecal-oral route.<sup>1</sup> Young animals are thought to be more susceptible to infection than adults.<sup>1</sup> Infected animals may appear clinically healthy for many years but are still capable of shedding bacteria and infecting other animals during this subclinical stage of the disease.<sup>2</sup> Clinically infected animals have non-specific signs that include weight loss and diarrhea.<sup>2</sup> Diagnosis of the disease is challenging due to the lack of antibody production until late in the infection, sporadic shedding of detectable numbers of bacteria, the difficulty of culturing the slow-growing organism, and minimal gross pathology in subclinical disease in some species.<sup>3</sup>

Not all animals exposed to *Map* develop clinical disease. It is not known whether this is because infection is never established or because the immune response controls or eliminates infection. Establishment of infection requires that large numbers of bacteria are taken up by macrophages and are able to evade the macrophages' bacteriocidal mechanisms. While the factors that influence the establishment of *Map* infection are not fully understood, it appears that dose, route of infection, strain variations, environmental factors, immune status and age of the host influence the development of disease.<sup>4</sup>

Historically, Johne's disease has been primarily a concern due to its economic impact. In the United States it is considered to be one of the most economically significant diseases in dairy cattle.<sup>5</sup> More recently there has been increasing concern about the disease as a possible zoonosis. *Mycobacterium avium* subsp *paratuberculosis* is hypothesized to be the cause of some cases of Crohn's disease, an inflammatory bowel disease of humans. There is currently insufficient evidence to determine whether there is a causal relationship between human *Map* infections and Crohn's disease.<sup>6</sup>

The *Map* host range includes both ruminant<sup>7</sup> and non-ruminant<sup>8</sup> wildlife species, and the spread of Johne's disease from domestic livestock to wildlife could significantly affect wildlife ecology. In addition, the establishment of Johne's disease in wildlife reservoirs could be detrimental to efforts to control or eradicate Johne's disease in domestic livestock.

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In 1998 a workshop was held on the diagnosis, prevention, and control of Johne's disease in non-domestic hoofstock.<sup>9</sup> One of the recommendations from this workshop was for zoos to establish a control or monitoring program according to risk category (low risk, high risk, or unknown Johne's disease status). It was recommended that Johne's management units (JMUs) be identified by each facility as part of the Johne's disease management plan. The workshop concluded that the prevalence of Johne's disease in a given JMU was the most valuable diagnostic information and that JMU surveillance should be the highest priority for all institutions with regard to management of Johne's disease.

In 2001 Manning and Ziccardi reported the results of a survey of all AZA accredited institutions with hoofstock species.<sup>10</sup> This survey found that one-third of the 133 respondents do not test for *Map* infection. Another third of the respondents test single animals only pre- or post-shipment. The authors conclude that there is insufficient surveillance for Johne's disease in up to 66% of the facilities participating in the survey. Human-animal contact areas were present in 80% of the responding institutions and domestic livestock were common in these exhibits.

The National Research Council recently published their report on the Diagnosis and Control of Johne's Disease.<sup>11</sup> This report was in response to increasing national concern about the apparent increase in prevalence of Johne's disease worldwide, a lack of national coordination of control programs, and public health concerns about the possible zoonotic risk of *Map*. The study concluded that Johne's disease represents a significant threat to animal health and warrants implementation of control programs. The study suggests that control programs for the dairy industry are the highest priority. The study also suggests that control programs for zoo animals and wildlife should be monitored to assure that a non-domestic animal reservoir does not compromise control efforts. The potential risk of Johne's disease to Crohn's disease increases the perceived importance of a Johne's disease control program.

In light of the increased concern about Johne's disease and its implications for animal health, the economy, and possibly public health, a re-examination of current surveillance and control programs for Johne's disease in zoological institutions is warranted.

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## THE IGUANA SPECIALIST GROUP VETERINARY UPDATE

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### Abstract

Iguanas are a unique group of animals inhabiting North, Central and South America, the Galapagos islands, the Antilles, and Fiji and Tonga. There are 3,000 species in 30 genera of seven families. The West Indian Iguana Specialist Group (WIISG) of the World Conservation Union (IUCN) was formed in the mid 1997s after a Population and Habitat Viability Analysis was held for the Jamaican iguana in 1993. The WIISG broadened its scope to become the Iguana Specialist Group (ISG) in 2000. An action plan was developed for the West Indian iguanas,<sup>1</sup> 10 of which are considered critically endangered, five endangered and the rest protected. Threats to their existence include loss of habitat primarily due to human activities such as tourism and introduction of exotic species including domestic cattle, sheep, pigs and goats, cats, dogs, rats and mongoose. The introduced species have altered the vegetation by overgrazing and caused reduced recruitment of new animals into populations though direct predation on eggs and juveniles. For some species, in addition to protection of habitat, it has been determined that recruitment should be augmented by head starting juveniles to a size that they can withstand predation is necessary. Head start programs are in place for four species. Veterinary involvement in the ISG and the *Cyclura* SSP has been largely to document the health status of free-ranging animals<sup>2</sup> in order to provide baseline information on populations, and to perform, document and refine procedures for pre-release health screening of head started animals.<sup>3</sup> Additionally, recommendations for health screening prior to translocations have been made. In a project funded by the Morris Animal Foundation, the following battery of tests were performed on free-ranging and captive prerelease animals: CBC, plasma biochemicals, minerals and vitamin D, fecal floatation and direct exam, bacterial culture of feces, and physical exam, morphometrics and ID. Data from five species of *Cyclura* has been directly compared and recommendations for pre-release health assessments were formulated. They include the recommendation that if 10 percent of the existing captive head start population is fully screened on a yearly basis, the individual animal screening need only include: Physical exam, weight, measurement of length, permanent ID, blood work to include total solids and hematocrit, white blood cell count, fecal exam (float and direct). Moving from an individual animal approach to a population based health program.

One of the strategies of the ISG is to develop action plans for species during annual meetings of the group in range countries. In addition, one of the challenges is to include and encourage participation of veterinarians in range countries as well.

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## ACKNOWLEDGMENTS

Veterinarians at more than 20 zoological institutions have been involved with and have contributed to the success of the ISG.

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Hudson Websites [www.iucn-isg.org](http://www.iucn-isg.org)

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## THE USE OF ANALGESICS IN SMALL MAMMALS

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### **Abstract**

All veterinarians engaged in the provision of veterinary care for small nondomestic mammals must possess a clinical knowledge of the behavioral manifestations of pain in each species. Equally important are a knowledge of the appropriate analgesic drugs for use in each species, and their response to each class of drugs so that an effective pain management plan can be developed for each patient.

The veterinary clinician engaged in a nondomestic species practice is further challenged by the lack of clinical studies regarding pain management in the species that he cares for. Practitioners providing care for such animals must rely on a diverse body of literature from both the human and veterinary fields to select the appropriate analgesic drug and management plan for the diverse species of animals that are under clinical care. The nondomestic practitioner must also be willing to be open to the inclusion of newer drug therapies in the development of pain management plans for his veterinary patients.

### **Assessment of Pain**

The assessment of pain in animals is primarily on the basis of behavioral response to painful stimuli.<sup>1</sup> The evaluation of pain in a small mammal is made all the more difficult by the fact that behavioral changes in small mammals maybe more subdued than those of domestic species.

Acute responses to pain are generally similar in all species (i.e., escape or avoidance of the source of the pain). Individual animals may vocalize and become aggressive, especially if they are restrained. Following a persistent painful situation, such as accidental injury or surgery, most small mammal reduces their level of spontaneous activity and try to hide. They will usually remain immobile until handled, and then they may try to escape.

In addition to behavioral changes the animals' external appearance may be altered. Small rodents may show a hunched posture, piloerection and soiling of the coat due to lack of grooming. Red colored porphyry secretions from the harderian gland may appear around the eyes and nose. If housed in groups, rabbits or rodents with pain may isolate themselves from their cage mates. Rabbits with acute pain may grind their teeth. As with other species, painful injuries to the limbs, spine or abdominal musculature may result in abnormal positioning of the body parts or a "tucked up" abdomen.

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A consistent sign of acute or chronic pain is reduced appetite (i.e., reduced food and water intake), and hence a loss in body weight. Daily body weight determinations one or more times daily will help the clinician assess the animals response to level of analgesia provided.

### **Analgesic Drug Selection**

The selection of a particular analgesic for a pain management plan is based on its regulated status, ease of administration, required frequency of administration and species sensitivity to the available drugs. Although the elevation of pain is the goal of the clinician, each class of analgesics has the potential to exert adverse effects. The clinician must look for adverse responses and institute an alternative pain management plan in the event the analgesic must be withdrawn.

A listing of some commonly used analgesics by species and categories is listed below in the tabular form (Table 1).<sup>2-5</sup> Where specific information is lacking, the clinician can usually extrapolate the clinical response from similar species of animals to the current species under consideration for analgesic therapy.

### **Summary**

The relief of pain in animals must be approached on the basis of one animal at a time. Since the responses of any one animal can be highly variable, all pain management programs should be based on the individual's response to therapy. Therefore, it is appropriate to reduce the dosage, increase the dosage, or shorten the period of treatment based on the response of the patient.

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**Table 1.** Analgesic drugs dosages for (mg/kg) small animals.<sup>a</sup>

Analgesic	Mouse, Gerbil Hamster	Chinchilla, Guinea Rat	Pig, Prairie dog	Rabbit	Ferret
<b>NSAIDS:</b>					
Acetaminophen	110-305 mg/p.o., i.p. (mouse)	110-305 mg/p.o., i.p.			
Aspirin	20 mg/s.c. (mouse)	20 mg/s.c. 100-120 mg/p.o.		100 mg/p.o.	
Carprofen	100-120 mg/p.o. 5 s.c. every 12 hr	5 i.m., s.c., p.o. every 24 hr	4 i.m., s.c. every 24 hr	4 i.m., s.c. every 24 hr	4 i.m., s.c. every 24 hr 1.5 p.o. every 12 hr
Flunixin	2.5 s.c. every 12 hr	2.5 i.m., s.c. every 12 hr	2.5 i.m., s.c. every 12-24 hr		
Ketoprofen		5 i.m., s.c., p.o. every 24 hr		3 i.m., s.c. every 24 hr	3 i.m., s.c. every 24 hr
Meloxicam	1-2 i.m., s.c., p.o. every 24 hr	1-2 s.c., p.o. every 12 hr		0.2 i.m., s.c., 0.3 p.o. every 24 hr	0.2 i.m., s.c., 0.3 p.o. every 24 hr
<b>Opioids</b>					
Buprenorphine	0.1, s.c. every 6-12 hr	0.05 i.m., s.c. every 8-12 hr	0.5 i.m., s.c. every 6-12 hr	0.01-0.05 i.m., s.c., i.v. every 6-12 hr	0.01-0.03 i.m., s.c., i.v. every 6-12 hr
Butorphanol	1.5 s.c. every 2-4 hr	2 i.m., s.c. every 2-4 hr	2 i.m., s.c. every 4 hr	0.1-0.5 i.m., s.c. every 4 hr	0.4 i.m., s.c. every 4 hr
Meperidine	10-20 s.c. every 2-3 hr	10-20 i.m., s.c. every 2-3 hr	10-20 i.m., s.c. every 2-4 hr	10 i.m., s.c. every 2-3 hr	5-10 i.m., s.c. every 2-4 hr
Morphine	2-5 s.c. every 4 hr	2-5 i.m., s.c. every 4 hr	2-5 i.m., s.c. every 4 hr	2-5 i.m., s.c. every 4 hr	0.5 i.m., s.c. every 4-6 hr
Oxymorphone	0.2-0.5 s.c. every 6-12 hr	0.2-0.5 i.m., s.c. every 6-12 hr	0.2-0.5 i.m., s.c. every 6-12 hr	0.05-0.2 i.m., s.c. every 8-12 hr	0.05-0.2 i.m., s.c. every 8-12 hr

<sup>a</sup>Adapted from the Heard.<sup>2</sup>

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## PAIN AND ANALGESIA IN FISH: UNANSWERED QUESTIONS

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### Abstract

### Introduction

Pain and analgesia has been documented in fishes. Reviewed research illustrates the similarities of fish nociception to that of mammals. A three-phase study evaluating novel methods for assessing analgesia in rainbow trout (*Onchorhynchus mykiss*) and determining pharmacokinetics of two common analgesic drugs is discussed.

### Nociception in Fish

Fishes possess mammalian-like anatomic, biochemical and functional components for the transmission, mediation, and central processing/modulation of painful stimuli.<sup>2</sup> Teleost fishes possess peptides, similar to corticotropin-releasing-factor in mammals, that regulate the hypothalamic-pituitary-adrenal axis, activate opioid receptors, and are key integrators of the neuroendocrine and immune systems.<sup>21,22</sup> Proteins analagous to mammalian GABA-benzodiazepine receptors have been documented.<sup>14</sup> Substance P-like immunoreactivity, likely contributing to nociception, is present and distributed similarly in fishes as in mammals.<sup>3,31</sup> Additionally, many studies in fishes have confirmed the presence of  $\mu$ ,  $\delta$ , and  $\kappa$  opioid receptors, and endogenous opioid activity that modulates response to stressors.<sup>6,24,26,30</sup> Social stressors in teleosts produce a marked opioid-mediated immunosuppression, lessened by administration of opioid antagonists.<sup>13,24</sup>

Prostaglandin-mediated inflammatory mechanisms in fishes also appear to be equivalent to those in mammals. Teleosts possess cyclooxygenase (COX) enzymes identical to those of mammals,<sup>16,18,27</sup> which are inhibited by COX-2-inhibiting non-steroidal anti-inflammatory drugs (NSAIDs). Prostaglandins exert a protective effect on the gastric mucosa and are decreased by COX-2 inhibitors in fishes.<sup>12</sup>

Goldfish show an escape response to electric shock, which is lessened by the administration of morphine and increased by naloxone.<sup>11,17</sup> Cod and steelhead react to electric stimulation, but were found to react less after intranasal administration of opioids and NSAIDs.<sup>5</sup>

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## Nociception or Pain?

Although fishes have been shown to avoid known sources of pain, suggesting a “pain memory” and thus a coordinated response,<sup>4,23</sup> there is disagreement over whether central processing or “awareness” of pain occurs, which is necessary for the experience of fear or suffering.<sup>7,25</sup> This debate centers on the presumed lack of a “consciousness” in fishes, due to an absence of areas in the cerebral cortex responsible for these functions in humans.<sup>29</sup> In contrast, several recent studies in the Netherlands have addressed the welfare aspects of fish slaughter methods.<sup>20,28</sup> The authors believe that, regardless of the presence of fish consciousness and despite this semantic controversy, humane medical practice dictates the incorporation of appropriate analgesics into medical treatments and clinical procedures for fishes. Despite documentation of analgesia produced by chemical agents<sup>5,11</sup> there is a paucity of information about effects, pharmacokinetics, and dosages for commonly available analgesic agents.

## New Methods to Evaluate Analgesia in Fish

Most studies assessing pain and analgesia in fishes have relied on behavioral avoidance responses to noxious stimuli, primarily electric shock.<sup>5,11,17</sup> In other species, the ability of an analgesic drug to reduce the minimum alveolar/anesthetic concentration (MAC) of an inhalant anesthetic agent has been used as an objective measurement of the drug’s analgesic properties.<sup>8,9,19</sup> To the authors’ knowledge, no study has attempted a similar study design using aquatic anesthetic agents with non-air breathing animals.

We have completed the first phase of a study, which determined a “minimum gill concentration” (MGC) of MS 222 in rainbow trout (*Onchorhynchus mykiss*) to determine the effect of varying dosages of butorphanol and ketoprofen on this MGC. During this MGC study, fish were placed in one or more of five tanks filled with MS 222 in varying concentrations. Once immobilization was achieved, a 22-gauge hypodermic needle was inserted into the caudal peduncle near the caudal fin as a noxious stimulus. If there was an escape response associated with the application of the stimulus, the fish was transferred to an increased concentration of MS 222 and the process repeated. This titration process continued until the concentration of MS 222 required to prevent a response to the stimulus was determined (MGC). One half of the fish were then given butorphanol, and the remainder given ketoprofen, both by i.m. injection in the caudal epaxial musculature.

Measurable relevant plasma biochemical changes in response to pain or other stressors in teleosts include an increase in cortisol, glucose, and lactate.<sup>1,10,15</sup> Sodium, potassium, and chloride concentrations have been variously reported to increase or decrease with stress.<sup>10,15</sup> The degree of changes in these parameters in response to stress is cumulative and depends upon the severity and duration of the stressors.<sup>1</sup> The second phase of our study will evaluate whether antinociception in rainbow trout is reflected by reduced alterations in these parameters. If so, fish that receive an effective analgesic before a noxious stimulus should exhibit significant differences in these biochemical parameters compared with a control group, implying a reduction

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in stress. Analgesic medication being the sole variable, this reduction in stress would be defined as analgesia.

### **Effective Analgesic Dosages in Fish**

Since opioid receptors and mediators and prostaglandin-mediated inflammatory processes have been found to be similar in fishes and mammals, we expect that butorphanol and ketoprofen will have analgesic effects in fish. No pharmacokinetic studies for either drug have been reported in non-mammalian species. The third phase of our study will determine the pharmacokinetics of both butorphanol and ketoprofen in rainbow trout.

### **Conclusions**

Despite a common assumption that the capacity to perceive pain is related to an animal's position in the phylogenetic hierarchy, research has shown that pain perception in fishes and other non-mammalian vertebrates is likely to be analagous to that of mammals. It is imperative that we develop appropriate protocols for the inclusion of analgesics in piscine treatments.

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**COMPARATIVE ANESTHETIC AND CARDIOPULMONARY EFFECTS OF PRE- VS. POST-OPERATIVE BUTORPHANOL IN SEVOFLURANE ANESTHETIZED HISPANIOLAN AMAZON PARROTS (*Amazona ventralis*)**

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**Abstract**

The anesthetic and cardiopulmonary effects of pre- vs. post-operative intramuscular butorphanol were determined and compared in eleven, adult sevoflurane-anesthetized Hispaniolan Amazon parrots (*Amazona ventralis*) undergoing coelomic endoscopy for gonadal evaluation. In two trials, 21 days apart, birds were randomly assigned to receive either pre-operative (n = 11) butorphanol tartrate (Torbugesic®, Fort Dodge Animal Health, Fort Dodge, IA 50501, USA) (2 mg/kg, i.m.) 20 min prior to induction of anesthesia with sevoflurane (SevoFlo™, Abbott Laboratories, North Chicago, IL 60064, USA) or immediately post-sevoflurane (n = 11) anesthesia. Baseline heart and respiratory rates were recorded in all birds prior to each study. Following induction of anesthesia with sevoflurane in 100% oxygen via face mask, each bird was intubated and anesthesia was maintained with sevoflurane for 40 min. Heart rate (HR), respiratory rate (RR), relative arterial oxygen saturation (SpO<sub>2</sub>), and end-tidal CO<sub>2</sub> concentration (EtCO<sub>2</sub>) were measured every minute for the first 5 min and every 5 min thereafter. No differences were seen in time to induction of anesthesia between birds administered butorphanol (40 ± 8 sec) and those induced with sevoflurane alone (38 ± 8 sec). In pre-operative butorphanol treated birds, heart rates were significantly higher at 25 and 30 min (399 ± 37 beats/min) when compared to baseline values and to birds anesthetized with sevoflurane alone (334 ± 53 beats/min). Amazons anesthetized with sevoflurane alone had significantly lower heart rates at 15, 20, 25 and 40 min of anesthesia when compared to baseline values. Respiratory rates were significantly lower in both groups throughout the anesthetic event when compared to baseline values. Birds receiving butorphanol prior to anesthesia had significantly lower respiratory rates at 5 and 40 min (18 ± 6 breath/min) of anesthesia compared to Amazons under sevoflurane (26 ± 3 breath/min) anesthesia alone. SpO<sub>2</sub> values were >90% throughout the anesthetic event in both groups, and no differences were noted over time or between groups. EtCO<sub>2</sub> values were significantly higher in butorphanol-sevoflurane anesthetized birds at 2 and 3 min compared to sevoflurane-anesthetized birds. For the remainder of the anesthetic event, EtCO<sub>2</sub> values did not change significantly over time, and no significant changes were present between groups. In both groups, EtCO<sub>2</sub> values were within clinically acceptable limits for 35 min of anesthesia, however at 40 min mild hypercapnia (> 45 mm Hg) was noted in all birds. Recoveries in both groups were

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rapid and smooth, and no differences in recovery time were seen between birds receiving pre-operative butorphanol ( $3.8 \pm 1.4$  min) and birds receiving post-operative butorphanol ( $4.6 \pm 1.5$  min). Administration of pre-operative butorphanol (2 mg/kg, i.m.) as part of a preemptive analgesic regimen appears to be safe and effective and will not cause significant changes in anesthetic and cardiopulmonary parameters in sevoflurane anesthetized Hispaniolan Amazons parrots.

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## EFFICACY OF LONG-ACTING LIPOSOME-ENCAPSULATED BUTORPHANOL IN *Amazona ventralis*

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### Abstract

Pain management is an essential part of both post-surgical recovery and treatment of chronic disease for animals. Pain control improves recovery rates and survival times of humans and laboratory animals.<sup>1,2</sup> Birds, especially parrots, are common companion animals and appropriate analgesia is a critical part of veterinary care for these species. Several recent advances in the study of avian analgesia validate the clinical use of analgesic drugs for the psittacine patient.<sup>3-7</sup> However, there are still significant barriers to providing adequate pain relief for birds, including frequent dosing schedules and the associated stress of frequent injections. Opioids are the most effective class of analgesic drugs for post-operative pain. Reliable techniques have been developed to evaluate the efficacy of analgesic drugs in birds including isoflurane-sparing techniques and antinociceptive testing methods.<sup>4-8,11</sup> Opioid drugs with different receptor affinities have been evaluated in psittacines and pigeons, including butorphanol (mixed agonist/antagonist with kappa agonist activity), buprenorphine (mixed agonist/antagonist with mu agonist activity), and fentanyl (mu agonist).<sup>4-8</sup> Based on these studies, the order of analgesic effectiveness in psittacines is 2 mg/kg butorphanol (most effective), 0.1 mg/kg buprenorphine and 0.02 mg/kg fentanyl (least effective).<sup>4-8</sup> The results of pharmacokinetic studies using these opioids in psittacines indicate that 2 mg/kg i.m. butorphanol, 0.02 mg/kg i.m. fentanyl and 0.1 mg/kg i.m. buprenorphine have very short mean residence times (MRT) of 1.13 (+/- 0.46), 1.98(+/- 0.22) and 1.05 (+/- 0.14) hr respectively.<sup>8-10</sup> Therefore, butorphanol is an effective analgesic for birds, but its clinical usefulness is limited by a very short half-life.

Encapsulation into liposomes is one method of preparing long-acting formulations of opioid drugs.<sup>12-15</sup> LE-morphine administered to mice produces significant plasma concentrations for 6 days after a single s.c. injection, and both LE-morphine and LE-oxymorphone administered to rats provided effective analgesia for 7 days following a single s.c. injection.<sup>12,14</sup> However, these products will not be optimal for use in avian patients because morphine is a poor analgesic for birds. Encapsulating butorphanol into liposomes would be an efficacious preparation for use in avian and laboratory species.

This study was undertaken to evaluate the analgesic efficacy and pharmacodynamics of a clinically relevant liposome encapsulated butorphanol tartrate formulation (LEBT) in the

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Hispaniolan parrot (*Amazona ventralis*). An ELISA method was used to detect butorphanol and its metabolites in parrot serum. Following a single i.m. dose of standard butorphanol tartate (2 mg/kg i.m.; n = 4), serum concentrations of butorphanol peaked at approximately 1 hr and were rapidly cleared. In contrast, following a single dose of LEBT(15 mg/kg ; n = 10) or 10 mg/kg n = 4), serum concentrations of butorphanol were elevated for 5 and 8 days respectively. No detrimental side effects were observed in any bird subject.

Analgesimetry data were collected using both thermal and electrical noxious stimuli delivered to the plantar surface of the foot. Latency to foot withdrawal was measured using a modified perch design. Compared to controls receiving liposomes without butorphanol (LE), administration of a single dose of LEBT (15 mg/kg s.c.) maintained an increased threshold to the noxious stimulus for 3 days based on thermal thresholds and 6 days based on electrical thresholds. There were no significant changes in foot withdrawal latencies after administration of LE alone. These data suggest that liposome-encapsulated butorphanol administered at 15 mg/kg s.c. is analgesic for 3-5 days after a single dose.

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## PHARMACOKINETICS OF ORAL CARPROFEN IN THE CALIFORNIA SEA LION (*Zalophus californianus*)

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### Abstract

Racemic carprofen (Rimadyl™ Pfizer) is a veterinary non-steroidal anti-inflammatory drug (NSAID) of the propionic acid class that includes ibuprofen, naproxen, ketoprofen, and vedaprofen.<sup>9</sup> It is licensed in the United States for use in dogs to help control inflammation and pain associated with surgery and osteoarthritis.<sup>5,12</sup> Forms of the drug have been used in other domestic species, including cattle,<sup>7</sup> horses,<sup>6</sup> cats,<sup>11</sup> and rats.<sup>10</sup> Ketoprofen has been used to good effect in Asian elephants (*Elephas maximus*),<sup>4</sup> llamas,<sup>8</sup> and camels.<sup>1</sup> There is a growing precedent for the off-label use of carprofen in California sea lions (*Zalophus californianus*; W. Van Bonn, personal communication),<sup>2</sup> and sea otters (*Enhydra lutris*).<sup>3</sup> However, little is known about the use of carprofen in California sea lions and no controlled study that describes the pharmacokinetics or clinical effectiveness of analgesics and/or NSAIDs in pinnipeds has been performed to date. Understanding the pharmacokinetics of a drug can improve the likelihood of establishing dose parameters that will allow the practitioner to alleviate pain and reduce inflammation, while minimizing untoward side effects. Improved pain control is necessary as we continue to develop and improve the comprehensive medical and surgical care of sea lions in rehabilitation and display facilities.

California sea lions (n = 10) with traumatic injuries, osteoarthritis, pneumonia, or keratitis resulting in blepharospasm that were eating on their own, appeared to be of normal hydration status, and that during rehabilitation would have received analgesic/anti-inflammatory therapy for their disease, were entered into the study. Study animals received an admit examination including a comprehensive physical exam, complete blood count (CBC) and serum chemistry analysis, and a pre-drug heparinized plasma sample. Those animals were then started on a treatment course of carprofen at the recommended canine dose of 3-4 mg/kg orally once daily for 5 days. Study animals were randomly assigned to two of ten possible time points (0.25 and 0.5, 1, 1.5, 2, 3, 4, 5, 8, 12 hr) for post-drug heparinized plasma collection on the first day of carprofen treatment. These time points were then summarized to reconstruct a drug elimination curve. Heparinized plasma, a CBC and serum chemistries were also collected on day five of treatment and again five days later. Plasma concentrations of carprofen were determined by high-performance liquid chromatography. Hematology and serum chemistries were assessed for significant changes during and after treatment. Daily clinical assessments (SOAPs) were made to document any improvement (or lack thereof) in the animals' condition. Specific attention

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was paid to lameness exams, mobility and activity, appetite, and interaction with pen mates, to subjectively determine analgesic efficacy.

The maximum plasma concentration, elimination half-life, and systemic availability of carprofen were determined. No adverse changes that could be correlated with carprofen administration were found on hematology or serum chemistries. There were no documented clinical deleterious side effects associated with drug administration. All study animals continued to eat and interact with pen mates. Animals with trauma or osteoarthritis-associated lameness showed improved mobility and there was documented reduction in blepharospasm in those study animals with corneal disease.

These data suggest that racemic carprofen is an acceptable drug to use for the alleviation of pain and inflammation associated with trauma, osteoarthritis, and corneal disease in California sea lions. The authors caution that the use of this drug in sea lions is considered “off-label”, and while there were no deleterious side effects seen in this study, some have been reported in other species.<sup>11</sup>

#### ACKNOWLEDGMENTS

The authors wish to thank the staff and volunteers of The Marine Mammal Center for their skill and support in the care of stranded marine mammals, and the veterinary pharmacology departments of UC Davis and NCSU for their help and advice.

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## THE USE OF MELOXICAM IN EXOTIC FELIDS AT THE CALGARY ZOO

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### Abstract

Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) of the oxicam family. It has potent anti-inflammatory, analgesic and antipyretic properties. It acts by inhibition of the enzyme cyclooxygenase (COX) which in turn inhibits the biosynthesis of prostaglandins and other autocooids. These substances are effective biologic mediators that are involved in many physiologic functions as well as pathologic conditions.<sup>1</sup> Meloxicam has 3 fold greater activity against the inducible inflammatory response form of cyclooxygenase (COX-2) than the constitutive form (COX-1). Inhibition of COX-1 is most commonly associated with deleterious gastric and renal effects.<sup>3,6</sup> In Canada, meloxicam is licensed for use in dogs at an initial dosage of 0.2 mg/kg once daily, followed by maintenance dosage at 0.1 mg/kg, and in humans at a total dose of 7.5 or 15 mg once daily.<sup>1,2</sup> The veterinary formulation is available as an injectable solution (5 mg/ml) or an oral solution (1.5 mg/ml) (Metacam, Boehringer Ingelheim, Burlington, Ontario, Canada, L7L 5H4), while the human formulation is oral tablets of 7.5 or 15 mg strength (Mobicox, Boehringer Ingelheim, Burlington, Ontario, Canada, L7L 5H4). To decrease volume of administration we have meloxicam compounded to a 5 mg/ml suspension by a compounding pharmacy in beef, banana and strawberry flavoured bases.

During the last few years at the Calgary Zoo meloxicam has been used extensively as the NSAID of choice in over 140 species ranging from amphibian and reptile species to bird and mammal species (Table 1). Clinical applications include management of surgical pain, treatment of suspected myalgia, and as supportive treatment in cases of injury, osteoarthritis, fever, illness, or cases of "ADR" (which we have coined "suspect meloxicam deficiency"). Meloxicam has been used for acute management such as perioperative pain control (1-7 days) as well as in the management of chronic pain over periods of weeks to months. One group of particular interest has been the exotic felids at the zoo- African Lion (*Panthera leo*), Bengal Tiger (*Panthera tigris tigris*), Canadian Lynx (*Lynx canadensis*), Cougar (*Puma concolor*), Siberian Tiger (*Panthera tigris altaica*), and Snow Leopard (*Uncia uncia*). For acute pain management, we have found an initial dosage of 0.1-0.2 mg/kg either orally or subcutaneously, followed by oral dosage of 0.1 mg/kg every 24 hr for up to 5 days to be effective and well tolerated in these species. For cases of chronic pain, such as neoplasia or osteoarthritis, a dosage of 0.1 mg/kg orally three times weekly up to every 48 hr has been effective and well tolerated.

There have been two cases where meloxicam has been used over periods of weeks to months in large cats with no observed clinical side effects. In the first case, a 14-yr-old, 190-kg male Siberian tiger was treated at a dosage of 0.1 mg/kg administered orally three times weekly for

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several weeks. This was in response to a diagnosed calcific tendonitis causing lameness in his left foreleg. Within 2 mo, meloxicam was administered again at the same dosage every other day in response to a lameness associated with an aggressive chondrosarcoma of the right distal radius and carpus. While meloxicam treatment did improve the mobility and activity level of this tiger, more palliative treatment was required as the tumour progressed, and a better level of analgesia was obtained when codeine monohydrate-sulfate trihydrate (200 mg p.o. every 24 hr; Codeine Contin, Purdue Pharma, Pickering, Ontario, Canada, L1W 3W8) was added to his therapy. The tiger was observed to sleep more often, however was much more fluid when moving on or off his sleeping platform or when ambulating while under this treatment. Other than the observed mild grogginess, there were no adverse reactions to the codeine such as excitement or constipation. When the decision to euthanize was reached, there were no gross lesions associated with meloxicam administration in the gastrointestinal tract or the kidneys. Histopathology revealed lymphoid hyperplasia of the stomach mucosa with no evidence of ulcerative change. Age related renal changes were observed, involving proteinaceous and fibrous accumulations in a few glomeruli, however no tubular pathology which may have been associated with the use of an NSAID was noted.

The second case involves a mature, approximately 8-yr-old, 170-kg white Bengal tiger which arrived at the Calgary Zoo in late October, 2003. This animal presented with a moderate lameness of the right hindleg with associated pronounced muscle atrophy of the pelvic region; there was no history of any treatment or diagnostic information with regards to this lameness from the previous institution. On radiographs during his quarantine exam, there was moderate to marked osteodegenerative changes to the right femoral head and acetabular rim, with mixed osteophytic-osteolytic arthritic changes to the neck of the right femur. The left femoral head and acetabular rim had much milder changes with mild calcification of the tendon insertion points of pelvic-appendicular ligaments. Serum chemistry values were within normal limits. Initial management consisted of injections of a semi-synthetic polysaccharide (3.3 mg/kg s.c. every 7 days; Cartophen Vet, Arthroparm, Ottawa, Ontario, Canada, K1G 3Y6) and meloxicam (0.1 mg/kg p.o. three times weekly). Meloxicam therapy has continued for several months, and while some clinical improvement in the degree of lameness was noted, a pronounced limp was still apparent. Four months after the initiation of meloxicam, the tiger was anesthetized for a bone biopsy to rule out an infectious or neoplastic cause for the degenerative changes in his right femur. At this time, osteoproliferation of the right femoral neck was still apparent, with focal areas of radiolucency, however there was a significant improvement seen when compared with the quarantine radiographs from November, 2003. Osteophytic production of both hip joints was decreased, there was increased bone density of the femoral neck, and a cleaner edge to the acetabular rim. There also was decreased calcification of tendon insertion points on the left side. Serum chemistry values were within normal limits. Our conclusion was that by controlling the pain and inflammation associated with the osteoarthritis, there was a corresponding increase in activity and weight bearing on the hindlimbs, leading to bony remodeling and improved health of the coxofemoral joints in the intervening months.

Side effects are not common in canine species treated with meloxicam, with doses up to 5x the recommended dosage administered for 26 wk not causing any clinical abnormalities. Suspected

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or potential adverse effects in dogs include gastrointestinal changes such as vomiting, diarrhea, inappetance, hematemesis, melena and ulceration, mild CNS or behavioural changes, elevated creatinine and blood urea nitrogen, dermatologic changes, elevations of liver enzymes, or immune-mediated anemia and thrombocytopenia.<sup>2,4</sup> In domestic cats, meloxicam has proved quite effective and safe for short term use in cats following surgery, for radiation induced stomatitis, or in treating painful locomotor disorders.<sup>5,7-9</sup> However, tolerance studies in cats resulted in the death of 2 of 12 cats after 8 days of treatment in the highest dose range (0.6 mg/kg SID) from duodenal ulceration and associated peritonitis, and after 9 days, all cats in the treatment groups had evidence of gastrointestinal ulceration. At lower doses, meloxicam was tolerated by cats, but the conclusion was drawn that the therapeutic index for meloxicam in cats is very narrow.<sup>4</sup> Our experience with exotic felids is that short term or chronic use has not been associated with adverse side effects, however we still recommend close monitoring of biologic and haematologic parameters in all patients on meloxicam therapy.

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**Table 1.** Taxonomic groups in which meloxicam has been used at the Calgary Zoo.

<u><b>Amphibians</b></u>	<u><b>Birds</b></u>	<u><b>Mammals</b></u>
Anura	Anseriformes	Bovidae
Caudata	Charadriiformes	Bradyrodidae
	Ciconiiformes	Callitrichidae
<u><b>Reptiles</b></u>	Columbiformes	Cavidae
Agamidae	Coraciiformes	Canidae
Boidae	Cuculiformes	Cebidae
Chamaeleonidae	Galliformes	Cercopithecidae
Colubridae	Gruiformes	Cervidae
Gekkonidae	Falconiformes	Chiroptera
Testudinidae	Passeriformes	Dasyproctidae
Varanidae	Piciformes	Equidae
	Phoenicopteriformes	Erethizontidae
	Psittaciformes	Erinaceidae
	Struthioniformes	Felidae
	Strigiformes	Hominidae
		Hylobatidae
		Hystricidae
		Lemuridae
		Loridae
		Macropodidae
		Mustelidae
		Pongidae
		Procyonidae
		Sciuridae
		Suidae
		Ursidae
		Viverridae

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**LONG TERM MEDICAL MANAGEMENT AND MONITORING OF TYPE 2 (NON-INSULIN DEPENDENT) DIABETES MELLITUS IN A SUMATRAN ORANG UTAN (*Pongo pygmaeus abelii*)**

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**Abstract**

A 21-yr-old male orang utan was diagnosed as having Type II (non-insulin dependent) diabetes mellitus in 1996. In the ensuing 8 yr, his case has been managed by a combination of hypoglycaemic medication, daily insulin injections and dietary manipulation. This paper will outline the approach to monitoring and treatment, the introduction of daily insulin injections and blood glucose testing, the use of oral hypoglycaemic medications, and the decision process for making dose changes.

**Diagnosis of Type II Diabetes Mellitus and Initial Dietary Manipulation**

“Hsing Hsing,” a male Sumatran orang utan, has been in the Perth Zoo collection since 1983, when he arrived at the age of 9 yr. Prior to 1996, there had been no clinical or physical evidence of diabetes mellitus. The disease was first suspected when hyperglycemia (18 mmol/L), and marked (4+) glycosuria were detected from samples taken under anaesthesia during routine physical examination. Subsequent regular testing of voided urine via dipstick (Multistix urinalysis reagent strips, Bayer Australia Ltd., Pymble, NSW) revealed that the glycosuria was persistent.

In 1996, Hsing was 21 yr old, and of good general health. Prior to arrival at Perth Zoo, he had been on a highly inappropriate and unregulated diet, which included regular access to confectionery and sugary drinks. Although he did not appear overweight when he arrived, his parents, being on a similar diet, were certainly obese. It was later discovered that Hsing’s father was also diagnosed as having diabetes, although specific details of his clinical progress are not available.

Hsing’s diet in 1996 consisted of a mixture of fruits and vegetables, eggs, bread and primate pellets, with regular access to browse. Foods used for enrichment included nuts, sultanas, peanut butter and jam. In response to the suspicion of diabetes, Hsing’s diet was altered immediately to decrease its total caloric and sugar content. This was achieved by increasing browse and vegetables, restricting fruit (particularly tropical varieties), and eliminating behavioural enrichment foodstuffs except for diet jams.

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After 2 wk on the revised diet, no changes were seen in Hsing's voided urine glucose levels. Further information on Hsing's clinical condition was required in order to identify more specific treatment options. The following tests were performed on blood collected under anaesthesia in November 1996.

*Fasting C-peptide levels:* within normal human limits (0.44 nmol/L, reference range 0.20-0.90). This suggested that Hsing was producing insulin precursors at the normal rate.

Assays for antibodies to pancreatic  $\beta$ -islet cells and glutamic acid decarboxylase (GAD): negative. A negative result indicates an absence of pancreatic autoimmunity, which in turn suggests normal pancreatic function.

Attempts to separate a glycosylated hemoglobin fraction (HbA<sub>1c</sub>), which becomes elevated in the presence of ongoing (>4 wk) hyperglycemia, were unsuccessful. The findings were considered by diabetologists to be consistent with a slowly-evolving, type 2 (non-insulin dependent) diabetes mellitus.

### **Initial Treatment With Oral Hypoglycemics**

Hsing was not showing any overt clinical signs of diabetes at the time of deciding to start him on oral hypoglycaemic medication. His appetite was the only evidence of a potential subclinical condition, being only fair to moderate, which was reflected by his average to lean body condition, and slightly low body weight of 81 kg; however, he was not showing evidence of polydipsia or polyuria

Given the absence of clinical manifestations of diabetes, diabetologists felt that there was scope to manage Hsing's condition without insulin therapy. Treatment was begun with metformin (Diaformin 500 mg tablets, Alphapharm Pty. Ltd., Glebe, NSW) in early 1997. Metformin acts to enhance the effect of insulin. It promotes increased glucose uptake in the presence of insulin, but does not elicit hypoglycemia.<sup>1</sup> It was considered that metformin would constitute a means of safely initiating a decrease in blood glucose levels without the risk of hypoglycemia. Hsing readily accepted tablets crushed up and mixed with diet cordial.

In selecting a medication regime, there was concern about the limited alternatives for monitoring Hsing's response to medication. At this point, he was not trained to tolerate conscious blood sampling, so the only clinical pathology parameter available for quantifying his condition was dipstick urinalysis of voided urine.

Prior to treatment, Hsing's urine dipsticks showed a consistent, marked glycosuria (3-4+), occasional traces of protein, and occasional ketones. The medication aim was to reduce the glycosuria to 2+, and eliminate the episodes of ketonuria. The limitations of monitoring via

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dipstick urinalysis were recognised, and keepers began working on conditioning Hsing for injections and blood sampling, using the traditional principles of operant conditioning.

The metformin dose was gradually increased from 125 mg s.i.d. to 500 mg s.i.d. over 3 wk, in order to observe for any untoward side effects of the medication, such as gastrointestinal upsets. After 1 mo on metformin, Hsing was anaesthetised for reassessment. While his general health continued to be good, his body weight had declined to 76.5 kg. Hematology and serum biochemistry were normal, with the exception of continued hyperglycemia (11 mmol/L) and elevated total bilirubin (28.0  $\mu$ mol/L). The hyperglycemia was less marked than previously noted. However, urine dipstick glucose was unchanged at 3-4+, although there had been no evidence of ketones reappearing in the urine.

A second oral hypoglycemic agent was added to Hsing's medication regime in May 1997. Gliclazide (Diamicon 80 mg tablets, Servier Laboratories Aust. Pty. Ltd., Hawthorn, Vic.), a sulfonylurea hypoglycemic agent, stimulates insulin secretion from functional pancreatic  $\beta$ -cells, and also increases their sensitivity to glucose stimulus.<sup>1</sup> Sulfonylurea agents also have extrapancreatic effects on lowering blood glucose. The initial dose of gliclazide was 20mg sid, given together with 500 mg s.i.d. of metformin. Over the ensuing month, this dose was increased to 40 mg s.i.d. due to continuing 3-4+ glycosuria.

By mid-1997, Hsing was on 60 mg of gliclazide and 500 mg metformin daily. Although his body weight had increased to 81.5 kg, his blood glucose was still very high (15.7 mmol) at anaesthesia in early 1998. Keepers were now making progress with conditioning Hsing to present his fingertip for blood sampling, and his shoulder for injections, using recognized operant conditioning techniques.<sup>2,3</sup> Hsing's gradual weight gain, and the continued absence of ketonuria, indicated some improvement in his condition.

Although the hyperglycemia persisted, the decision was made to avoid insulin therapy at this point. Hsing still had no overt polyuria or polydypsia, and his daily oral medication doses were still much lower than the daily maximum doses recommended for humans (1000 mg t.i.d. of metformin and 160 mg b.i.d. of gliclazide). Furthermore, Hsing's cooperation with presenting his shoulder for injections was still unreliable, so insulin could not be considered immediately. Veterinarians elected to manage his condition by gradually increasing his oral medication dosages, provided that no side effects were seen.

### Clinical Manifestations of Diabetes Emerge

By July 1999, keepers had Hsing trained to tolerate finger pricking for blood glucose monitoring (Esprit Glucometer, Bayer Australia Ltd, Pymble NSW). Hsing's blood glucose levels were usually between 20-30 mmol/L, and never below 10 mmol/L. Hsing's HbA<sub>1c</sub>, measured from finger prick blood samples, increased from 12.5% in August 1999 to 13.6% in November 1999. Although normal HbA<sub>1c</sub> levels have not been determined in orang utans, these levels are

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considered overtly diabetic according to the analyzer specifications for humans (DCA 2000 Hemoglobin A<sub>1c</sub> Reagent Kit and Analyzer, Bayer Corporation).

At this time, Hsing began to exhibit symptoms of sweating, restlessness and erratic behaviour. These symptoms were interpreted as clinical manifestations of persistent, chronic hyperglycemia, and reflected his elevated blood glucose readings and increasing HbA<sub>1c</sub>. Hsing's gliclazide dose was increased to the daily maximum of 160 mg bid. As these symptoms persisted, gradual increases in the doses of metformin were also instigated, reaching 1000 mg b.i.d. in late 1999. In spite of these relatively high medication doses, Hsing's blood glucose readings persisted at 15-30 mmol/L.

In the light of the emergence of clinical signs, and the poor response to high doses of oral hypoglycemic medication, it was decided that treatment with insulin was now unavoidable. Hsing was anaesthetised to review his general health, and to examine him for side effects of chronic hyperglycemia.

Hsing's fasting insulin levels were 6 mU/L (normal human range 3-26 mU/L), indicating a normal level of production of insulin. C-peptide levels continued normal, and there was once again no evidence of anti-islet cell or GAD antibodies. Examination by a veterinary ophthalmologist did not detect any evidence of diabetes-related retinopathy. Serum microalbumen assay did not demonstrate any evidence of renal damage associated with chronic diabetes. His body weight at this time was 86kg.

#### Initiation of Insulin Therapy

Insulin medication commenced in March 2000. Due to the difficulties in coordinating insulin treatment with Hsing's daily routine, an intermediate acting human insulin, with a duration of effect of 24 hr, was selected (Protaphane, isophane human insulin injection, Novo Nordisk Pharmaceuticals Pty. Ltd. Australia, North Rocks, NSW). The onset of effect of the isophane insulin was 1.5 hr, with a maximum effect at 4-12 hr.<sup>1</sup> The insulin was administered to Hsing immediately before his evening meal.

Unfortunately, Hsing became refractory to blood sampling at this time, forcing a reliance on dipstick urinalysis and behavioural cues to try and detect a response to treatment. The starting dose of 4 U s.i.d. was increased in 4 U increments on a weekly basis. At the onset of insulin therapy, the gliclazide dose was halved to 160 mg s.i.d. rather than 160 mg b.i.d., since the combination of a high gliclazide dose with insulin therapy increased the risk of hypoglycemia.<sup>1</sup>

By May 2000, the insulin dose was 20 U s.i.d. At this time, urine glucose levels registered 2-3+ consistently rather than 3-4+ as had been seen on previous dose regimes. Gliclazide and metformin doses continued at 160 mg s.i.d. and 1000 mg b.i.d. respectively.

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The clinical response to the insulin treatment was significant. Hsing became less irritable, and his activity and alertness increased. Polyuria and polydipsia had resolved completely by May 2000, and there was a significant improvement in his appetite. By June his weight was 90 kg, and by early September had reached his target weight of 95 kg.

Incremental increases of 4 U in the insulin doses continued until a dose of 24 U was reached in May 2000. By mid-June 2000, keepers had regained compliance for blood glucose testing. Shortly after this, an excessively low evening blood glucose level of 2.7 mmol/L was registered. It was thought that the combination of the morning glipizide dose with the insulin therapy was resulting in the potential for evening hypoglycemia, so the daily gliclazide dose was halved to 80mg.

Now that a therapeutic dose of insulin had been reached, the aim of treatment was to maintain blood glucose levels at 5-10 mmol/L. Hsing's blood glucose levels tended to be lower in the evening (6-7 mmol/L) than in the morning (13-14 mmol/L). Since the evening glucose levels were still too high, an insulin dose increase was warranted, but this carried some risk of eliciting morning hypoglycemia. Therefore, from June 2000, the gliclazide dose was gradually reduced, and the insulin dose increased, until August 2000, when gliclazide therapy was withdrawn completely. The insulin dose had been increased to 34 U by this time. Since gliclazide can cause side effects of gastrointestinal disturbance and biochemical abnormalities (including elevations in CK, ALP, AST, BUN and bilirubin),<sup>1</sup> this was considered to be an improvement in Hsing's longterm medication plan.

A review of the metformin dose was conducted in November 2000. Because this drug increases the effectiveness of insulin,<sup>1</sup> an extra 500mg midday dose was initiated. This brought the total daily dose of metformin to 2500 mg. It was hoped that this would reduce the requirement for further increases in the insulin dose. This did seem to have some effect, and the insulin dose was not increased again for another month. The insulin dose rose by a further four units through 2001 to 38 U per day.

#### Introduction of a New Oral Hypoglycemic Agent

In late 2001, another oral hypoglycemic agent was added to the medication regime. Glipizide (Minidiab 5 mg tablets, Pharmacia & UpJohn Pty. Ltd., Rydalmere, NSW) acts on  $\beta$  islet cells to stimulate production of endogenous insulin.<sup>1</sup> This was likely to elicit a response in Hsing's case, since insulin assays and anti-islet cell antibody assays indicated that he had a functional  $\beta$ -islet cell population. Glipizide was initiated at 2.5 mg s.i.d. in the morning, increased to the therapeutic dose of 5 mg s.i.d. when no side effects were seen after 14 days. The use of glipizide seems to have been successful in stimulating endogenous insulin production, in that Hsing's insulin dose has increased by only 6 U in the 2.5 yr subsequent to the introduction of glipizide.

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## Discussion

Predisposing factors in the development of type II diabetes include age, obesity and genetics.<sup>5</sup> While Hsing was never obese, he certainly had a very high sugar diet in his early years, which may have prompted the expression of his genetic predisposition for type II diabetes. In the early stages of development of type II diabetes, peripheral insulin resistance induces hyperinsulinemia, which eventually gives way to hypoinsulinemia as the pancreas fails to maintain adequate insulin secretion.<sup>5</sup> The development and manifestation of Hsing's disease was consistent with that of a "borderline" type II diabetic: hyperglycemia, weight loss, polyuria and polydypsia developed gradually over 3 yr. C-peptide and insulin assays did not detect a hyperinsulinemic phase, but this may have occurred prior to diagnosis.

Although it was not possible to monitor blood glucose in the early stages of oral medication, it appears that Hsing's diabetes did not improve dramatically until insulin therapy was initiated. As well as the decline in his blood glucose levels, his improvement was marked by significant increases in alertness, activity, appetite and body weight.

At the time of writing, Hsing's medication regime consisted of isophane insulin at 44 U/day (given in the evening), metformin at 2500 U/day (divided into 8 a.m. 1000mg; 12 noon 500 mg; 4 p.m. 1000 mg), and glipizide 5 mg each morning. His insulin levels have not increased since January 2003, and oral hypoglycemic doses have not changed since 2001. His blood glucose is checked by keepers every third day, in the morning and evening, and the veterinary department perform a regular review of his average blood glucose levels, originally on a monthly basis but now generally every 2-3 mo. Current blood glucose levels range from 6-13 mmol/L, with evening levels generally slightly lower than morning levels. As has been the case with the longterm management of diabetes in other nonhuman primates,<sup>4</sup> there is some variability in Hsing's response to insulin, and occasional hyperglycemic spikes are seen. Often, the cause remains unidentified, although there is some correlation with stressful episodes such as hot weather.

A feature of Hsing's longterm management has been the establishment of contingency plans for managing his diabetes in the event of changes in his routine. Veterinarians have worked with diabetologists, dietitians and diabetes nurses to formulate contingency plans for keepers and veterinary staff. Ongoing communication with these experts continues to be an important feature of the management of Hsing's case. Contingency planning is now in place for the following events:

*Hypoglycemic episodes:* In the initial stages of treatment, when blood glucose monitoring was not possible, it was feared that glipizide medication might trigger a hypoglycemic episode. A protocol was generated, outlining the signs of hypoglycemia, first aid actions for keepers, and veterinary emergency procedures.

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*Non-compliance:* If Hsing refuses to eat in the morning, glipizide medication is withheld. Because metformin does not elicit hypoglycemia, this is given regardless of his appetite. If he refuses to comply with insulin injection, keepers are instructed to persist for as long as possible. Missing the occasional dose of insulin is unlikely to be life-threatening in the type II diabetic, but fortunately Hsing's compliance has been 100% since May 2000. If Hsing refuses his evening meal subsequent to receiving his insulin injection, he is left with access to food overnight. All episodes of non-compliance are managed on a case-by-case basis in consultation with Perth Zoo veterinarians.

*Elective GA management:* In the case of elective anaesthesia, metformin treatment is stopped 3 days before surgery to prevent lactic acidosis; glipizide and insulin are continued as per normal; procedures are scheduled for early morning; consideration is given to an intra-operative dose of insulin if indicated by high blood glucose readings. Metformin is reinstated 2 days post-GA.

#### **ACKNOWLEDGMENTS**

The authors would like to sincerely thank all the diabetes specialists who have donated their time and resources to Hsing's case, particularly Mrs Beryl Marsh, Dr David Hurley and the other staff of the Diabetes Clinic at Royal Perth Hospital; Mr Stuart Moody (Bayer Australia); and the staff of the primate section and veterinary department at Perth Zoo.

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## MEDICAL MANAGEMENT OF A GERIATRIC BULL ELEPHANT (*Elaphus maximus*) WITH MULTIPLE PROBLEMS, A CASE REPORT

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### Abstract

A 50-yr-old male Asian elephant (*Elaphus maximus*) “Murugan” was suffering from chronic joint disease and recurring bouts of colic. Specifically the right elbow and both carpal joints were affected, which was confirmed by infra-red thermography. The animal was kept on low doses of phenylbutazone orally. Colic was treated with metamizol + scopolamine injections and dietary measures.

Early 2002 his condition deteriorated and he started to develop severe ventral and preputial edema. No diagnoses for the edema could be made and the condition was treated with furosemide and cortico-steroids. Urine samples and blood analysis revealed diabetes mellitus. When Murugan developed melena, showed great discomfort and refused medication he was humanly euthanatized.

On post mortem severe cartilage degeneration of multiple joints, stomach ulceration, fat necrosis, and pancreatic changes were found. To the authors knowledge this is the first time that diabetes mellitus was diagnosed in an elephant.

### Case Report

A 50-yr-old male Asian elephant (*Elaphus maximus*) “Murugan” weighing approx. 5000 kg, was suffering from (suspected) chronic joint disease and reoccurring bouts of colic. In particular the right elbow and both carpal joints were affected. Murugan showed different degrees of lameness over the years and occasionally snapping sounds were heard from the joints. He sometimes seemed to have difficulty in bearing weight on his right front leg and “snapped through” his carpal joint. The elephant needed regular hoof and nail trimming via “Target Training” (under the guidance of Mr. Alan Roocroft, Elephant Business, Ramona, CA). As part of the treatment he was made to stand in a plastic tub with lukewarm water and soap to help to soften the toe abscesses. Particular digits III and IV of the right front foot needed

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repetitive treatment due to necrotic laminitis with resulted in entire nails being removed. This treatment has been described by numerous authors.<sup>1,3</sup>

When Murugan showed discomfort and pain he orally medicated with phenylbutazone powder (Equipalazone Powder<sup>®</sup>, Arnolds Veterinary Products Ltd, Shrewsbury, Shropshire, SY1 3TB UK). Starting dose 4 mg/kg BW twice daily (20 sachets b.i.d.) for 2 days, 2 mg/kg twice daily (10 sachets b.i.d.) for 2 days and thereafter 1 mg/kg (five sachets) s.i.d. After 1 wk the dose was reduced to 1 mg/kg on alternate days as long as needed. The drugs were administered in bread mixed with beet or cane sugar syrup. The horse dose was used and extrapolated for the animal's weight.<sup>7</sup>

In July 2002 Murugan was examined using an "infra-red thermograph" camera (Dr. Sabine Hilsberg, Frankfurt Zoo, Germany). The thermographs confirmed the suspicion of joint disease by showing intensive "hot spots" in the carpal and right elbow joint.<sup>5</sup>

Apart from regular recurring lameness, Murugan had bouts of vague signs of colic. These signs were thought to be caused by constipation of the colon and treated successfully with laxatives, liquid paraffin (Eurovet BV, Bladel, the Netherlands), dietary measures and a combination of metamizol and scopolamine injections (Buscopan Compositum<sup>®</sup>, Boehringer Ingelheim BV, Alkmaar, the Netherlands; containing 500 mg/ml metamizol-sodium and 4 mg/ml butyl-scopolamine). The recommended dose was used and extrapolated to the elephants' weight thus receiving 250 ml in total (125 g metamizol and 2 g scopolamine)

Late Summer of 2002 - Spring 2003

Murugan appeared to have increasing joint pain. The oral dose of phenylbutazone was raised again (with increasing risk of stomach mucosa ulceration, as seen in the horse<sup>2</sup>). But other oral NSAIDS like vedaprofen (Quadrisol, 100 mg/ml oral paste, Intervet, Boxmeer, the Netherlands) was refused by the animal.

X-rays were taken from his toes (with the help of Dr. Willem Schaftenaar of Blijdorp Zoo, Rotterdam) following the technique as described by Gace<sup>4</sup> did not reveal involvement of periost or articular surfaces of phalange. Carpal joint dimensions prohibited X-ray assessment. Murugan developed severe ventral and preputial edema, which responded well to oral hydrochlorothiazide and dexamethason treatment (Diurizone<sup>®</sup> 20 gram powder, Vétoquinol S.A., 70204 Lure, Cedex, France, 25 mg dexamethason and 7.5 g hydrochlorothiazide per 100 g powder). Starting dose 400 g on D1, followed by 200 g on D2-4 and slowly cut back to 50 g every second day. However, as soon as treatment was stopped the edema returned. The cause of the ventral and preputial edema could not be established by physical and/or laboratory analysis.

Therapy was than changed to 30 g oral furosemide s.i.d (Sigma - Aldrich Chemie BV, Zwijndrecht, the Netherlands) and 140 mg dexamethason injection (Voreen<sup>®</sup>, Boehringer Ingelheim BV, Alkmaar, the Netherlands), followed by 2.5 g s.i.d oral encapsulated prednisolon

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(Alfasan Nederland BV, Woerden, the Netherlands, 150 mg per capsule). After 4 days the dose was halved and later administered on alternate days.

Blood work revealed the following abnormalities (values were compared with the ISIS data<sup>6</sup> for Indian elephants) (Table 1). As from March 2003 urine analysis showed high and rising glucose levels and some protein. Repeated blood analysis shown in Table 2. As a result the dexamethason was stopped. However glucosuria remained unchanged (>100 mg/ml).

### Spring 2003

Physical symptoms of debilitation and discomfort got worse. Murugan suffered from pain despite the NSAID treatment (butazolidine) and difficult to train because of “introvert” behavior. He refused his medication more and more.

By the end of May his stool turned black (melena) and were sometimes covered with frank blood. Stomach or duodenal ulceration was feared. In addition snapping sounds in the joints increased and the elephant had more and more difficulty to stand on his right front leg.

Due to the severity of the signs and the fatal prognosis Murugan was humanly euthanatized on the 4th of June 2003, using 6 ml LA-Immobilon<sup>®</sup> (2.45 mg etorphine and 10 mg/ml acepromazine, Veticore, Marlow, Bucks, SL7 1FJ, UK) and 750 ml of pentobarbital (Euthasate<sup>®</sup>, 200 mg/ml, Sanofi Santé BV, Maassluis, the Netherlands).

Since Murugan – a wild caught bull – had no offspring, an effort was made to obtain semen by electro-ejaculation carried out by Drs. Hildebrandt and Göritz, of the IZW in Berlin, Germany. However no life sperm could be collected.

### Post Mortem Findings

Good bodily condition. No molars found in the mandible. Severe fat necrosis in entire abdomen, associated with pancreatitis. Severe stomach ulcer and ptechia in mucosa associated with blood loss. Swollen liver and spleen (from euthanasia solution?), enlarged right ventricle of the heart with aortic stenosis and “jet lesions” in aortic wall. Endocardiosis of left AV-valve. The pancreas felt too firm.. The testicles were small and atrophied.

The articular surface of the right carpal joint was almost entirely gone with a 2 × 3 × 3 cm free floating cartilage body. Arthritis in left carpal joint, right elbow and between cervical vertebra C<sub>1</sub> and C<sub>2</sub> was also diagnosed.

### Histology

Generalized chronic arthritis with severe cartilage lesions in multiple joints. Generalized fat necrosis. Pancreas: very irregular, sometimes very small islets of Langerhans, some

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vacuolization of the endocrine cells of islets and of the epithelium of the smaller ducts. A layer of connective tissue surrounds some of the islets.

Irregular glomeruli with interstitial inflammatory reaction in both kidneys. Testicles: brown pigment in interstitial macrophages and sperm production in seminiferi tubuli.. Liver: iron pigment in Kupffer cells and some in hepatocytes. Multifocal infiltration of round nuclear cells in vessel walls periportally with some amyloid disposition. Multiple ulcers and mucosal erosions in stomach. Focal necrosis of some heart muscle fibres. Aorta; increased number of vessels and plamacellular infiltrates in intima:

Blood analysis carried out on samples taken just before the euthanasia (Table 3).

### **Conclusion**

The fact that only very small remnants of the cheek teeth were found makes difficult food uptake likely.

Murugan suffered from severe arthritis of multiple joints as seen commonly in old elephants.<sup>1,3</sup> Mainly the left and right carpal joints (front feet) were affected as well as arthritis of two cervical inter-vertebral joints. His colossal head and tusks may have contributed to this phenomenon. Because of the continuous analgesic therapy stomach erosion and ulceration occurred resulting in abdominal pain, inappatence, and melena. As seen more often<sup>8</sup> in elephants the ventral edema could not be explained.

Pancreatitis (very painful on its own in humans) could have contributed to the bouts of colic, leading to fat necrosis and diabetes mellitus type I (pancreatic insufficiency). This explains the polyuria-polydipsia as was seen in the animal. diabetes mellitus has (to the best of the authors' knowledge) never been described before in elephants.

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**Table 1.** Results of blood analysis from November 2002.

Date	Parameter	Value	Interpretation
29.Nov. 02	TP	76 g/L	
	Alb.	22 g/L	↓
	Glob	54 g/L	↑
	A/G	0,4	}inflammation
	LDH	1056	↑
	Potassium	6.3 mmol/L	↑; erythrolysis (mild)

**Table 2.** Results of blood analysis from March 2003.

Date	Parameter	Value	Interpretation
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20.Mar 03	TP	72 g/L	
	Alb.	23 g/L	↓
	Glob.	49 g/L	↑
	A/G	< 0,5	inflammation
	Al Phos	134 U/L	↑
	GGT	30 U/L	↑
	GOT	70 U/L	↑ mild liver damage
	Potassium	7.6 mmol/L	↑; erythrolysis
	Glucose	10.89 mmol/L	↑↑, Diabetes Mellitus, Cushing, or as result of therapy (dexamethason)
	Insulin	180 pmol/L (=25.0 μU/L)	low-normal, but far too low for rising glucose level; non-responsive pancreas
	Fructosamine	212 μmol/L	normal

**Table 3.** Results of blood abalysis from June 2003 just prior to euthanasia.

Date	Parameter	Value	Interpretation
04.June 03	Hb	6,5 mmol/L↓	because of hemolysis due to storage conditions a few parameters were changed and more difficult to interpret
	Ery's	2,5 × 10 <sup>12</sup> /L↓	
	Creatinine	169 μmol/L↑	↑↑↑↑ severe pancreatic damage / pancreatitis
	Pancreas-specific Amylase	16.563 IU/L	
	LDH	5187 U/L	↑↑↑ partly due hemolysis, partly due to pancreatitis
	Peptide C	↓↓	precursor for insulin; too low for glucose level >> non-responsive pancreas

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## TREATMENT OF ANESTRUS DUE TO HYPERPROLACTINEMIA WITH CABERGOLINE IN A CAPTIVE ASIAN ELEPHANT (*Elephas maximus*)

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### Abstract

### Introduction

Hyperprolactinemia (serum prolactin concentrations  $\geq 15$  ng/ml) has been identified in 13 of 31 African (*Loxodonta africana*) and one of six Asian elephants (*Elephas maximus*) by the Conservation and Research Center (CRC) Endocrine Research Laboratory, Smithsonian Institution, National Zoological Park, Front Royal, VA. Most of these elephants are flat-liners with low serum progesterone ( $<0.1$  ng/ml) but some are cycling normally. Treatment regimens for anestrus due to hyperprolactinemia has been reported in humans and several others species with a dopamine agonist such as bromocriptine or more recently cabergoline.<sup>3,9</sup> The most common cause of hyperprolactinemia is a slow-growing, benign, prolactin-secreting pituitary adenoma,<sup>5,10</sup> although other pathologies can elevate serum prolactin concentrations. An anestrus Asian elephant with hyperprolactinemia was successfully treated and began normal cycling after 6 mo of cabergoline therapy.

### Case Report

A 28-yr-old Asian elephant at Busch Gardens Tampa Bay (BGT) was diagnosed with hyperprolactinemia by CRC in October 1997. At this laboratory, serum prolactin concentrations in hyperprolactinemic females averaged  $34.0 \pm 18.8$  ng/ml (range 15.4-71.1 ng/ml) as compared to concentrations for non-cycling flatliner elephants with 'normal' prolactin levels ( $6.7 \pm 4.1$  ng/ml; range 1.8-11.9 ng/ml). The BGT elephant was unusual in that elevated serum progesterone concentrations (0.5-1.0 ng/ml) were present in contrast to the traditional flat-liners identified by CRC. Repeated rectal ultrasound exams confirmed that neither pregnancy nor mummified fetus was present.

In May 2000, the elephant was diagnosed with *Mycobacterium tuberculosis* from a vaginal discharge. A standard course of treatment was instituted per the USDA Elephant Tuberculosis Guidelines and concluded in January 2002. Serum prolactin concentrations had started to decline, but were still elevated, and serum progesterone remained elevated; the elephant was not cycling. In March 2002, a course of cabergoline (Dostinex®, Pfizer Inc., Eastern Point Road,

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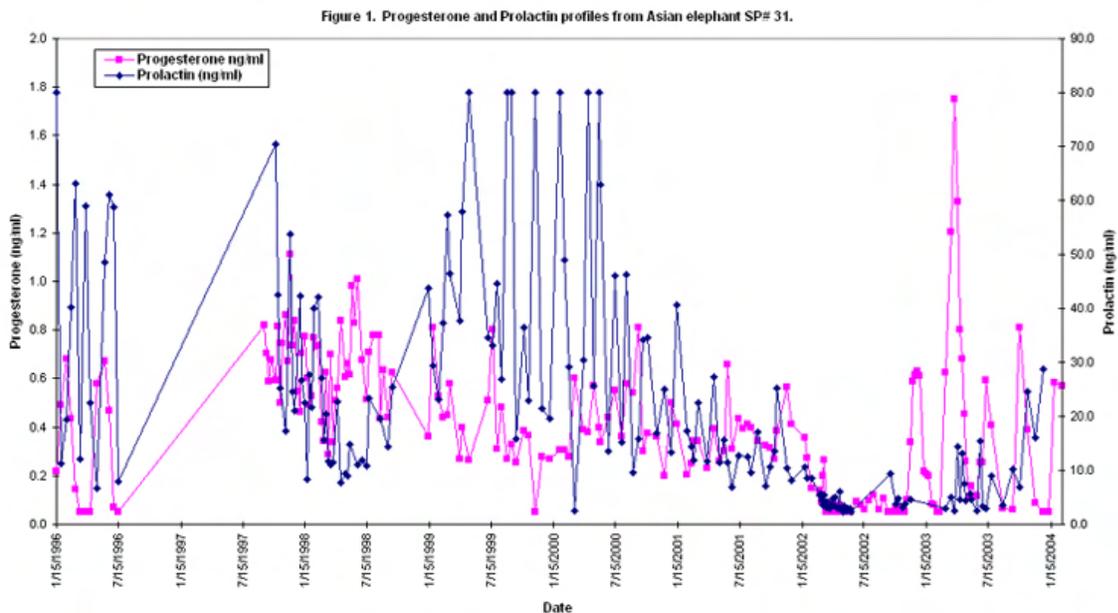
Groton, CT 06430, USA), 1mg orally twice weekly for 6 mo, was instituted following human guidelines.<sup>9</sup> Serum prolactin concentrations declined almost immediately after the first treatment and were followed about 1 mo later by return to baseline of progestins. Progestin secretion remained low until November 2002 when normal cycling resumed with observation of a normal luteal phase. From November 2002 through January 2004, the elephant has exhibited four normal estrous cycles. Prolactin concentrations have remained within the normal range for elephants, over 1 yr after treatment withdrawal. In March 2003, she again had a vaginal discharge that was culture positive for *Mycobacterium tuberculosis*. This culture was a multi-drug resistant strain that had an identical molecular fingerprint to the original strain in May 2000. A revised treatment protocol was instituted and continues as of this writing. From November 2003 through mid-February 2004, prolactin concentrations were tending to rise to pre-cabergoline therapy concentrations (Fig. 1)

Cabergoline has been used to treat anestrus in humans<sup>8</sup> and dogs<sup>3</sup> and to control prolactin secretion in various mammals.<sup>4</sup> Cabergoline is a long-acting dopamine receptor agonist with a high affinity for D2 receptors. It exerts a direct inhibitory effect on the secretion of prolactin. Anti-tuberculocidal drugs, especially rifampin, are known to increase serum prolactin in humans (D. Ashkin, pers.comm.), but this is not consistent with the timing of the treatment in this case. Transient increases in prolactin can be caused by sleep, anorexia, protein ingestion, hypoglycemia, stress, and chest wall stimulation or trauma.<sup>4,10</sup> The cause of the hyperprolactinemia in this elephant is suspected to be due to a pituitary tumor. Relapse of hyperprolactinemia is more common in humans with micro- or macroprolactinomas.<sup>2</sup> Other evidence for a pituitary tumor in this elephant includes the elevated progesterone concentrations observed. This is documented in humans with prolactinomas.<sup>7</sup> Prolactin is recognized as a cytokine and, with persistent immune activation, it may become elevated.<sup>6</sup> One other African elephant with hyperprolactinemia was diagnosed with *Mycobacteriosis bovis* post mortem. Further examination of a possible association between tuberculosis in elephants and hyperprolactinemia is certainly warranted.

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**RADIOGRAPHIC EVALUATION OF CARDIAC SIZE IN FLYING FOX SPECIES  
(*Pteropus rodricensus*, *P. hypomelanus*, AND *P. vampyrus*)**

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**Abstract**

Dilated cardiomyopathy is relatively common in captive flying foxes. Quantitative measurements were determined that could be used to diagnose cardiac disease and describe the appearance of normal and abnormal thoracic radiographs in these animals. Lateral and ventrodorsal thoracic radiographs from 66 apparently healthy flying foxes of three species (Rodriguez island flying fox, *Pteropus rodricensis*, n = 18; small island flying fox, *P. hypomelanus*, n = 16; Malaysian flying fox, *P. vampyrus*, n = 32) were evaluated. Absolute and relative cardiac dimensions were measured and the radiographs subjectively evaluated to describe the cardiac appearance in relation to other thoracic structures. The same methods were applied to radiographs from nine flying foxes with confirmed cardiomyopathy. The following ratios were most efficient in categorizing cardiac silhouette size: in the ventrodorsal projection, heart width to thoracic width and heart width to clavicle length, and in the lateral projection, heart width compared to thoracic height. On the ventrodorsal projections, heart width was on average 55% the width of the thorax and 95% the length of the clavicle. On a lateral projection, heart width was 75% thoracic height and heart length was 110% thoracic height. When radiographs from the bats with known dilated and acute cardiomyopathy were compared to those of apparently healthy animals, the apicobasilar heart length compared to thoracic height and heart width compared to thoracic height on lateral films were the most sensitive ratios for diagnosing cardiomegaly.

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## OUTBREAK OF TULAREMIA IN BORNEAN ORANGUTANS (*Pongo pygmaeus borneo*)

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### Abstract

In the summer of 2003, numerous rabbits (*Sylvilagus floridanus*) were found dead on grounds of the Topeka Zoo, including the outdoor orangutan exhibit. Several rabbit carcasses were tested for various potential diseases, including tularemia (*Francisella tularensis*) and West Nile virus, but results were negative for pathogens.

In August, one female and one male Bornean orangutan (*Pongo pygmaeus borneo*), became lethargic and anorexic. Both animals were chemically restrained with medetomidine (Domitor, Pfizer Animal Health, Exton, PA 19341, USA; 0.05 mg/kg i.m.), and ketamine (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA 50501, USA; 3 mg/kg i.m.), reversed with atipamezole (Antisedan, Pfizer Animal Health, Exton, PA19341, USA; 0.25 mg/kg i.m. for physical examination and to obtain baseline clinical pathology. Abnormal laboratory findings included a leukocytosis (36 and  $16.8 \times 10^3$ /dl, respectively) characterized by neutrophilia and monocytosis, and a mild elevation of albumin (2.8 and 3.4 g/L) and LDH (1401 and 396 U/L). Both orangutans were administered i.v. and s.c. balanced electrolyte fluids, dexamethasone (Dexamethasone Sodium Phosphate, American Pharmaceutical Partners, Los Angeles, CA 90024, USA; 2 mg/kg i.v.), and a course of doxycycline (Doxy 100, American Pharmaceutical Partners, Los Angeles, CA 90024, USA; 5 mg/kg, p.o., b.i.d.). Doxycycline was selected because of its efficacy for many bacterial diseases and as it is related to tetracycline, a drug of choice to treat tularemia.<sup>2,4</sup>

Two days later, a second adult female orangutan, died without prior clinical signs. Post mortem findings revealed a septicemia but cultured only hemolytic *E. coli*, *Citrobacter* sp., and *Streptococcus pneumoniae* from the lungs, liver, and spleen. Samples were again submitted for tularemia and West Nile virus. At this time, the two previously examined orangutans developed signs of pneumonia with fever (103°F/39.6°C) and coughing. Following chemical immobilization with the same anesthesia protocol used previously, both animals were administered balanced

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electrolyte fluids and doxycycline i.v. Doxycycline was continued p.o. as before, and over the next few days the condition of both animals improved. Both orangutans were considered fully recovered within 17-18 days.

Despite the lack of signs characteristic of tularemia in the two surviving orangutans during the first examination and the negative results for tularemia from the rabbit carcasses, tularemia remained high as a differential diagnosis because of the history of these orangutans handling dead rabbits. The spleen of the dead orangutan was tested for tularemia by culture and immunohistochemistry while samples of the previously submitted rabbit carcasses were retested for tularemia. Laboratory results from both the orangutan and the dead rabbits were positive for *Francisella tularensis*. Serum samples from the two initial clinical orangutan examinations had negative serology for tularemia. However, it was suspected that these animals were sampled in early onset of the disease before potential seroconversion. Serum samples collected since the presentation have shown seroconversion to tularemia.

As result of these findings, the remaining two orangutans in this group were administered oral doxycycline for 2 wk and a small-mesh wire fence was placed around the outdoor exhibit to prevent rabbits from entering the enclosure.

Outbreaks of tularemia in nonhuman primates are rather uncommon.<sup>2,3,5,6</sup> Sources of infection are mainly rodents with access to holding units.<sup>2,3,5,6</sup> The only case of tularemia in great apes was reported in a Western lowland gorilla (*Gorilla gorilla gorilla*) where the source of infection was never determined.<sup>1</sup> This is the first report of tularemia in orangutans.

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## MID-METACARPAL AMPUTATION IN A JAGUAR (*Panthera onca*)

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### Abstract

An adult female jaguar (*Panthera onca*) (45 kg) was assessed for multiple severe puncture wounds to the right forelimb inflicted by a conspecific during an introduction. The pair was initially introduced with minimal aggression 1 wk prior to presentation. During this period, the animals were separated in the evening, with visual and tactile contact through chain link fence. The evening before presentation, the male was able to grab the female's right front foot underneath this shift door, producing the initial injury.

The following morning, due to severity of the wounds, the jaguar was immobilized with medetomidine 40 µg/kg (Dormitor, Pfizer, Exton, PA 19341, USA) and ketamine 4.4 mg/kg (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA 50501, USA) i.m. by dart via a CO<sub>2</sub> injection pistol (Dan-inject-Wildlife Pharmaceuticals, Fort Collins, CO 80524, USA). The jaguar was then maintained using isoflurane (Isoflo, Abbott, Chicago, IL 60064, USA). Physical examination confirmed multiple severe puncture wounds and crushing injury to the distal right front limb at the metacarpus and traumatic dermatitis to both forelimbs. One of the punctures of the right front limb entered dorsally and exited at the palmar aspect of paw. Several lacerations on the right carpal and metacarpal pads were present, including one which exposed the proximal phalanx of digit 1. Radiographs of the distal right forelimb revealed no orthopedic abnormalities. The wounds were surgically debrided, the first digit was amputated at the metacarpophalangeal joint, and primary closure with 2.0 polyglyconate (Maxon, Tyco Healthcare, Norwalk, CT 06856, USA) in a cruciate pattern was made to reduce exposure of the extensor tendons over the distal metacarpophalangeal joint. The milder injuries to the left forelimb were thoroughly irrigated with dilute chlorhexidene solution. The jaguar was treated with penicillin 60,000 IU/kg s.c. (Han-Pen B-Hanford Pharmaceuticals, Syracuse, NY 13201, USA) and enrofloxacin 2.5 mg/kg i.m. (Baytril, Shawnee Mission, KS 66216, USA). The animal was reversed with atipemazole 5 mg i.m. (Antisedan, Pfizer) and recovered uneventfully. Meloxicam, 0.1 mg/kg, p.o. s.i.d (Mobic, Abbott) was administered the next day as well as marbofloxacin 50 mg, p.o., b.i.d (Zeniquin, Pfizer) post-procedurally. With the extent of skin injury to the right forefoot, viability was questionable and sedation for reassessment was scheduled in 3 days.

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The jaguar was immobilized as before to assess the wound. The right distal limb at the mid-metacarpus had involved soft tissue that was cold, gray, and the digital nail beds associated with this limb were purple. The wounds were thoroughly cleaned and a spoon splint was placed to stabilize the limb against further injury. The same day, the animal was immobilized a second time as previously described for transfer to Oklahoma State University, College of Veterinary Medicine (Stillwater, OK) for limb sparing surgery. Pre-operatively, the distal right forelimb was ultrasounded to assess blood flow on the dorsal and palmar aspect of the limb at the metacarpus. No visible vasculature was identified ultrasonographically. Due to clinical presentation, a mid-metacarpal amputation was performed in an attempt to salvage the remainder of the right front limb. The metacarpal pad had dubious viability, but was left in place as a weight-bearing surface upon recovery. A wet-to-dry dressing was placed and covered with a modified Robert Jones bandage on the right forelimb. Bacteriologic culture and sensitivity of the excised tissue revealed *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Micrococcus* spp. susceptible to most antibiotics. At that point, amoxicillin/clavulanate 25 mg/kg b.i.d., p.o. (Clavamox, Pfizer) was initiated in addition to the prior regimen.

The morning following surgery, the animal had removed the distal aspect of the bandage, exposing the avulsed metacarpal pad. The animal required another sedation by the prior route for cast placement. However, the cast was removed that same day by the jaguar. A second attempt to securely bandage the affected area was made to provide as much limb as possible for future reconstructive efforts. A modified 5-gallon bucket and leather collar were fashioned into an Elizabethan-collar to prevent access to the bandaged limb. However, by the next morning, the cast had slipped, the surgical wound was exposed, and the first collar was cracked. Further sedation was needed to lavage the wound, apply a lighter wet-to-dry bandage and to replace the Elizabethan-collar. Elastikon (Johnson and Johnson, Summerville, NJ 08876, USA) stirrups were applied with ether spray (Pyroil, Valvoline, Lexington, KY 40509, USA) to further secure the bandage by creating an "ether-patch" to the skin. The bandage remained in place for 3 days. Twice weekly bandage changes were instituted to surgically debride the wound and replace the wet-to-dry bandaging. Propofol 40-60 mg i.v. (Propoflo, Abbott) was added to the protocol in several of the immobilizations to improve sedation plane during the bandage changes. At each recheck examination, the amputation site was viable and granulation tissue quickly developed but moderate amounts of purulent material persisted on granulation bed. During these biweekly immobilizations, several bandages techniques were attempted to provide support for the amputation site without compromise to the adjacent healthy skin. Following, one re-bandaging procedure, a small amount of brown exudate was observed in the jaguar's pharynx upon extubation. With the frequency of procedures, stress-induced gastritis and ulceration was a concern and managed preventively with omeprazole, 1 mg/kg p.o., s.i.d. (Prilosec, AstraZeneca, Merck, Whitehouse Station, NJ 08889, USA) and sucralfate, 1 g, p.o., t.i.d (Carafate, Warrick Pharmaceuticals, Reno, NV 89506, USA).

Five weeks after the initial injury, the granulation bed was appropriate to attempt reconstructive surgery to provide a more appropriate weight-bearing surface of pad at the amputation site. Autografts of pad from the right metatarsal pad, all four right rear digital pads, left metacarpal

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pad, and two of the left digital pads were sutured with simple interrupted absorbable sutures in a rosette pattern on the distal end of the amputation site.

Bandage changes were reduced to once weekly under sedation and included a shoulder strap to secure the bandage to the right front leg. The feet (left front and right rear) that provided tissue for the reconstructive procedure were lightly bandaged with nonadherent bandage material to cushion weight bearing and prevent severe contamination. A week prior to the autograft, the jaguar was started on acepromazine, 2 mg/kg p.o., b.i.d. (ProAce, Fort Dodge Animal Health) in an attempt to decrease the anxiety of the immobilizations and bandages. The jaguar appeared less anxious between immobilization periods with initiation of the acepromazine.

To the authors' knowledge, no reports of mid-metacarpal amputation and pad reconstruction in large cats have been documented. This technique may prove to be effective as an alternative to whole leg amputation for severe traumatic distal limb wounds in large felids.

#### **ACKNOWLEDGMENTS**

We thank Dr. John P. Hoover for his expertise and Dr. Martha Larry and the Large Carnivore staff at the Tulsa Zoo for their assistance.

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## DRUNKEN ASIAN ELEPHANTS (*Elephas maximus*) FROM RYEGRASS HAY

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### Abstract

Copenhagen Zoo has maintained a herd of Asian elephants (*Elephas maximus*) since 1878. In 1999, the herd (2.3) was housed with separation of males and pairs of females. The elephants were fed concentrate, hay, vegetables, fruit, bread, sugar beets, and browse.

On the day of presentation, keepers reported that the alpha female demonstrated pronounced body wide ataxia. Due to concerns of the dry moat, the elephant was moved into an indoor enclosure. On a visual examination, the elephant was found somewhat more cooperative than usual and severely ataxic. Rectal temperature was normal (36.9°C). Auscultation demonstrated normal peristaltic sounds. Oral mucosa was pink and moist. Keepers reported that the wrapped ryegrass hay had smelled oddly when the elephants were fed in the preceding days. However, the elephants had eagerly consumed it, even before their concentrate. The hay was inspected and clearly smelled of alcohol. A tentative diagnosis of ethanol intoxication –“drunken elephant” - was made.

Elephants reportedly have a taste for alcohol and will readily eat fermented hay. The local police department was contacted for assistance. After some persuasion, they agreed to bring a Breathalyzer (Lion Alcolmeter, model S-300, Sweden) to the zoo to assess the elephant. Approximately 15 kg of hay was put into a plastic bag for 30 min to allow accumulation of any vapors. This was conducted three times and in the third attempt, enough air was trapped to process and obtained a reading over 0.1% alcohol concentration. Direct analysis of exhaled air from the elephant was unsuccessful. A blood sample was collected from an ear vein and submitted for alcohol concentration analysis to a human forensic lab (Forensic Department, University of Copenhagen, Denmark). These tests provided a result of very low alcohol content (0.0024% – legal limit for humans in Denmark driving a vehicle is 0.05%). This amount of alcohol would not account for the ataxia.

The following day, the ataxia had not changed despite diet changes that returned the elephants to normal dry hay. After reviewing the literature and considering hay composition, the diagnosis was changed to ryegrass staggers.<sup>1,2</sup> At this time, the elephant had a normal appetite but remained confined indoors due to ataxia. After 10 days, the keepers judged that although the elephant was somewhat ataxic, it was stable enough for outside access. However, it promptly staggered and fell into the moat. The elephant was dragged from the moat by an unaffected elephant, assisted by the keepers. After another 4 days, a noticeable improvement was present in the ataxia. After 16 days, the elephant was released safely outside.

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Surprisingly, 4 days after the initial case presented, a second female elephant demonstrated signs of ataxia, then the 6-ton breeding bull became ataxic the following day. This was unexpected because the suspected hay had been promptly removed from all animals' diet. The bull was severely affected and made a slow recovery – 18 days before the ataxia resolved completely.

If the mycotoxicosis had worsened, the elephants would have been treated with acepromazine or diazepam.<sup>2</sup> In summary, the long onset of intoxication from ingestion of the fermented hay to the onset of clinical signs was surprising. The protracted recovery phase called for patience in both keepers and the veterinarian.

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## PERINEAL URETHROSTOMY AND POST-OPERATIVE MANAGEMENT IN A CHIMPANZEE (*Pan troglodytes*)

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### Abstract

A 20-yr-old male chimpanzee (*Pan troglodytes*) was presented with a history of amputation of the distal 2 cm of the penis by a conspecific 4 yr previously. Since this injury, a progressively enlarging swelling of the ventral penis was observed which was approximately 2 cm diameter at the time of presentation. The animal demonstrated progressively more severe dysuria and, by the time of presentation, urination was a slow stream accompanied by prolonged abdominal straining. Endoscopy revealed a urethral diverticulum, and this was opened by transurethral “unroofing” which was performed using a human pediatric resectoscope.<sup>1</sup> Unfortunately, postoperative infection of the surgery site caused extensive necrosis. Subsequent loss of the majority of the penis occurred and a perineal urethrostomy was required to allow urination.

Perineal urethrostomy was achieved using the procedure described for humans following total penectomy,<sup>2</sup> although compared with human surgery, a relatively longer length of urethra was required to pass through the deep layer of subcutaneous fibrofatty tissue of the chimpanzee perineum. Recovery was uneventful and despite some narrowing of the meatus, no further treatment was required for the following 18 mo. At this time, stenosis caused an apparently acute onset urethral obstruction that was successfully managed by atraumatic dilatation using a fine bougie (filliform) and connecting progressively wider bougies (followers, and van-Buran sounds). Obstruction due to insertion of a piece of straw into the meatus was observed after 24 mo and suspected on several subsequent occasions. The development of polyp-like outgrowths of the distal urethra caused obstruction after 26 mo.

Re-examination using endoscopy and dilatation are currently performed every 3-6 mo. To the authors’ knowledge, neither a urethral diverticulum or perineal urethrostomy have been reported previously in a nonhuman primate.

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

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### Abstract

The Family Bucerotidae (hornbills) is characterized by a unique anatomic structure known as the casque. To date, nine great Indian hornbills from multiple US zoological institutions have presented with fatal, invasive squamous cell carcinoma (SCC) originating in the casque. Multiple treatment protocols have been attempted with limited success including: radical surgical resection, radiation, and photodynamic agents.<sup>3,10</sup> In this case, antiangiogenic treatment, OLCAT-005a, was selected to suppress neoplasia growth in addition to radiation treatment. The bird eventually succumbed to complications associated with a prosthesis, but this is the first application of topical antiangiogenic agents in avian SCC.

### Introduction

The great Indian hornbill (*Buceros bicornis*) is at risk for invasive SCC of the casque, which in the case literature has been invariably fatal. Nine specimens have been diagnosed, with no survivors. Of the nine affected individuals, six were male, suggesting a gender predisposition. Limited information on the structural and functional anatomy of the casque is documented, but case reports indicate that SCC of the casque is resistant to conventional treatments used in humans and other mammals.<sup>8,12,14</sup>

Angiogenesis (neovascularization) is a critical event in neoplasia formation, allowing for rapid neoplasm expansion and metastases.<sup>1,5</sup> Microvasculature density correlates positively with malignant progression in squamous neoplasia, and cutaneous neoplasms show increased neovascularization.<sup>4,7</sup> Antiangiogenic therapy is a new treatment modality designed to suppress tumor blood vessel growth, halt neoplasia progression, and improve overall survival.

### Case Report

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In June 2003, a 33-yr-old male great Indian hornbill presented for examination because of a malodorous smell. Physical exam revealed weight loss and a soft necrotic area on the cranial casque, containing large amounts of pus and secondary myiasis. Blood was collected for baseline complete blood count, chemistry panel, and *Aspergillus* titers (Table 1). Lateral and ventrodorsal skull radiographs revealed a large soft tissue density occupying the entire casque, extending to the supraorbital rim of the cranium. Multiple punch biopsies were collected for culture and histopathology (NWZP, Northwest Zoo Path, Monroe, WA 98272, USA) revealed invasive SCC. The wound was aggressively debrided. *Proteus* but no fungal organisms were cultured from the debrided material. Enrofloxacin (Baytril, Bayer Corporation, Shawnee Mission, KS 66225, USA, 10mg/kg i.m., s.i.d., for 7 days) was initiated.

One week post-presentation after preliminary histopathology returned with SCC, the bird was transported to LSU to undergo radical casque resection. The bird was anesthetized with sevoflurane (SevoFlo, Abbott Laboratories, Chicago, IL 60064, USA). The dorsal casque was resected in a semi-circle using a bone saw, resulting in exposed spongy bone. The wound was lavaged thoroughly with 0.5% chlorhexidine (Nolvasan, Fort Dodge, Fort Dodge, IA 50501, USA) and bandaged with Tegaderm (3M, Saint Paul, MN 55144, USA). Recovery from anesthesia was uneventful. Enrofloxacin was continued orally twice daily and Benebac (Bird Benebac, Pet Ag Inc. 261 Keyes Ave., Hampshire, IL 60140, USA; 2 g p.o., s.i.d.) was initiated. Following this surgery, the bird appeared bright and alert, with a good appetite and weight maintenance.

One week post surgical resection, radiation, using a 6MV linear accelerator, was initiated while the bird was in sedated as before, placed in sternal recumbency, and aligned using orthogonal positioning lasers. Prior to each of the three weekly radiation treatments, the casque was aggressively debrided and cleaned with 0.5% chlorhexidine solution. To allow for an adequate radiation build-up zone, 1 cm of tissue equivalent material was placed over the neoplasm bed. The total field size was 6.1 cm × 19.4 cm and included a 3-cm margin. Radiation (10 Gy) was administered through lateral parallel opposed fields, with the prescribed dose delivered to the midline. The post-radiation wound was bandaged with combination of a Tegaderm and Duoderm (Conva Tec Ltd, Bristol Myers Squibb, Princeton, NJ 08543, USA). Twice weekly assessments were performed and, as needed, wound debridement, lavage, and bandage changes. Blood was collected every 14 days to monitor clinical pathology profiles (Table 1). Leukocytosis prompted blood cultures that demonstrated *Pseudomonas* bacteremia. Systemic antibiotics were resumed (Enrofloxacin, 10 mg/kg, p.o., b.i.d. for 5 days).

The hornbill was returned to LSU for ablation with a CO<sub>2</sub> laser, to remove the casque base and dorsal rhinothecal sinus, before radiation treatment was resumed. After 2 wk, surgical excision was performed to remove the neoplasm visible on ventrodorsal and lateral skull radiographs. One third of the dorsal rhinotheca was removed by blunt dissection and electrocautery. Histopathology revealed multifocal osteolysis, fibroplasia, and inflammation with small nests of SCC embedded in inflamed and necrotic tissue. Although the animal appeared clinically

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improved 8 days after surgery, skull radiographs revealed lysis of the supraorbital ridge while visible neoplasm was again apparent on the casque.

At this time, topical antiangiogenic therapy was initiated using the regimen OLCAT (Off-Label Combinatorial Antiangiogenic Therapy)-005a developed by the Angiogenesis Foundation using FDA-approved drugs that show biologic activity on vascular endothelial cells or neovascularization.<sup>9</sup> The OLCAT-005a regimen includes imiquimod 5% cream, tretinoin 0.1% microsphere gel, calcipotriene 0.005% ointment, diclofenac 3% gel in hyaluronic acid, and hydrocortisone valerate 0.2% ointment. Drugs were mixed together in equal parts prior to applying a thin layer over the affected tissue. The frequency of administration was governed by the Individualized Maximal Tolerated Dose (IMTD<sup>SM</sup>) algorithm developed by the Angiogenesis Foundation<sup>17</sup>: 2 × /wk × 2 wk, 3 × /wk × 2 wk, Monday through Friday × 2 wk, then daily (total: 12 wk of ≥3 × /wk).

Decrease in neoplastic lesion size was visibly apparent after only 1 wk of topical antiangiogenic therapy. Two weeks after topical therapy was initiated, the first negative biopsy returned. Radiographs at this time suggested no apparent neoplasia and clinical pathology had returned to normal limits.

Due to the multiple casque resections, the rhinotheca became weakened and multiple procedures were attempted to enhance the structural support. The antiangiogenic treatments were decreased in frequency to minimize excessive stress while the LSU Dental service and a local artisan were consulted for the creation of a prosthetic casque.

One week following cessation of the 12-wk topical protocol, visible regrowth of the neoplasm in the left rostrum was noted. As this area had not been treated directly with the topical agents and biopsy for histopathology revealed SCC, topical treatment was re-instituted. With the prosthesis nearing completion, final adjustments included correcting the weight in comparison with the original casque and adding portals that would allow application of topical treatments. The prosthetic composition was a rigid Urethane polymer (GTS-850, Industrial Polymer Inc., Houston, TX 77047, USA).

At 15 wk post-treatment, the bird was manually restrained for prosthetic placement. At this time, visible re-growth of neoplasm was debried, the wound lavaged with dilute chlorhexidine, and topical treatment resumed. Skull radiographs revealed lytic and proliferative lesions cranial to the frontal bone. The heated prosthesis was appropriately molded and screwed into the soft palate with a titanium plate. When returned to holding facility, the bird appeared slightly off balance, yet was attempting to catch grapes. Within 36 hr of placement, the bird managed to dislodge the prosthesis. A second prosthetic was attached with a larger titanium plate that was removed by the bird within days.

The bird was assist-fed until transported to LSU after this event for the final surgical resection and prosthetic placement. The remaining affected casque was removed and the third prosthesis was attached using 18<sup>g</sup> gauge cerclage wire looped through the remaining viable casque. Of

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note, this prosthetic was heavier than the first two. Three days post-operatively, the bird appeared depressed with cervical ventroflexion observed. The bird was manually restrained to remove part of the prosthesis (350 g). Severe bruising of the hocks and subcutaneous hematomas were visible. The following day, the bird remained depressed in mentation so supportive care and assist feeding were required. The bird expired hours after this treatment with a presumptive diagnosis of exertional myopathy with necropsy revealing severe visceral gout and pectoral zonal myositis. However, no apparent signs of neoplastic growth or metastasis were observed grossly while histopathology of the rhinotheca revealed SCC with no vascular invasion.

## Results and Discussion

SCC in the great Indian hornbill is an extremely aggressive disease that does not respond completely or consistently to conventional treatments such as surgical resection, radiation, and photodynamic agents. On presentation, an affected bird may appear as a severe fungal or bacterial casque infection. Skull radiographs and multiple deep punch biopsies are required for diagnosis. It is recommended that all great Indian hornbills have multiple (lateral, ventrodorsal, rostrocaudal) radiographic views of the skull performed once to twice yearly to aid in early detection ([www.coraciiformestag.com](http://www.coraciiformestag.com)).

Neoplasia is dependent upon angiogenesis for expansion and metastases. Currently, there are more than 70 antiangiogenic drugs in human oncology clinical trials. In this particular case, a novel treatment was developed (OLCAT-005a) by the Angiogenesis Foundation suppressed malignant growth. Each drug component in the OLCAT-005a protocol has discrete antiangiogenic mechanisms, as well as synergistic biologic effects.<sup>6</sup> Imiquimod exerts antiangiogenic activity by up-regulating of local production of interferons and interleukin-12 which have anti-proliferative and pro-apoptotic effects on endothelial cells.<sup>13,16</sup> Retinoids such as tretinoin, vitamin D analogs such as calcipotriene, and COX inhibitors such as diclofenac, have been shown to have antiangiogenic activity.<sup>9,11,15</sup> The combined additive and synergistic actions of these components in OLCAT-005a have biologic and clinical activity in treating SCC.<sup>16</sup>

This is the first case of successful application of antiangiogenic therapy to an avian SCC. During the 12-wk course of topical treatment, neoplasia suppression was noted in treated areas previously recalcitrant to surgery and irradiation. Additionally, the neoplasm re-appeared in the sites in which the topical agents were not applied or unable to penetrate completely. The neoplasm recurred after cessation of the OLCAT-005a although the final histopathology revealed no signs of vascularization within the recrudescing mass. Therefore long-term angiostatic treatment may be beneficial to suppress SCC growth to convert this otherwise fatal disease into a manageable condition. The agents appears safe, well tolerated, and demonstrated efficacy for neoplasia suppression in avian SCC. As more data regarding the anatomic structures and functions of the Bucerotid casque are determined, diagnostics and preventive medicine will improve for this structure.

## ACKNOWLEDGMENTS

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The authors thank the dedication of the Audubon Bird and Veterinary Departments.

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**Table 1.** Primary focus of clinical pathology reported every 2 wk.

WBC K/ $\mu$ l	Mean <sup>2</sup>	Lymph K/ $\mu$ l	Mean <sup>2</sup>	Hets K/ $\mu$ l	Mean <sup>2</sup>	PCV %	Mean <sup>2</sup>	CPK IU/L	Mean <sup>2</sup>	UA mg/dl	Mean <sup>2</sup>
38,000	11,650	11,400	4,420	25,460	6,231	35	46	1,276	1,674	5.1	0.446
13,000		4,810		8,060		42		1,500		2.6	
24,000		7,680		15,840		39		1,196		3.1	
31,000		8,060		22,010		44		700		3.9	
49,900		8,483		43,120		23		1,635		7.1	
85,000		28,050		56,100		21		844		3.1	
39,000		12,480		24,570		34		1,276		1.6	
15,000		3,750		11,100		46		1,012		2.3	
27,000		6,750		19,710		40		908		1.5	
24,000		7,440		15,840		42		1,216		2.1	
20,000		4,200		15,000		40		1,216		4.8	
17,000		8,670		7,990		18		13,920		6.8	

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## MANAGEMENT OF FUNGAL DERMATITIS AND PYODERMA IN A GROUP OF SHORT-NOSED ECHIDNAS (*Tachyglossus aculeatus*)

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### Abstract

The post mortem diagnosis of dermatophytosis in a short-nosed echidna (*Tachyglossus aculeatus*) prompted the clinical investigation of Perth Zoo's remaining echidnas. All presented with dermatopathy of varying severity. The lesions were diagnosed histologically as dermatophytosis, probably caused by *Microsporum gypseum*, with secondary pyoderma. All clinical cases responded to a combination of systemic antibiotics, topical treatment and bathing, and environmental change.

### Case Study

#### Case 1: Detection of Dermatophytosis in an Echidna at Necropsy

An individual from Perth Zoo's collection of short-nosed echidnas (*Tachyglossus aculeatus*) was submitted for veterinary examination after being found laterally recumbent and severely depressed. Traumatic excoriations on the snout and forelimbs suggested that the animal had become entrapped while burrowing, and produced the injuries when attempting to struggle free. Almost all the spines on the cranial third of the body were broken, and remaining spines were friable and easily damaged. Although hospitalized and given subcutaneous fluids in 20ml boluses throughout the afternoon, the echidna was found dead the following day.

At post mortem examination, the echidna (Echidna 1) was found with very brittle spines, marked ventral alopecia, and excessive waxy exudate on the ventral skin and in the ears. Histopathologic examination of the skin revealed a severe pyoderma and marked dermatophytosis. Fungal hyphae were evident within the superficial keratin and serocellular crust, and the surface keratin was also heavily infested with bacteria, including gram positive cocci and bacilli and some gram negative bacilli. The skin pathology was characterised by parakeratotic and hyperkeratotic hyperkeratosis, dermal infiltration with polymorphonuclear cells, and pustules.

#### Case 2: Physical Examination and Diagnostic Sampling of Echidna Collection

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The findings of Echidna 1 prompted the veterinary examination of the five remaining echidnas in the collection. Although the echidnas were routinely examined by keepers every 4-6 wk, close inspection of the skin was made difficult by the accumulation of soil and debris in the spines, and the echidnas' tendency to curl up when restrained. Veterinary examination necessitated manual restraint after removal from their burrows. All five were found to have varying degrees of dermatitis, characterized by alopecia, fragile and broken spines, and dark, waxy exudate on the ventral skin and in the ears. Due to the possibility of dermatophytosis, all the animals were transferred from their heavily mulched outdoor enclosure to a dry, lightly mulched, indoor enclosure. At transfer, skin scrapes and swabs were collected from all individuals for microscopy, fungal and bacterial culture and sensitivity. Spines and hairs from the most affected individuals were submitted for potassium hydroxide examination and histopathology.

Microscopy of skin scrapes and potassium hydroxide examination of hair shafts in affected individuals revealed a few fungal hyphae, not infecting hair shafts. The absence of hair shaft involvement suggested that the hyphae were soil contaminants, rather than pathogens. No ectoparasites were detected on skin scrapes. Wet microscopy and gram stains were characterised by occasional fungal hyphae, with some gram negative bacilli and gram positive cocci. Culture from four individuals (Echidnas 2, 3, 4 and 5) grew a moderate to heavy mixed growth of a number of organisms, including *Enterobacter* spp., *Klebsiella* spp., coagulase negative *Staphylococcus*, non-hemolytic *Streptococcus*, and non-hemolytic *E.coli*.

Fungal culture of spines and skin scrapings was undertaken for the three most affected individuals (Echidnas 2, 3, and 5). Echidna 5 grew *Mucor* spp., Echidna 3 grew *Aspergillus fumigatus* and *Penicillium* spp., and Echidna 2 grew *Microsporium gypseum*. Given the histopathologic finding of dermatophytosis in Echidna 1, it was suspected that *Microsporium gypseum* was the primary pathogen in all the echidnas, and that opportunistic bacterial infection with commensal organisms resulted in the secondary pyoderma.

### **Initial Treatment of Echidna Group**

Topical treatment of echidnas is made difficult by their spines, burrowing behaviour and physical strength. A manual restraint technique of suspending them by their hind feet causes them to uncurl, exposing the head, ventral region and the non-spiny parts of the limbs. While waiting for results of diagnostic sampling, each animal was restrained in this manner every 3 days for i.m. long-acting penicillin injection (Norocillin LA Injection: procaine penicillin 150mg/ml, benzathine penicillin G 112.5mg/ml: Norbrook Laboratories Australia Pty. Ltd., 1ml/10kg body weight). At each treatment, the animal was also sprayed topically with oxytetracycline (Terramycin Pinkeye Aerosol: oxytetracycline hydrochloride 2mg/g, Pfizer Animal Health, West Ryde, New South Wales). Although cultured *Enterobacter* and *Staphylococcus* species were resistant to the penicillin, treatments were continued for 2 wk due to concerns of stress related to more frequent treatment modalities.

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Because the animals were otherwise in good health and of good general body condition, specific antifungal treatment was not performed. It was hoped that the condition would be self-limiting if the echidnas were removed to another enclosure and secondary bacterial infection was controlled. Manual restraint and veterinary examinations were scheduled once every 3 wk to monitor the progress of alopecia and damage to spines.

### **Examination, Sampling and Treatment Under Anaesthesia**

Over the ensuing month, the skin condition of the worst affected echidna (Echidna 2) deteriorated further, so it was anesthetized for a more detailed workup and treatment. The animal was induced in an induction chamber using isoflurane (Isoflurane Inhalation Anaesthetic, David Bull Laboratories, Mulgrave, Victoria) then maintained by facemask.

Skin lesions were characterised by generalized alopecia, with scaling on the ventral mandible and accumulation of skin debris and crusting at the base of the spines. Many spines were broken, and the shafts of the broken spines were hollow, rather than being solid keratin. Waxy seborrheic discharge was present around the head and in the external ear canals.

Wedge skin biopsies were taken for bacterial and fungal culture, and histopathology. The echidna appeared to be in good body condition, and no other abnormalities were found on physical examination. Hematology and serum biochemistry parameters were within ISIS reference ranges for the species.

After diagnostic samples were taken, the echidna was placed in dorsal recumbency in a warm water bath, to which an antibacterial, keratolytic, antifungal shampoo was added (Sebolyse Medicated Foam: miconazole nitrate 20 mg/ml, chlorhexidine gluconate 20 mg/ml, selenium sulfide 2.5 mg/ml; Dermcare-Vet Pty. Ltd., Springwood, Queensland). A long-handled dishwashing brush was used to scrub the skin and the spines. This process was effective in removing scurf, dirt and seborrheic debris. Topical treatment with miconazole aerosol powder (Daktarin, 2% miconazole nitrate; Janssen-Cilag Pty. Ltd., North Ryde, New South Wales) was initiated following the bath. This product was sprayed over the spines and ventrum at a distance of 15 cm from the animal, once daily for 3 wk. Sebolyse baths were given every 2 wk for a total of four treatments.

Histopathology of the skin biopsy from Echidna 2 showed evidence of heavy fungal infection and secondary pyoderma. Bacterial culture of the skin biopsy once again demonstrated a heavy mixed growth. No pathogenic fungal species were detected on culture of the skin biopsies, although soil commensal species of *Fusarium* and *Rhizopus* were identified.

Two of the remaining four echidnas (Echidnas 3 and 4) developed signs that warranted bathing under anesthesia, but the Echidnas 5 and 6 resolved without further treatment. Only Echidna 2 was treated with topical miconazole spray. Manual restraint and veterinary examination of all echidnas was continued every 4-6 wk for 6 mo, to assess the need for further bathing. Echidna 2

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underwent Sebolysse baths on another two occasions during the 6 mo, but all the others continued to improve without further topical treatment.

## Discussion

The monitoring, diagnosis and treatment of disease in echidnas is a challenge. Conscious manual restraint is difficult and potentially stressful to the animal. Echidna which are not restrained appropriately assume a defensive posture; in these cases, this caused keepers to underestimate the extent and severity of skin lesions during routine weighing and handling, prior to the death of Echidna 1.

There is limited information in the literature regarding pathologic skin conditions in echidnas.<sup>1-3,5-7</sup> A hyperkeratotic condition is described in juvenile echidnas, which has been associated with the presence of pox virus particles.<sup>1,3,5,7</sup> *Staphylococcus granulomata*,<sup>5</sup> and nodular masses caused by plerocercoids of *Spirometra erinacei*,<sup>7</sup> are also reported. There is one report of *Microsporum gypseum* being isolated from the skin of a captive echidna with fractured, friable spines, but there are few published details of this case.<sup>1-3, 6, 7</sup>

Histopathologic examination of biopsies Echidnas 1 and 2 confirmed the diagnosis of dermatophytosis. It seems likely that the causative agent was *Microsporum gypseum*, given that this organism was grown from one of the quill samples taken from Echidna 2. The failure to isolate *M.gypseum* from the other cases is not surprising, since saprophytic fungal overgrowths are a common complication of dermatophyte culture.<sup>4</sup> Fungal soil commensals were detected on skin scrapings and fungal culture from several individuals. *Mucor* sp., which were cultured from Echidna 5, are also known to be potentially pathogenic, and have been known to cause systemic disease and ulcerative dermatitis in platypuses.<sup>1,3,6</sup> However, the non-ulcerative nature of the echidnas' skin lesions, the histopathologic appearance, and the lack of systemic illness, were more consistent with dermatophytosis than mucormycosis.

The secondary pyoderma was responsive to long acting antibiotics in most cases, but the most severely affected animals required regular topical treatment under anesthesia to resolve the problem. The baths were highly successful in removing debris and seborrheic buildup, as well as providing a means of medicating the skin with antibacterial and antifungal compounds.

The use of antibiotic and antifungal drugs in echidnas is limited by the lack of reliable pharmacokinetic data. In this case study, no adverse effects were seen with the use of long-acting penicillin, topical miconazole, topical Sebolysse or topical oxytetracycline spray.

Eighteen months after the death of Echidna 1, the entire echidna group was moved from their temporary holding enclosures into a new outdoor display enclosure. The new enclosure features a sand and leaf litter substrate, and custom built, easily cleaned burrows containing jarrah sawdust as bedding. There have been no relapses since the echidnas were removed from their original enclosure. It seems likely that the origin of the skin condition was environmental, and

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may have been linked to the use of a new type of soil substrate that was put in the enclosure about 10 wk prior to the death of Echidna 1. *M. gypseum* is known to survive for long periods in soil,<sup>4</sup> and the echidnas burrowed extensively in the soil of their old exhibit, only emerging from their diggings to feed. In the new enclosure, they are inclined to use the pre-built burrows rather than digging their own, and this is likely to contribute significantly to the persisting good condition of their skin and spines.

#### ACKNOWLEDGMENTS

The authors would like to thank the staff of the Perth Zoo Veterinary Department and Australian Section for their dedication in the management of these cases. Thanks also to Dr. John Jardine of Vetpath Laboratory Services for his comments on the histopathology and culture results.

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## TESTICULAR NEOPLASIA IN A WHITE RHINOCEROS (*Ceratotherium simum*): FERTILITY AND ASSISTED REPRODUCTIVE MEASURES TO MINIMIZE GENETIC LOSS

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### **Abstract**

Reproductive assessment has become an established method to evaluate breeding fitness in male white rhinoceros (*Ceratotherium simum*). Ultrasonography and electroejaculation are routinely used to characterize the reproductive organs and evaluate semen quality. Despite reported increased connective tissue in the testicular parenchyma in aged males, no substantial reproductive disorder has been described to date that could potentially compromise male fertility. Reduced semen quality and limited breeding activity were mostly accounted to mate choice problems and social stress of the males in captivity.

This case report describes diagnosis of a unilateral testicular neoplasm in a 20-yr-old white rhinoceros. Ultrasonography characterized the accessory sex glands and left testis with dimensions as seen in male rhinos with good semen quality. In the right testis, neoplasia compromised one third of the testicular volume. The abnormal structure was solid, with a well-defined border and moderate blood flow. Histopathology confirmed the neoplasia as a seminoma. Adjacent zones of tissue necrosis and mineralization suggested a malignant character of the neoplasm. However, despite the size and invasiveness of the mass, the collected ejaculate contained appropriate concentration and high percentage of motile sperm. Sperm was cryopreserved in 0.5 ml straws using a DMSO egg yolk extender before further neoplastic growth might have ceased spermatogenesis. Post thaw motility of >50% demonstrated that the testicular neoplasia in this individual did not preclude the production of good quality semen as it had been detected at an early stage. This case of a testicular neoplasia describes that reproductive lesions in male rhinoceros may be an underlying cause in single individuals for absent reproductive success.

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## PRELIMINARY RESULTS OF MEDETOMIDINE-KETAMINE-BUTORPHANOL FOR ANESTHETIC MANAGEMENT OF CAPTIVE WHITE-NOSED COATI (*Nasua narica*)

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### Abstract

### Introduction

The white-nosed coatimundi (*Nasua narica*) is one of two members of the genus *Nasua* in the raccoon family, Procyonidae. With the exception of a few reports, detailed published data regarding species-specific anesthesia protocols and physiologic parameters for the coati genus is lacking. Most published protocols include high doses of dissociative agents, ketamine or tiletamine in combination with zolezepam.<sup>1,4,6</sup> These agents have been associated with inadequate neuromuscular relaxation, tonic/clonic convulsions, and prolonged, rough recoveries. Use of these agents is less desirable because of the lack of specific reversal agents.

Alpha<sub>2</sub>-adrenergic receptor agonists have been used in combination with ketamine to anesthetize a wide array of non-domestic mammal species.<sup>3</sup> The combination of  $\alpha_2$ -adrenergic receptor agonist medetomidine and its specific receptor antagonist, atipamezole, has the advantage of increased  $\alpha_2$ -adrenergic receptor specificity and excellent reversibility leading to rapid, smooth recoveries. The addition of medetomidine to ketamine regimens has substantially reduced ketamine dosages needed, diminishing many of the adverse effects seen with higher doses of dissociative agents. The addition of butorphanol to medetomidine-ketamine protocols has been reported in several carnivore species.<sup>5,7-9</sup> While a few reports of medetomidine-ketamine in procyonids are published,<sup>2</sup> minimal to no data is documented regarding the success of medetomidine-ketamine-butorphanol in procyonids or, *Nasua* spp. more specifically. This report details the preliminary results of medetomidine-ketamine-butorphanol (MKB) at three separate zoological institutions (with and without additional isoflurane supplementation) to facilitate common medical and surgical procedures in captive white-nosed coatis (*Nasua narica*).

### Methods

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Thirteen anesthetic events were performed in four adult white-nosed coatis (one male, three females), weighing 3-13 kg, using  $57.3 \pm 6.5$   $\mu\text{g}/\text{kg}$  medetomidine (Domitor®, Pfizer Animal Health, Exton, PA 19341, USA),  $5.6 \pm 2.1$   $\text{mg}/\text{kg}$  ketamine (Ketaset®, Fort Dodge Laboratories, Inc., Fort Dodge, IA 50501, USA), and  $0.35 \pm 0.1$   $\text{mg}/\text{kg}$  butorphanol (Torbugesic®, Fort Dodge Laboratories, Inc.) combined and administered i.m. by a 3 ml pressurized plastic blowdart (Telinject USA, Inc., Saugus, CA 91350, USA) with a  $1.1 \times 30$  mm needle or hand injected via syringe. Ten of these events were supplemented with isoflurane (IsoFlo®, Abbott Laboratories, North Chicago, IL 60064, USA) at a range of 0.5-2.5% of oxygen flow (average 1-2 L/min) for maintenance. In five events, inhalant anesthesia was maintained via endotracheal tube. In the other five events, isoflurane was provided via facemask. No additional injectable drugs were required to alter the anesthetic plane for the types of procedures performed, once the animal became recumbent in all events.

Heart rate, respiratory rate, rectal temperature, and pulse oximetry data were collected throughout each procedure and recorded at 5-min intervals. Monitoring data and physiologic values for the injectable combination alone and the injectable combination plus isoflurane are summarized (Table 1). Procedures performed with the aid of these anesthetic combinations included physical examination, phlebotomy, vaccinations, radiography, abdominal ultrasonography, manual bladder expression for urine collection, dynamic endocrine testing, skin biopsy, wound lavage and bandage change, mass removal, and ovariohysterectomy. The injectable protocol alone was only used for physical examination, phlebotomy, and skin biopsy.

All anesthetic events were reversed with atipamezole (Antisedan®, Pfizer Animal Health). Eleven anesthetic events were reversed with  $0.26 \pm 0.1$   $\text{mg}/\text{kg}$  atipamezole i.m. while in the remaining two events, atipamezole was administered half s.c. and half i.m. In three events, naltrexone (Trexonil®, Wildlife Pharmaceuticals, Inc. Fort Collins, CO 80524, USA) reversed the butorphanol component at 10 mg/1 mg of butorphanol, with half administered s.c. and half i.m. Complete recovery occurred in all anesthetic episodes.

## Results and Discussion

Mean time to first effect and mean time to recumbency for all anesthetic events was  $3.5 \pm 1.6$  min and  $5.8 \pm 3.2$  min, respectively. Mean time from darting to intubation in the events using supplemental inhalation anesthesia via endotracheal tube was  $23.3 \pm 8.1$  min. Total length of procedure was considered as the time from the initial darting to a return to sternal recumbency. Average length of procedure for the MKB plus isoflurane events was  $148.3 \pm 82.4$  min (range 66-325 min) while the average length of MKB events alone was  $51.7 \pm 19.3$  min (range 30-67 min). The mean time to arousal was  $8.5 \pm 4.5$  min (range 1-14 min) in the MKB plus isoflurane events and  $5.4 \pm 4.0$  min (range 1-9 min) in the MKB events. Times from administration of reversal agents to sternal recumbency were variable in the MKB plus isoflurane events at  $35.5 \pm 33.2$  with several taking over 50 min. Removal of one outlier event, involving a very prolonged recovery following isoflurane maintenance ( $> 110$  min from first arousal to sternal), provided a

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mean time from reversal to sternal recumbency of  $25.9 \pm 13.8$  min. In the MKB events, the mean time from reversal to sternal recumbency was  $10.7 \pm 8.0$  min.

Undesirable neuromuscular activity was observed in one MKB sedation. In this event, the animal began spasmodic jerking at 10 min post-injection followed by a 20 sec seizure at 40 min that necessitated reversal with atipamezole and naltrexone. No seizures were noted post reversal. Myoclonic activity was noted in two other MKB plus isoflurane events. One episode of bradycardia was observed in an MKB plus isoflurane event.

No seizures were reported when using MKB with supplemental isoflurane; however, increased visible neuromuscular activity was observed. Intermittent muscular twitching is an occasional reported effect of medetomidine used alone in domestic dogs.<sup>10</sup> The paroxysmal neuromuscular twitching observed in two isoflurane-supplemented cases was attributed to this type of effect.

This is the first report of seizures with MKB in a non-domestic species. Addition or substitution of a benzodiazepine into the protocol may provide more consistent, safe use for injectable use alone. Overall, medetomidine-ketamine-butorphanol with supplemental isoflurane was effective for anesthesia; however, recovery times were considered prolonged in 30% of the events. More experience with this protocol in coatis is necessary to assess the anesthetic effects relative to previous ketamine and tiletamine-zolezepam anesthetics in these species. Preliminary results of medetomidine-ketamine-butorphanol without isoflurane show variable success, but do not support its use alone for longer procedures (>45-60 min) in this species due to the potential for seizure activity.

#### ACKNOWLEDGMENTS

The authors would like to thank Drs. Julio Mercado, Tom Curro, and Doug Armstrong (Omaha Henry Doorly Zoo) and Dr. Nancy Lung (Fort Worth Zoo) for their support and help with this project. We thank the respective veterinary and animal care personnel at all of the institutions for their help with data collection. We would also like to thank Dr. Barbara Wolfe (North Carolina Zoological Park) for the sharing of anesthesia records.

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**Table 1.** Physiologic monitoring data for medetomidine-ketamine-buttorphanol with isoflurane (MKB + isoflurane) and medetomidine-ketamine-buttorphanol alone (MKB). Data is reported as the mean  $\pm$  standard deviation with ranges of measurements listed in the following row.

	n	Mean respiratory rate (breaths/min)	Mean SpO <sub>2</sub> (%)	Mean heart rate (beats/min)	Mean rectal temperature (°C)
MKB + isoflurane	10	35.2 $\pm$ 9.3 range: 18-80	93.5 $\pm$ 6.0 range: 76-100	101.8 $\pm$ 23.2 range: 57-173	36.8 $\pm$ 1.2 range: 34.9-38.8
MKB	3	33.4 $\pm$ 16.1 range: 16-72	88.0 $\pm$ 8.5 range: 72-99	118.1 $\pm$ 35.7 range: 79-220	38.0 $\pm$ 0.3 range: 37.6-38.4

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## A MULTI-SPECIES OUTBREAK OF ORF WITHIN A ZOOLOGICAL COLLECTION

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### Abstract

During July and August 2001 the Minnesota Zoological Garden experienced an outbreak of proliferative dermatitis in three different ungulate species in three separate areas of the zoo. Nine musk oxen (*Ovibos moschatus*), eight Shetland sheep (*Ovis aries*) and two of four Sichuan takin (*Budorcas taxicolor tibetana*) were clinically affected. Disease severity and extent of lesions was markedly different between the affected species. However, histopathology and electron microscopy were consistent with a parapoxvirus infection in all individuals. Restriction enzyme analysis and partial gene sequencing of isolated virus suggest the outbreak was due to infection by a single orf virus (ORFV) strain.

### Introduction

Orf (contagious ecthyma) is caused by a highly infectious epitheliotropic poxvirus of the genus Parapoxvirus which includes the closely related bovine papular stomatitis virus, pseudocowpox virus and parapoxvirus of red deer.<sup>3,4,10,11</sup> Tentative members of the genus include Auzyk virus, chamois contagious ecthyma virus, red squirrelepox virus and sealpox virus.<sup>11</sup> Orf virus has a worldwide distribution and is a common cause of disease in domestic sheep and goats and can affect a wide range of wild artiodactylids.<sup>11</sup> Orf virus is readily transmitted to humans. Infection typically occurs when abraded skin contacts infected animals or fomites. Although there is no evidence for latent carriers of the disease,<sup>11</sup> lesions can persist for months. In a dry environment at room temperature, ORFV is capable of surviving for 15 yr.<sup>9</sup>

### Case Report

On 28 July 2001, 8 of 9 (6.3) members of the musk ox (*Ovibos moschatus*) herd were observed with crusty lesions on their muzzles, lips or nostrils. The herd was comprised of animals ranging from 2 to 9 yr of age. One bull, housed separately from the herd, was noted to have lesions on 2 August 2001. A quarantine area was established around the musk ox area and personnel caring for the animals wore protective clothing including coveralls, latex gloves, and rubber boots. Footbaths containing quaternary ammonia disinfectant (NPD Unicide 256, Brulin and Company,

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Inc., Indianapolis, IN 42606, USA) were utilized prior to leaving the area. Waste products from the animal enclosures were handled with separate equipment and taken to a separate compost area from that used for waste of other animals in the collection.

Animals developed multilobulated cauliflower-like papillomatous lesions on their muzzles and lips. Most also had proliferative lesions on the buccal aspect of oral mucous membranes. Severely affected animals had marked mucous membrane thickening and facial edema. Some animals had stertorous breathing due to partial occlusion of nares. Multilobulated, cauliflower-like, papillomatous lesions were noted around eyes and the tarsal and carpal joints of some animals. Diagnostic testing of biopsies confirmed parapoxvirus virus based on histopathology and electron microscopy. Older animals (greater than 5 yr) were less severely affected and their lesions were significantly decreased within 1 mo of onset of clinical signs.

Severely affected animals were given supportive medical care including some or all of the following throughout the course of the disease: intravenous fluids, penicillin G benzathine and penicillin G procaine (Crystiben, Fort Dodge, Fort Dodge, IA 50501, USA), ceftiofur sodium (Naxcel, Pharmacia & Upjohn Company, Kalamazoo, MI 49001, USA), B vitamins (Vitamin B Complex, Bimeda, Inc., Riverside, MO 64150, USA), flunixin meglumide (Banamine, Phoenix Pharmaceutical, Inc., St. Joseph, MO 64014, USA) iron dextran (Iron Dextran Injection, Durvet, Inc., Blue Springs, MO 64014, USA), stanozolol (Winstrol-V, Pharmacia and Upjohn Company) and dexamethasone sodium phosphate (Phoenix Pharmaceutical, Inc.) Lesions were cleaned topically with dilute povidone-iodine solution (Prodine Solution, Phoenix Pharmaceutical, Inc.) and lavaged with water from a garden hose. To reduce myiasis, animals were treated with permethrin pour on insecticides (Ultra Boss, Schering-Plough Animal Health Corp., Union, N.J. 07083 USA or Cyence, Bayer Corp., Shawnee Mission, KS 66201, USA) and pyrethrin/piperonyl butoxide fly gel (Pet-Guard, Virbac, Inc., Fort Worth, TX 76161, USA). Some of the animals became anorectic due to the severity of the disease. Even with supportive care, two animals died. A third severely affected animal was euthanatized 2 October 2001 due to its deteriorating condition.

All eight Shetland sheep (*Ovis aries*) housed at the zoo's farm were observed with lesions on their lips on 16 August 2001. These animals were located approximately 160 meters from the nearest musk ox. These sheep were housed in the same building, but in separate pens from 39 pygmy goats (*Capra hircus*) and one Oberhasli goat (*Capra hircus*). Because of the zoonotic potential of the disease and to prevent transmission to other animals, the exhibit was immediately closed and all animals were moved to a quarantine area away from public contact. The sheep developed only mild, focal proliferative lesions on their lips. One of the sheep was euthanatized and submitted for necropsy; histopathology and electron microscopy of affected tissue confirmed parapoxvirus lesions. None of the goats developed any lesions. Lesions in the sheep resolved in about a month. However, the animals were not returned to the disinfected barn for 2 mo to decrease the risk of transmission to zoo patrons.

On 26 August 2001, two of the zoo's four Sichuan takin (*Budorcas taxicolor tibetana*) were observed to have "wart-like" lesions on their lower lips. These animals were housed at least 0.5

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kilometers from either musk ox, or sheep exhibit areas. One of the affected animals was a 9-yr-old cow with a nursing calf. The cow's lesion did not become more severe and the calf never developed lesions. The other affected animal was a 16-mo-old male. This animal developed multiple papillomatous lesions on its muzzle, nose, and oral mucous membranes; some of the lesions became ulcerated. Lesions were biopsied and electron microscopy of the fresh tissue revealed parapoxvirus. This animal continued to eat well and the lesions were nearly resolved a month later.

The first musk ox to die was markedly emaciated while the other two submitted for necropsy showed moderate emaciation. Gross skin lesions were previously described. The two animals, which died exhibited, moderate enlargement of retropharyngeal and submandibular lymph nodes. All three animals had acute moderate pulmonary congestion and acute diffuse alveolar pulmonary edema. The euthanatized musk ox had a solitary, approximately 2 cm diameter chronic ruminal ulcer. Lesions on the Shetland sheep were restricted to the lips.

Skin samples obtained from all affected species revealed similar histologic lesions that consisted of epidermal hyperplasia with marked ballooning degeneration of superficial keratinocytes; lymphoplasmacytic dermatitis and numerous bacterial colonies. In all cases superficial keratinocytes contained variable sized cytoplasmic eosinophilic inclusion bodies, which were consistent with Bollinger bodies. Electron microscopy of fresh samples of the affected epidermis confirmed the presence of characteristic ovoid-shaped parapoxvirus virions approximately 200nm × 160nm in all affected species. Confirmation of these results was done by amplification of parapoxvirus DNA by the polymerase chain reaction.

Virus isolation was performed on skin lesions from all species and virus was isolated from musk ox and Shetland sheep samples, but not from Sichuan takin samples. Lack of virus isolation in takin samples was likely due to small sample size.

Because all parapoxviruses have similar virion morphology, DNA characterization was performed to confirm that this disease outbreak was the result of an ORFV. Viral DNA collected from lesions of animals affected in this outbreak was compared to known strains of ORFV.<sup>2</sup> By using a variety of techniques including DNA band pattern analysis and sequencing of particular genes, it was concluded that the proliferative dermatitis seen in the various ruminant species at the Minnesota Zoological Garden was caused by a single strain of ORFV.<sup>2</sup>

## **Discussion**

An outbreak of orf affecting animals belonging to three ruminant species (musk oxen, sheep and takin) is described. The occurrence of this outbreak during summer likely made disease control and supportive care of the animals more difficult due to myiasis and flies acting as potential mechanical vectors for the virus. This might account for the spread of the disease to different locations within the zoo as zoo personnel immediately implemented protocols, described previously, to control spread of the disease. The hot summer weather increased the

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risk of animals becoming hyperthermic while being immobilized for supportive care. Unfortunately, the long duration and severity of lesions caused anorexia and secondary bacterial infection, which contributed to the decline of the animals. Cidofovir (Vistide, Gilead Sciences, Foster City, CA 94404, USA) has shown marked in vitro inhibitory effects for parapoxviruses and may be useful as a treatment for ORFV infected animals in the future.<sup>8</sup>

The source of the ORFV in the outbreak reported here could not be determined although several possibilities exist. In 2000, the zoo opened a working farm that exhibits dairy cattle, sheep, goats, pigs, draft horses, poultry and rabbits. All animals acquired by the zoo are isolated from other animals during a quarantine period of at least 30 days. During this time animals are examined for evidence of health problems and communicable diseases and are not allowed to enter the collection if diseases are detected. No evidence of orf-like lesions had been seen in these domestic animals during quarantine or at any time since the farm exhibit opened. Although there is no evidence of latent virus infection in animals recovered from ORFV<sup>11</sup> it is possible some of the small ruminants recently introduced to the farm may have acted as physical carriers of the virus. Animal caretakers or visitors to the zoo could also have carried the virus into the zoo on clothing.

The zoo is located adjacent to a nearly 2000 acre county park, which contains numerous white-tailed deer (*Odocoileus virginianus*). Orf has not been reported in wild white-tailed deer; however, experimental exposure did cause clinical disease.<sup>6</sup> Although the suburbs have taken over the agriculture land which surrounded the zoo when it opened in 1978, in 2001 residents remained within a few miles of the zoo who had horses and quite likely sheep or goats. Therefore, the possibility that dry scab from affected wildlife or livestock in nearby areas was blown by the wind onto zoo premises cannot be eliminated as the potential source of this outbreak. The virus can survive for years in dry scab<sup>7,9</sup> and a remote possibility exists that it survived in a protected location within the musk ox exhibit since an outbreak occurred at the Minnesota Zoological Garden in September 1983. That outbreak was confined entirely to the musk ox herd and eight of the twelve animals in the herd were noted to have lesions.<sup>12</sup>

Although orf is a relatively common disease in sheep and has been reported in musk oxen,<sup>1,5,12</sup> this is the first reported case of orf in Sichuan takin. The introduction of ORFV (*Parapoxvirus ovis*) into a zoo creates a considerable risk for various ruminant species and visitors. As the present report shows, the range of ruminant species known to be susceptible to ORFV is growing and possibly all nondomestic ruminant species should be considered susceptible to the disease.

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## CONCURRENT EPIZOOTIC HEMORRHAGIC DISEASE AND BLUETONGUE OUTBREAK IN WHITE-TAILED DEER (*Odocoileus virginianus*) AND LIVESTOCK IN CENTRAL IDAHO, 2003

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### Abstract

Hemorrhagic disease (HD), which is caused by several related *Orbivirus*, including epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV), is a common disease of deer in the central and southern regions of the United States. Both viruses can infect cattle and sheep and are spread by *Culicoides* spp. Gnats. Both EHDV and BTV have been associated with severe disease in white-tailed deer (*Odocoileus virginianus*). In domestic livestock, only BTV has been associated with severe disease in sheep.

A large scale outbreak of EHDV-2 was confirmed in central Idaho along the Clearwater and Salmon Rivers in August and September 2003. Diagnosis was confirmed through necropsy, gross lesions, and virus isolation. Although mortality was widespread, it varied considerably in local areas with known or estimated mortality rates ranging from 20-90+%. About 10% of the total WTD population in central Idaho was affected with approximately 3000-5000 white-tailed deer estimated to have died. At least one mule deer was also confirmed to have died from EHDV-2.

Livestock, including sheep and cattle are present within most of the outbreak area and a flock of sheep in this area developed clinical signs consistent with BTV infection during the same time period. Serologic titers were found to both EHDV and BTV, however, virus isolation indicated BTV-17 as the cause of the clinical signs and limited mortality. EHDV-2 was isolated from one of 17 sheep in the flock. Domestic cattle in the outbreak area were not clinically affected. However, serology and virus isolation from two cattle herds in outbreak area indicated that the cattle were exposed to both BTV and EHDV, but only BTV-17 was confirmed by isolation.

An interesting aspect of this outbreak relates to the differences in virus isolation results between white-tailed deer and livestock species that reside within the same area. All of the virus isolations from white-tailed deer were confirmed as EHDV-2 while all but one isolation from sheep and cattle were identified as BTV-17. Further investigation is continuing and further monitoring of the deer population as well as gnat studies will be done in the near future.

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## ***Mycobacterium avium* ss. *paratuberculosis* IN FREE-RANGING BIRDS AND MAMMALS ON LIVESTOCK PREMISES**

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### **Abstract**

The paradigm that *M. paratuberculosis* (*Mptb*) infection is primarily a predicament for ruminants was broken when the organism was isolated from lagomorph, canid, mustelid, corvid, and murid species captured on John's disease-positive dairy farms in Scotland<sup>1</sup>. Whether any or all of these non-ruminant species represent reservoirs of the infection vs. dead end hosts is not yet known.

To better elucidate the epidemiology of *Mptb* infection under domestic agricultural husbandry protocols typical of two regions in the United States, we caught close to 100 individual wild animals on John's disease test-positive farms (7) and test-negative farms (2) in Wisconsin and Georgia. Approximately 50 different wild species (of which 20% were birds) typical of dairy or beef farming habitats were captured. One fecal sample and three gastrointestinal tissue samples from each animal were processed for radiometric culture within 24 hr of collection. The mycobacterial isolates were identified by mycobactin dependency, HPLC and genetic insertion sequence patterns. Analysis of multiple polymorphic genetic markers will be completed to characterize the phylogenetic similarity of *Mptb* strains isolated from wildlife and cattle on each premise. Histopathology will be completed for *Mptb* culture-positive tissues.

Samples are still incubating. To date, *Mptb* has been isolated from 28 animals representing diverse species such as domestic cats, raccoons, starlings plus an armadillo, skunk, shrew, sparrow and a Norway rat. A number of these animals were shedding the environmentally hardy organism raising two possibilities: (1) once established, a cycle of infection may be maintained within a variety of wildlife species independent of further exposure to infected domestic livestock and (2) infected wildlife may represent a risk factor for susceptible livestock (< 6 mo) through contamination of feed or forage.

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## ***Brucella abortus* AND ELK (*Cervus elaphus*): WHERE DO WE GO FROM HERE?**

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### **Abstract**

In the United States, elk (*Cervus elaphus*) serve as a reservoir for *Brucella abortus*, and pose a significant problem for wildlife managers and the cattle industry. Over the last 27 yr, seroprevalence among female elk surveyed on feedgrounds in the Greater Yellowstone Area has averaged approximately 34%.<sup>9</sup> Ballistic vaccination programs of feedground elk with *B. abortus* strain 19 (s19) were implemented in the 1980's and continue with notable success.<sup>9</sup> However, research has been ongoing to produce an alternative vaccine regimen to s19, given the fact that it produces erroneous positive results on routine brucellosis surveillance tests in animals that have received the vaccine.<sup>3</sup> *B. abortus* strain RB51 (sRB51) is an attenuated rough mutant that is currently being used in domestic cattle as a calfhood vaccine. sRB51 does not induce antibodies to the lipopolysaccharide O-side chain epitopes and thus does not elicit false positives on serologic tests.<sup>8</sup> The results of research conducted by many of our colleagues in the last decade have indicated that, although safe, parenteral sRB51 is not efficacious in protecting elk from abortion in the face of challenge with virulent *B. abortus*.<sup>1,2,5,6</sup> This observation comes despite this species' ability to mount a significant IgG antibody response to the vaccine.<sup>6,7</sup>

At the National Animal Disease Center, we have recently investigated the immune response to both sRB51 and s19 in captive elk, specifically looking at total IgG titers, as well as at antigen stimulated proliferation of peripheral blood mononuclear cells (PBMC) in vaccinated animals. Results show that IgG levels appeared to peak 4-6 wk after both single vaccination and booster given 65 wk post-prime. Proliferation of PBMC in the face of both RB51 and S19 antigens was predominantly of the B cell type. These data indicate that elk produce an immune response to current *Brucella* vaccines that is clearly of a humoral quality. It is widely believed that elimination of *Brucella abortus* infection requires a strong cell mediated immune (CMI) response.<sup>7</sup> Therefore, a vaccine regimen capable of producing such a response will likely be more effective in protecting elk from *Brucella*-induced abortions. Future work to be done at the National Wildlife Research Center (NWRC) includes the use of an adjuvant (AdjuVac) developed at the NWRC, consisting of modified Johne's vaccine in an oil and water emulsion, to determine whether sRB51 administered with this adjuvant may elicit the desired CMI response with long-lasting duration. Various recombinants of RB51 will also be tested for their efficacy in elk as well.

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## CHARACTERIZATION OF IMMUNOLOGIC RESPONSES TO BRUCELLOSIS AND TUBERCULOSIS VACCINES IN WILD UNGULATES AND DOMESTIC CATTLE

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### Abstract

Estimates for the seroprevalence of brucellosis in bison (*Bison bison*) in Yellowstone National Park, or elk (*Cervus elaphus*) on feedgrounds in the Greater Yellowstone Area, are between 40 and 50%. In a similar manner, in Michigan the prevalence of tuberculosis in white-tailed deer (*Odocoileus virginianus*) in some areas is as high as 10-15%. The high prevalence of these diseases in wildlife reservoirs is of concern due to the possibility of transmission of these pathogens to domestic livestock in which they have almost been eradicated by regulatory programs. Long term protection against intracellular pathogens such as *Brucella* spp. or *Mycobacterium bovis*, is predominantly through cell-mediated immunity. In a series of studies conducted at the National Animal Disease Center, immunologic responses of bison, elk, white-tailed deer, cattle, and reindeer (*Rangifer tarandus tarandus*) were evaluated after vaccination with brucellosis or tuberculosis vaccines, or following experimental infection with virulent strains of *B. abortus* or *M. bovis*. Bison and cattle have robust immunologic responses to brucellosis vaccines with strong humoral and cell-mediated responses, although the temporal interferon- $\gamma$  (IFN- $\gamma$ ) responses differed. In a similar manner, white-tailed deer develop robust humoral and cell-mediated responses following vaccination with *M. bovis* bacille Calmette-Guerin (BCG) or infection with a virulent strain of *M. bovis*. In comparison, elk and reindeer develop strong humoral responses following vaccination with BCG or brucellosis vaccines, but measurements of cellular immunity suggest very poor responses that are transient, and associated with low levels of IFN- $\gamma$  production. Our data suggests that immunologic responses may markedly vary between species. We hypothesize that these immunologic responses explain differences in susceptibility to infection and vaccine efficacy, and may reflect evolutionary selection against natural pathogens. Our findings may have implications for management decisions on programs to control or eliminate intracellular pathogens within captive or free-ranging populations.

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**EXPERIMENTAL INFECTION OF REINDEER (*Rangifer tarandus*) WITH *Mycobacterium bovis*: PATHOLOGIC AND IMMUNOLOGIC FINDINGS**

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**Abstract**

In the United States all species of Cervidae are included in the USDA's uniform rules and methods for the eradication of bovine tuberculosis and; therefore, are subject to regulations regarding intradermal tuberculin testing. In reindeer, infection with *M. bovis* is exceedingly rare. The objectives of the present study were to describe the pathologic changes associated with *M. bovis* infection in reindeer and evaluate the effectiveness of intradermal tuberculin testing and an in vitro blood based assay for interferon- $\gamma$  (IFN- $\gamma$ ) as means of diagnosis of tuberculosis in reindeer. Eleven reindeer were inoculated intratonsilarly with  $2 \times 10^4$  CFU of *M. bovis* while four non-inoculated reindeer served as negative controls. The comparative cervical test (CCT) was done on all reindeer 3 and 8 mo after inoculation. Blood was collected monthly for IFN- $\gamma$  analysis. Thirteen months after inoculation, all reindeer were euthanatized and examined. Various tissues were collected for bacteriologic culture and microscopic examination. All experimentally inoculated reindeer developed lesions in the medial retropharyngeal lymph nodes. Tracheobronchial, mediastinal and mesenteric lymph nodes, tonsils and lungs were less frequently affected. The CCT accurately identified *M. bovis* inoculated reindeer, but false positive results were common among negative control reindeer. Modifications in the USDA's method for interpretation of the CCT decreased false positive results without increasing false negative results. An in vitro blood-based assay to measure IFN- $\gamma$  production showed that mycobacteria-specific IFN- $\gamma$  responses from *M. bovis*-infected reindeer exceeded those of negative control reindeer. However, positive IFN- $\gamma$  responses to *M. bovis* purified protein derivative (PPDb) were also detected in negative control reindeer. ESAT-6 and CFP-10 are antigens unique to *Mycobacteria* spp. within the tuberculosis complex. While use of these antigens did not diminish detection of *M. bovis*-infected reindeer, it did decrease false positive results in negative control reindeer. Reindeer are susceptible to infection with *M. bovis*; however, lesions are fewer in number, less severe in nature and less widely disseminated than those seen in white-tailed deer similarly inoculated. Comparative cervical skin testing of reindeer can be highly sensitive, but has low specificity. Specificity can be improved by modification of criteria for interpretation of the CCT. A blood-based IFN- $\gamma$  assay may prove useful for tuberculosis

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diagnosis when recombinant CFP-10 or ESAT-6 / CFP-10 antigens are used to enhance the specificity of the IFN- $\gamma$  assay.

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## MANAGEMENT OF BOVINE TUBERCULOSIS IN RIDING MOUNTAIN NATIONAL PARK, CANADA

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### Abstract

Bovine TB caused by *Mycobacterium bovis* is becoming a disease of major concern in free-ranging ungulate populations in North America. An adaptive management program to determine the prevalence and eventually eradicate bovine TB in elk, white-tailed deer and cattle has been instituted in the region of Riding Mountain National Park in south-western Manitoba, Canada. Measures to help reduce the prevalence of bovine TB have included barrier fencing for hay storage yards, legislation changes to make baiting cervids outside the park illegal, enhanced surveillance of cattle and farmed bison surrounding the park, increased harvest of elk through liberalized hunting seasons, and targeted herd reduction of elk within the park. The apparent prevalence of bovine TB in elk in the western half of RMNP based on preliminary blood sampling using net gun capture and parallel interpretation of a lymphocyte stimulation test (LST), fluorescence polarization assay (FPA) and polymerase chain reaction (PCR) is approximately 35%. Selective removal of 87 elk, including 37 elk with suspicious blood results, followed by necropsy and bacterial culture of tissues confirmed bovine TB in 15% of elk. Ongoing studies will attempt to determine the effectiveness of management actions, determine the prevalence of bovine TB in the RMNP regional elk and white-tailed deer populations, identify potential spillover hosts in and around the park, and improve methods for the diagnosis of bovine TB.

### Introduction

A focus of bovine tuberculosis (*Mycobacterium bovis*) has recently been identified in free-ranging elk (*Cervus elaphus manitobensis*), white-tailed deer (*Odocoileus virginianus*) and domestic cattle in the area of Riding Mountain National Park (RMNP) in south-western Manitoba, Canada.<sup>1</sup> Historically, cattle grazing was common in the park up until 1970 and tuberculosis was known to be common in the area in the early 1900's. Bovine tuberculosis was considered to be not uncommon in cattle in the 1950's and 1960's and there were four outbreaks in a total of eleven cattle herds around RMNP Since 1991.<sup>2</sup> This resulted in the creation of the Riding Mountain TB Eradication Area (RMEA) to detect and control the spread of bovine TB in cattle herds in January of 2003. In 1992 an adult bull elk was found dead which tested positive

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for bovine TB after which time a passive surveillance program using samples provided by hunters identified an additional eight elk and one white-tailed deer with bovine TB.<sup>1</sup> In response the Interagency TB Task Group was formed in 2000 consisting of personnel from the Canadian Food Inspection Agency, Parks Canada Agency, Manitoba Agriculture and Food, and Manitoba Conservation with input from local stakeholder groups. A Wildlife Health Action Plan was developed consisting of a 5-yr TB management strategy and implementation plan. The vision is to eradicate bovine TB from the greater Riding Mountain ecosystem and the long-term goals are to achieve and maintain bovine TB-free status in domestic cattle; to eradicate bovine TB in wildlife that may pose a risk to agriculture; to minimize wildlife-livestock interactions in the Riding Mountain region; and to minimize unnatural cervid herding behavior which occurs where cervids feed on agricultural produce, thereby minimizing the potential for disease transmission. The following paper will describe management actions and research initiated since 2000 to reduce the spread of bovine TB and to understand the epidemiology of bovine tuberculosis in RMNP.

## Methods

Three hundred-and-three elk were captured to determine disease prevalence and geographic clustering within the park by helicopter net gunning<sup>3</sup> in 2002, 2003 and 2004. Forty elk were captured in 2002, 113 in 2003 and 150 in 2004. Elk captured in 2002 and 2003 were primarily from the west side of RMNP while in 2004, 50 elk were captured on the west side and 100 were captured on the east side of RMNP. GPS or VHF radio collars or ear tag transmitters were affixed to animals to determine habitat use and movement within and around the park.<sup>4</sup> Blood was drawn by jugular venipuncture into sterile Vacutainer™ tubes with lithium heparin, sodium EDTA or nothing. Samples were kept at room temperature until analyzed within 48 hr or serum was separated by centrifugation and frozen. Parallel interpretation of three different blood tests was used to evaluate *M. bovis* status; a lymphocyte stimulation test (LST), a fluorescence polarization assay (FPA) and a polymerase chain reaction (PCR). Although blood was collected from all elk captured, only 229 samples were of sufficient quality or quantity to permit completion of all three tests. The remaining 74 samples were analyzed with one or two tests only. Forty of 153 elk captured from February 2002 to March 2003 tested positive on at least one of the three blood tests. Thirty-seven of these elk were re-captured in April 2003 and killed by anesthesia and intravenous potassium chloride administration. A sub-sample of fifty of 110 elk that tested negative on the blood tests were re-captured in December 2003 and killed by captive bolt gun and exsanguination. A complete necropsy was performed on all killed elk. All identifiable lymph nodes from the head, thorax, abdomen and peripheral limbs were harvested fresh and in 10% buffered formalin. Bacterial culture and acid-fast staining were completed on lymph nodes and any other tissues with visible lesions suggestive of bovine TB. Animals were considered positive if *M. bovis* was isolated in pure culture from at least one lymph node or suspicious tissue sample or a positive PCR result was obtained from tissue.

## Results and Discussion

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Several management actions were initially undertaken to attempt to reduce the prevalence and further spread of bovine TB within the RMNP ecosystem. A barrier fencing program was initiated in 2002 to fence winter haystacks around the park and prevent contact between cattle and elk. A total of 72 8-foot high page wire barrier fences were erected with funding provided by the province of Manitoba and Parks Canada which has now fenced approximately 90% of hay storage yards within 1.6 km of the park boundary. An ongoing education program has also been actively encouraging landowners bordering the park to remove hay bales from fields prior to the onset of winter and clean up hay storage yards. Hunting seasons in the two game hunting areas (GHA 23 and 23A) surrounding the park were lengthened and additional permits given out to help in herd reduction efforts.

Legislation was also changed within the Manitoba Wildlife Act in 2002 to make the baiting of elk with either intentionally placed bait or the use of natural forage as bait outside the park illegal. Baiting and supplemental feeding of white-tailed deer has been implicated as a major risk factor in increasing the prevalence of bovine TB in white-tailed deer in Michigan.<sup>5</sup> Provisions were made to allow wildlife officers to determine what was reasonably considered “bait” and to take measures to either fence or remove the bait. Hunting is not allowed within 800 metres of a cervid bait site within Manitoba Game Hunting Areas (GHA) 23 and 23A which surround the park on the north and south. Provisions were also made to provide additional protection for wolves (*Canis lupus*), which are the main predators of elk in RMNP to allow natural predator-prey mechanisms to remove diseased animals. Ongoing studies are in progress to further understand the predator-prey dynamics and dispersal of wolves within the RMNP ecosystem. Prescribed fire was also used as a management tool to improve elk forage habitat within the park and keep animals within the park boundaries during the winter months when forage availability is limited.

Special regulations were enacted to restrict the movement of cattle in and out of the RMEA in January 2003 in which a special TB management zone was created. Within the RMEA there are 55,000 breeding cattle on approximately 650 premises representing approximately 10% of Manitoba’s cattle herds and approximately 1% of Canadian cattle herds.<sup>6</sup> In Manitoba, the RMEA is classified as TB-Accredited-Advanced according to current Canadian standards while the rest of Manitoba and Canada have been classified as TB-Free since 1997. A movement permit, based on a negative herd test and/or individual animal testing, has been required since January 1, 2003 to remove farmed bovids and cervids from the RMEA into other areas. From 1997 to 2002, cattle surveillance had involved the testing of all cattle herds in a 10-kilometer zone around positive cervid cases, and the testing of previously untested herds in a 6-kilometer zone around the western boundary of the park. In the fall of 2002, surveillance testing was expanded to encompass the regular testing of all cattle and farmed bison herds located within the RMEA every 12 to 36 mo, resulting in the detection of three infected herds.<sup>2</sup>

An elk movement study was undertaken in 2002 to determine seasonal elk movements in and out of the park. Two broad groups of elk have been defined so far with Type I elk having been primarily located inside RMNP (<5% of locations outside of RMNP) and Type II elk having

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been primarily located both inside and outside the park (>5% of locations outside park).<sup>7</sup> Based on the first 2 yr of telemetry data, 61% of the collared elk have been located outside RMNP at least once with females using areas outside the park more than males. Elk have been documented using areas outside of RMNP more during the spring and summer months than in the winter months.<sup>4</sup>

Targeted herd reduction has been instituted within the park boundaries resulting in the removal of 87 elk in 2003 and another 27 elk in March 2004. Among animals that were removed in 2003, bovine TB was confirmed through culture or PCR on tissues in 13/87 (14.9%) animals, 8 (61.5%) which were adult females and 5 (38.5%) which adult males. These animals were primarily from the west end of the park where a geographic focus of bovine TB in elk had been previously identified.<sup>1</sup> The apparent prevalence (AP) of bovine TB in elk captured on the west side of RMNP based on parallel interpretation of the three blood tests was 30/86 (34.9%), while in sampling throughout the entire park in 2004 it was 23/143 (16.1%). Test results from the 2004 removals are pending.

Passive surveillance of hunter-killed and road and predator-killed elk, white-tailed deer and moose (*Alces alces*) was also carried out in the areas surrounding the park since 1992. Samples from 10 of 1463 (0.7%) elk, 1 of 1079 (0.09%) white-tailed deer and none of 557 (0%) moose were culture positive for *M. bovis* between 1992 and 2002.<sup>1</sup> Prevalence of bovine TB in the two rural municipalities on the west end of the park was estimated to be 2.5% and 2.9% respectively based on this type of sampling.<sup>1</sup> As previously identified, this type of surveillance seems to underestimate the true prevalence of disease especially when animals are not collected directly from within geographic foci within in the park. In contrast, passive surveillance for bovine TB has been successfully used to determine trends in prevalence in Michigan in free-ranging white-tailed deer and elk with very little bias due to the ability to directly sample all areas uniformly.<sup>8</sup>

Continuing research will focus on the role of wolves in the spread and control of bovine TB, improving bovine TB diagnostic methods in wolves and elk, further clarification of the geographic extent of the infection as well as the identification and role of other species as reservoir and spillover hosts for bovine TB in the region.

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## EFFICACY OF LIVESTOCK PROTECTION DOGS FOR DETERRING DEER FROM CONTACTING CATTLE

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### **Abstract**

Bovine tuberculosis (*Mycobacterium bovis*) (TB) is established in wild white-tailed deer (*Odocoileus virginianus*) in the northeastern portion of Michigan's Lower Peninsula. The NWRC is developing and evaluating means to potentially minimize direct and indirect contact between potentially infected deer and livestock. One research endeavor has involved livestock protection dogs. Dogs have been used successfully for thousands of years to reduce predation on livestock, primarily sheep. We evaluated the ability of livestock protection dogs to minimize contact between wildlife and cattle. We conducted the study within the TB-endemic area of Michigan on 2 privately-owned deer farms. Both farms contained unnaturally high deer densities (243 deer/km<sup>2</sup> and 93 deer/km<sup>2</sup>), insuring a challenging evaluation of the dogs. Protected pastures contained a dog and 4 calves and unprotected pastures contained just 4 calves. We used 3 methods of data collection to establish how effective the dogs were: direct observations, motion-activated video, and track plots. Through direct observations, we documented deer using cattle feed 113 times in unprotected pastures and never in a protected pasture. Deer came within 5 m of cattle 79 times in unprotected pastures and 3 times in protected pastures. From video data, we found the dogs to be effective in virtually eliminating deer use of cattle feed (protected = 2 deer visits, unprotected = 303 deer visits), direct contact within 5m (protected = 0 deer visits, unprotected = 114 deer visits), and deer use of cattle pastures (protected = 3 deer visits, unprotected = 426 deer visits). Through the use of track plots, we also found less deer use of protected pastures (protected = 278 deer visits, unprotected = 1,020 deer visits). Currently, we are evaluating the efficacy of the dogs on actual cattle operations in the endemic area.

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## MOVING CONSERVATION *AHEAD* (ANIMAL HEALTH FOR THE ENVIRONMENT AND DEVELOPMENT): PROGRESS AT THE INTERSECTION OF PROGRAM AND POLICY

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### Abstract

Our organizations hosted a highly interactive forum at which invited Southern and East African and other experts shared their vision for conservation and development success at the wildlife / livestock interface with IUCN World Parks Congress attendees and invited representatives from bilateral and multilateral development agencies and other interested parties. African governmental and nongovernmental experts from Botswana, Kenya, Malawi, Mozambique, Namibia, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe participated.<sup>1</sup> Our goal was to foster a sharing of ideas among African practitioners and development professionals that will lead to concrete and creative initiatives that address conservation and development challenges related to health at the livestock/wildlife/human interface. The focus was, appropriately, on ongoing efforts and future needs in and around the region's flagship protected areas and conservancies and their buffer zones- the places where tensions and challenges at the livestock/wildlife interface are often greatest.

Discussions and planning focused on several themes of critical importance to the future of animal agriculture, wildlife, and, of course, people: competition over grazing and water resources, disease mitigation, local and global food security, zoonoses, and other potential sources of conflict related to the overall challenges of land-use planning and the pervasive reality of resource constraints. We have since been working to develop the most promising collaborative concepts that emerged from this forum into a suite of projects, grounded in real landscapes but cognizant of the critical need for policy reform, and based on the solid professional partnerships we believe are emanating from the *AHEAD* (Animal Health for the Environment And Development) enabling environment.

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<sup>1</sup> The WCS*AHEAD* website is at [www.wcs-ahead.org](http://www.wcs-ahead.org) and includes the complete agenda from the World Parks Congress (Durban) *AHEAD* launch, abstracts of presentations, the presentation slidesets themselves, biographical sketches and contact details for most of the invitees, as well as a range of downloadable video and audio clips from the forum.

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As we look around the world, impacts from interactions between livestock and wildlife (and habitat) are often profound. The issues at this interface represent an unfortunately all-too-often neglected sector of critical importance to the long-term ecological and sociopolitical security of protected areas and grazing lands worldwide. With its initial focus on Southern and East Africa and its diverse land-use mosaic, we believe the *AHEAD* initiative can help facilitate collaborative work with and among African partners to continue to bring sound science to bear on natural resource management decisions that directly affect the livelihoods and cultures of Africa's people, including those decisions that impact the future of Africa's protected areas and wildlife resources. As socioeconomic progress demands sustained improvements in health for humans, their domestic animals, and the environment, we recognize the need to utilize a "one health" perspective—an approach that was the foundation of our discussions at the World Parks Congress, and that has guided the follow-on work since.

Since the September 2003 program launch, *AHEAD* has helped catalyze the development of several innovative regional projects that focus on the health / conservation nexus. In addition, the importance of these issues was formally recognized by the IUCN World Parks Congress when it officially included "Disease and Protected Area Management" as a key emerging issue in its "Emerging Issues" documentation:

(<http://www.iucn.org/themes/wcpa/wpc2003/english/outputs/durban/eissues.htm>), which is the first time ecosystem health issues have been addressed like this in the Congress' 40-yr history. The text from the "Disease and Protected Area Management" section is below.

### **Disease and Protected Area Management**

The health of wildlife, domestic animals and people are inextricably linked.

Small improvements in the health of domestic and wild animals and thus their productivity can lead to dramatic improvements in human livelihoods and thus the reduction of poverty.

Alien invasive pathogens should be addressed with vigor equal to that devoted to addressing more 'visible' alien invasive species.

The role of disease in protected areas and the land-use matrix within which they are embedded must be recognized and addressed within the context of protected area and landscape-level planning and management.

Animal and human health-based indicators may reveal perturbations to natural systems not detectable by more commonly employed methodologies, thus improving the quantitative evaluation of trends in a protected area's health and resilience.

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## MOVING THE BOUNDARIES: ENABLING VETERINARY INVOLVEMENT IN CONSERVATION THROUGH EDUCATION

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### **Abstract**

There are many boundaries which may hinder the ability of veterinarians to contribute effectively to wildlife conservation initiatives. This paper discusses veterinary educational initiatives at Murdoch University that are moving disciplinary, institutional, cultural, experiential and educational boundaries to train veterinary students and graduate veterinarians in wildlife, zoo and conservation medicine. The collaborative partnership with the Veterinary Department at Perth Zoo, which resulted in the establishment of several of these educational initiatives, will be discussed.

### **Introduction**

Biodiversity conservation poses enormously complex challenges which are further compounded by the fact that in most cases decisions must be made quickly and in the face of incomplete data. The challenges can only be addressed through interdisciplinary approaches involving diverse expertise; effective communication and exchange of knowledge; the ability to reconcile polarised views and reach consensus; and the efficient use of limited finance and resources.<sup>3,8</sup>

Veterinarians have a significant role to play within interdisciplinary teams working on biodiversity conservation projects.<sup>3,8,11</sup> Wildlife veterinarians can contribute in a significant manner to the planning and implementation stages of wildlife conservation projects. Private veterinary practitioners can also have a pivotal role to play in biodiversity conservation, since they are literally 'at the coal face' dealing with members of the community on a daily basis. Private practitioners are not only in a position to tend to injured and ill wildlife clinical cases, but also can provide advice to ensure that wildlife rehabilitation efforts are ecologically sound and can collaborate with local government agencies and community-based conservation groups on wildlife conservation projects.

Veterinary involvement in biodiversity conservation projects may include: health assessment and monitoring of wildlife and/or domestic animal populations; health studies of zoonotic, anthrozootic and interspecies transmission of diseases, involvement with welfare, regulation and production aspects of wildlife utilization programs, training and capacity building in developing countries, interdisciplinary collaboration, data collection and management, research,

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development of diagnostic capabilities to improve identification of disease agents in wildlife, in-situ and ex-situ management of threatened species, planning of export and import procedures of wildlife species, and policy development at a local, national and international level.<sup>3,4,6,12</sup>

## **Discussion**

In Australia, although many veterinarians have been interested in wildlife conservation, the concept of active and worthwhile involvement in biodiversity conservation has seemed difficult to achieve.<sup>5</sup> There are many boundaries which may hinder the ability of veterinarians to contribute effectively to wildlife conservation initiatives.

This paper discusses veterinary educational initiatives at Murdoch University, in Perth Western Australia, that are moving boundaries to train veterinary students and graduate veterinarians in wildlife, zoo and conservation medicine. Several of these educational initiatives have been established as the result of collaboration with the veterinary department at Perth Zoo. The following educational initiatives will be discussed in this paper:

Postgraduate studies in conservation medicine (Master of Veterinary Studies in Conservation Medicine and Postgraduate Certificate in Veterinary Conservation Medicine).

Wildlife and zoo medicine clinical rotation for undergraduate students.  
Postgraduate residency program based at Perth Zoo.

Conservation medicine field trip to Indonesia for undergraduate students.  
Wildlife medicine externships for undergraduate students.

The boundaries that confront many veterinarians and that will be discussed in terms of these educational initiatives are disciplinary, institutional, cultural, experiential and educational boundaries.

### **Disciplinary**

Collaboration between scientists from a range of disciplines is required to address the biologic, political, economic and social aspects of biodiversity conservation problems.<sup>8</sup> Karesh, et al. (2002) emphasise the importance of constructing bridges “to connect castles of disciplinary knowledge” in order to ensure successful conservation outcomes. In order to effectively contribute to biodiversity conservation, wildlife veterinarians must be able to see the big picture and develop a global viewpoint.<sup>2,4,12</sup> Wildlife veterinarians should not only have a sound understanding of epidemiology, wildlife biology and management, but should also be familiar with principles from other disciplines that are relevant to biodiversity conservation.

Conservation professionals often find themselves exploring a diverse range of disciplines, which are new and unfamiliar to them, in order to implement conservation initiatives. The postgraduate

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programs in conservation medicine provide veterinarians with training and expertise, which can be applied in private practice, zoos and wildlife conservation projects. Students undertaking the Master of Veterinary Studies in Conservation Medicine are able to select electives from the disciplines of biologic, environmental and social sciences. The issues covered by these elective units are pertinent to biodiversity conservation, and often form the basis upon which the success of conservation projects is dependent.

Postgraduate students in the conservation medicine program also have the opportunity to undertake a field placement with a conservation project either in Australia or overseas. These placements enable graduate students to work in the field with wildlife veterinarians and biologists and therefore directly experience and appreciate the necessity of an interdisciplinary approach to wildlife conservation.

### Institutional

Institutional boundaries also inhibit collaboration on biodiversity conservation. It is crucial that institutions working towards similar conservation goals collaborate to ensure that expertise is shared and limited resources are used effectively.

The southwest of Western Australia is internationally recognised as one of 25 Global Biodiversity Hotspot regions. This region is also recognised for the threats to its wildlife and the severity of its environmental degradation, which pose significant challenges to biodiversity conservation. This places a special responsibility on the institutions involved with managing this biodiversity. Close collaborative links have been established between Murdoch University, Perth Zoo and the Western Australian Department of Conservation and Land Management in efforts to address some of these challenges, through long-term health monitoring research projects associated with several endangered fauna recovery programs and through these new educational initiatives.

Murdoch University and Perth Zoo are currently collaborating to establish and offer the postgraduate programs in conservation medicine, the undergraduate wildlife and zoo medicine clinical rotation, the postgraduate residency program and the wildlife externship program for undergraduates. In establishing the undergraduate Wildlife and Zoo Clinical Rotation, both institutions recognized the need to incorporate training in wildlife medicine in the undergraduate curriculum for veterinary students. As a result, a collaborative program was developed that involves all fifth-year students undertaking a rotation in wildlife medicine in a teaching facility which is based at Perth Zoo. The development of this clinical rotation, which represents an innovative teaching initiative in the Australasian region, exposes the students to their responsibilities in treating wildlife and trains them in the basic principles necessary to deal with sick and injured wildlife, which could be presented for examination and treatment by veterinarians in private practice.

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The on-line postgraduate programs in Conservation Medicine are offered to provide veterinarians in practice with training in the fields of wildlife and conservation medicine. Collaborative associations have also been established with a number of wildlife agencies, conservation projects and zoos in Australia and overseas, to enable postgraduate conservation medicine students to undertake a placement at one of these institutions.

### Interpersonal/Cultural

Veterinarians are usually respected and influential societal members within a community and are therefore in a position to engage in informed debate concerning environmental issues, with the primary goal being to effect change. Veterinarians can only effectively engage in such a debate if they have a holistic approach to veterinary medicine, good knowledge of the relevant issues, good inter-personal skills and are able to listen to differing points of view from stakeholders and reach consensus on important environmental issues.<sup>5,8,9</sup>

In developing countries, issues of poverty and rural development are intertwined with those of biodiversity conservation; and as such biodiversity conservation initiatives must address socioeconomic issues of the rural poor in developing countries if they are to have a chance of success. Foreign involvement in wildlife conservation projects in developing countries has been associated with the transfer of technology and practices from developed countries, which often turn out to be inappropriate, impractical and unsustainable within the context of a developing country.<sup>10</sup> Foreign veterinarians can play an important role in wildlife conservation projects in developing countries as long as they are culturally sensitive, have a good understanding of socioeconomic issues in these countries, and avoid the detrimental pitfalls of value judgments associated with ecological imperialism. Foreign veterinarians working in such projects should focus their efforts on “capacity building and education so that local people can become more involved in conservation programs and see the relevance of conservation in their own lives.”<sup>10</sup> The postgraduate programs in conservation medicine highlight the interdependence of environmental conservation and sustainable development, particularly in poor rural communities. The field placements, associated with the postgraduate programs, enable students to work collaboratively with local veterinarians in developing countries. The field trip to Indonesia gives students exposure to basic issues in conservation medicine in a developing country context.

### Experiential

Until recently, veterinarians in Australia have not been trained for involvement in biodiversity conservation projects and Keefe (1997) argues many of these veterinarians are “blinkerered” as a result.

The standard training that veterinarians receive in the areas of problem-solving, acquisition of technical knowledge, development of diagnostic plans and communication, provide them with skills and expertise which can be applied to wildlife conservation projects. However, there is often a nervous apprehension among private practitioners about their ability to treat wildlife

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patients and contribute effectively to biodiversity conservation issues. Keefe (1997) challenges veterinarians about these misplaced apprehensions and states, “if you must do but one violent action in your life, let it be the ripping off of those blinkers and the casting of them aside.”

The educational programs outlined here aim to provide veterinary students and graduate veterinarians with the basic expertise required to work in the fields of wildlife, zoo or conservation medicine.

### Educational

The postgraduate programs in conservation medicine are flexible in their program structure so that students can select electives that are relevant to work in private practice, zoos or wildlife conservation projects. These programs are offered in both internal and external mode; and can be undertaken by full-time or part-time study. The fact that these programs can be studied via distance education has provided greater access for veterinarians that are not able to leave the work place in order to take up full-time study. It is hoped that in addition to training specialists who will find full-time careers in wildlife medicine, this degree will enable veterinarians in rural and urban private practices to extend their work to include involvement in the development and implementation of effective wildlife conservation policy and practice.

Veterinary involvement is considered critical to the success of wildlife conservation programs that are planned and implemented by governmental and non-governmental organizations.<sup>1,7,13</sup> This paper has discussed several educational initiatives offered by Murdoch University. These educational programs are moving boundaries, which have traditionally impeded veterinary involvement in biodiversity conservation programs, and aim to train veterinarians to effectively participate in *in-situ* wildlife conservation projects.

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**TRANSLOCATING THE CRITICALLY ENDANGERED PO`OULI (*Melamprosops phaeosoma*)**

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**Abstract**

The Po`ouli, an endemic Hawaiian honeycreeper, was first discovered on the island of Maui in 1973.<sup>1</sup> At that time the population was estimated to number less than 200 birds.<sup>1</sup> Since its discovery, the species has declined to the point that surveys conducted between 1997 and 2000 estimated only three birds remained in the upland forests of east Maui,<sup>2</sup> each occupying a geographically distinct home range. In 1999 a joint State and Federal Environmental Assessment (EA), prepared by the Hawaii Department of Land and Natural Resources and the U.S. Fish and Wildlife Service, explored options for recovering this critically endangered species.<sup>3</sup> Molecular techniques and morphometric data used to determine the gender of each bird suggested the group was comprised of two females and one male.<sup>4</sup> Translocation of one female to the male's home range was chosen as the most appropriate management option.

In April 2002, one of the two females was captured and translocated to the male's home range. Radiotelemetry was used to monitor the bird's movements for a total of 10 days. Within 24 hr after its release, biologists discovered that the bird had returned to its original home range. Subsequent tracking and observations revealed no evidence that it had developed any association with its male conspecific. In this instance, translocation followed by a hard release failed to create a socially interactive pair of Po`ouli.

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## EPIZOOTIOLOGY AND MANAGEMENT OF FELINE LEUKEMIA VIRUS IN FREE-RANGING FLORIDA PANTHERS: PRELIMINARY RESULTS

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### Abstract

Routine testing for feline leukemia virus (FeLV) antigen has been negative in Florida panthers (*Puma concolor coryi*) since 1983. However, between November 2002 and March 2004, four of 43 (9%) free-ranging Florida panthers tested positive for FeLV antigen. Two had a peripheral lymphadenopathy and a moderate to severe non-regenerative anemia at capture; all died within 2 wk to 5 mo of diagnosis. Antibody titers were determined for serum collected from panthers captured between 1990 and 2003; 18 of 223 (8%) were positive, with the percentage of positive titers increasing sharply beginning in 1997. Preliminary results of polymerase chain reaction (PCR) performed on archived tissues indicate virus or provirus to be present in eight panthers. Positive antigen, antibody, and PCR results were primarily from panthers sampled in the northern portion of panther range. Genetic sequencing indicates the virus to be subgroup A. Positive antibody titers and PCR results in antigen negative panthers indicate that at least some panthers do not become persistently infected. A vaccination program in free-ranging panthers was begun in August 2003, and as of 19 March 2004, 18 free-ranging panthers have been vaccinated.

### Introduction

Feline leukemia virus (FeLV) is a retrovirus of domestic cats (*Felis silvestris catus*) that can cause anemia, neoplasia, and/or immunosuppression. Infection in wild cats is rare and has been primarily limited to case reports involving captive felids.<sup>13,15</sup> Reports in free-ranging non-domestic cats include a mountain lion (*Puma concolor*) from California (USA)<sup>6</sup> and a sand cat (*F. margarita*) from Saudi Arabia.<sup>10</sup> Ten to 24% of European wildcats (*F. silvestris silvestris*) were also FeLV positive,<sup>2,3</sup> although interbreeding with domestic cats occurs frequently in this subspecies.<sup>1</sup>

The Florida panther (*P. concolor coryi*) is a remnant population numbering approximately 80 individuals in southern Florida. This population has been the focus of intense research and

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genetic management since 1981. Routine testing for FeLV antigen in all captured free-ranging Florida panthers has been negative since 1983;<sup>11,12</sup> however, the finding of two FeLV antigen positive panthers during the 2002-2003 capture season prompted an investigation and management program. The objectives of this presentation are to describe the epizootiology of FeLV infection in the Florida panther and discuss efforts to eradicate the disease.

## Methods

Adult and juvenile Florida panthers and Texas cougars were captured by the Florida Fish and Wildlife Conservation Commission and National Park Service using trained hounds, chemically immobilized, and fitted with radio-collars. Neonatal kittens were handled at 1-3 wk of age in their natal dens. Biomedical samples collected from panthers included whole blood, skin biopsies, and hair. Other samples were taken as indicated.<sup>12</sup> Enzyme-linked immunosorbent assay (ELISA) antigen tests were performed at Cornell University (Ithaca, NY), and positive tests were confirmed by immunofluorescent antibody (IFA) at the National Veterinary Laboratory (Franklin Lakes, NJ). Immunohistochemistry of archived tissues from necropsied panthers is pending. ELISA antibody titers were determined at Hansen Veterinary Immunology (Dixon, CA). Polymerase chain reaction (PCR) and genetic sequencing were performed at the Laboratory for Viral Carcinogenesis (Frederick, MD).

To assess the safety and antibody response to vaccination, three Texas cougars and three Florida panthers underwent a vaccine trial while in captivity at White Oak Plantation. Test subjects were immobilized on three occasions at 3-4 wk intervals. Two ml of Fel-O-Vax® Lv-K (Fort Dodge Animal Health, Fort Dodge, Iowa, USA) were administered intramuscularly. Serum samples for ELISA antibody titers were collected at each immobilization and subjects were monitored for adverse reactions.

## Results and Discussion

Since October 2002, four of 43 (9%) panthers have tested FeLV ELISA antigen positive. All positive panthers were located in Okaloacoochee Slough (OKS) in the northern portion of panther range and had overlapping home ranges. Positives represented 50% (4 of 8) of panthers captured in OKS since October 2002. All were adults between 2 and 11 yr of age and most were positive at initial capture. One panther (FP109), an 11-yr-old adult, tested negative when captured in 2002 but positive when captured 1 yr later. Two (FP122, 123) of the four antigen positives tested IFA positive, and two were inconclusive. One panther (FP115) was also feline immunodeficiency virus (FIV) positive (approximately 28% of free-ranging panthers are FIV positive).<sup>9</sup> Coinfection with FIV in domestic cats may exacerbate the effects of FeLV infection.<sup>4</sup>

All antigen positive panthers have died. FP115 (with concurrent FIV and FeLV infections) lost 45 lbs and died of an *E. coli* septicemia 5 mo after capture. FP122 had a severe non-regenerative anemia (PCV 18%) and generalized lymphadenopathy at capture and died 2 wk later. Other than gross signs of severe dehydration, emaciation, and anemia, necropsy results were inconclusive,

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and death was likely related to FeLV infection. FP109 was moderately anemic and had a generalized lymphadenopathy at capture and was killed by another male 1 mo later. Finally, FP123, an adult male FeLV antigen/IFA positive panther, was in good condition at capture but was killed by another male 2 mo later. This male (FP132) was captured, tested FeLV negative, and was then vaccinated. Archived tissue from 8 panthers tested positive for FeLV by PCR (preliminary results), primarily in the northern portion of panther range. Positive PCR findings in some panthers may indicate latent infections. Positive findings in tissues collected at necropsy from FP109 and in experimentally aged bone marrow (sternum, FP109) indicate severely decomposed tissues may be suitable for PCR detection of FeLV. Detection of FeLV by PCR in feces collected at necropsy from FP115 indicates this technique may be useful for non-invasive monitoring for FeLV; this technique is currently being evaluated. Preliminary results of genetic sequencing indicate the virus to be subgroup A and is similar to that in domestic cats. Preliminary sequencing results also indicate that there may have been two separate introductions of the virus into the panther population. Infected domestic cats are the likely source.

ELISA antibody titers have been determined for archived serum from free-ranging panthers and Texas cougars captured between 1990 and 2003 (n = 223). FP109, 115, 100, and 15 other samples were positive; the two IFA positive panthers (FP122, 123) were antibody negative. Positives were concentrated in OKS and the northern portion of panther range; positive titers decreased to the south and were non-existent south of US41 including Everglades National Park. Positives have also increased dramatically since 1997 (Fig. 1). Positive antibody titers were more likely among males versus females and may indicate that interactions among males (fighting) are an important mode of transmission. Positive antibody titers also appeared to increase with age.

Following exposure, most domestic cats clear the virus although some may remain latently infected. Reactivation of latent infections is unlikely >1 yr following resolution of viremia.<sup>7</sup> Approximately 30% of exposed domestic cats become persistently infected although susceptibility decreases with age.<sup>5</sup> Although discordant test results can be difficult to interpret, in general, transiently infected cats will usually be antibody positive, may also be transiently antigen and PCR positive, but will remain IFA negative. Latently infected cats will have similar test results but will become PCR positive. Persistently infected cats will be positive on ELISA antigen, PCR, and IFA, although ELISA antibody titers may be negative. Preliminary antigen, PCR, and antibody results indicate that the outcome following exposure in Florida panthers is similar to that seen in domestic cats – with some panthers transiently infected, some latently infected, and some persistently infected. Two of the four antigen positive panthers were also positive by IFA indicating persistent infection. Interestingly, the two IFA positive panthers were ELISA antibody negative suggesting an inadequate immune response. The status of the two ELISA antigen positive, IFA inconclusive, panthers is unknown; however, ELISA antibody titers were positive. Transient infections may be indicated by antibody positive but antigen and PCR negative panthers – and represent approximately 50% of panthers sampled north of I-75 over the past 2 yr. Latent infections may be represented by antibody and PCR positive, but antigen negative, panthers.

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Despite the recent introduction of Texas cougars to south Florida as part of a genetic introgression program, many panthers, especially those in the northern portion of panther range, are inbred and have a reduced genetic variation. Genetic analysis is pending, however, based on morphologic traits, we suspect that all antigen positive panthers were pure (canonical) panthers. However, antibody titers and PCRs indicate that some pure panthers and Florida panther/Texas cougar intergrades have been exposed but did not become persistently infected. Inbreeding depression and/or reduced genetic variation may have resulted in an increased susceptibility of pure panthers to FeLV. This speculation is confounded by the uneven distribution of genotypes – there is a greater percentage of pure Florida panthers in the northern portion of panther range. An examination of relatedness, genotype, and genetic variation of infected and non-infected panthers is pending.

No adverse reactions were observed in the six cougars/Florida panthers undergoing a vaccine trial in captivity. Although most subjects seroconverted, antibody titers are not necessarily indicative of vaccine efficacy in domestic cats.<sup>14</sup> Vaccination followed by booster using Fel-O-Vax® Lv-K is safe and relatively effective in domestic cats (95-100% efficacy).<sup>14</sup> Although a single inoculation will provide some protection, boosters are necessary to adequately protect against FeLV infection. Ideally a booster is given between 3 and 6 wk post inoculation, however, a booster may be sufficiently protective given between 10 days and 8 wk post inoculation. Boosters given 1 yr or more after a single vaccination will certainly provide at least some protection, however controlled studies are lacking. Boosters are administered by treeing and darting the panther with the vaccine. Administering boosters is logistically difficult as well as dangerous to the panther. The decision to administer a booster depends on evidence of previous exposure, FeLV status, FIV status, location, and gender. Computer models indicate that 23-72% of domestic cats must be effectively vaccinated to achieve control in a population having a 10% prevalence of FeLV.<sup>8</sup> As of 19 March 2004, 18 free-ranging Florida panthers have been vaccinated – representing approximately 18-23% of the population.

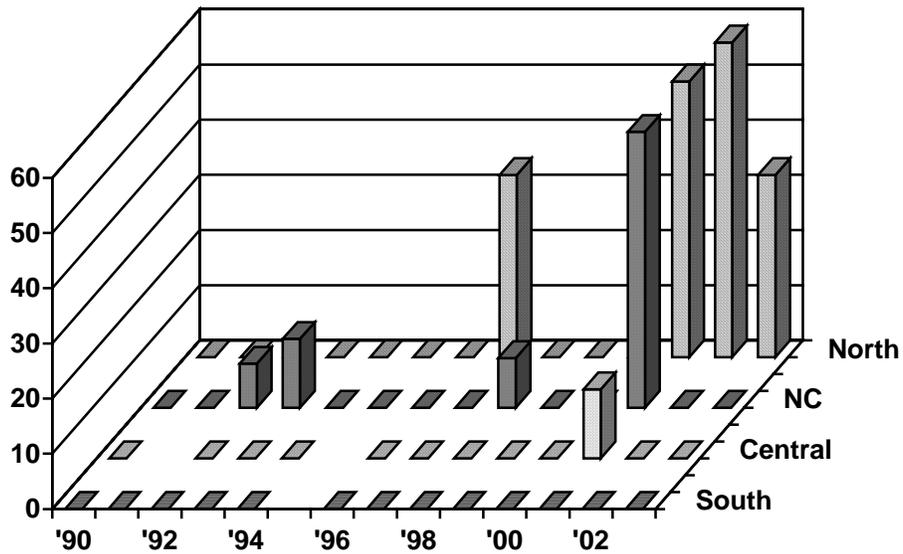
#### **ACKNOWLEDGMENTS**

We are indebted to R. McBride and M. Roelke who are largely responsible for the success of the Florida panther recovery project. We appreciate the capture efforts of the Big Cypress National Preserve including D. Jansen and E. Blankenship, as well as other researchers involved in the project including S. Bass, R. C. Belden, and M. Lotz. Finally, we greatly appreciate the advice and assistance of J. Levy, W. Hardy, L. Mathes, E. Hoover, J. Evermann, N. Pedersen, D. Jessup, C. Crawford, B. Ferree, and K. MacDonald. Funding for this study was provided through the Florida Panther Research and Management Trust Fund, Florida Nongame Wildlife Trust Fund, and the Federal Endangered Species Project E-1.

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S = All areas south of US41: Stair Steps Unit – Big Cypress National Preserve (BCNP), Loop Road Unit – BCNP, Everglades National Park, Southern Glades Wildlife Management Area; C = All areas between I-75 and US41; NC = Public lands north of I-75; N = Okaloacoochee Slough Wildlife Management Area, Big Cypress Seminole Indian Reservation, private lands north of I-75.

**Figure 1.** Percentage of panthers FeLV ELISA antibody positive by year and location 1990-2003.

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## CONSERVATION MEDICINE AND ITS APPLICATION TO NORTHERN PRAIRIES: A VETERINARY PERSPECTIVE

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### **Abstract**

The Turner Endangered Species Fund (TSEF) is dedicated to conserving biodiversity by ensuring the persistence of imperiled species and their habitats. Our efforts focus on carnivores, grasslands, plant-pollinator complexes, species that historically ranged on properties owned by R.E. Turner, and dissemination of reliable scientific and policy information of biodiversity conservation. The overall organizational goals are directed at protecting environments and habitats, although most research efforts often address the endangered or threatened species or populations within that habitat. TSEF now integrates all health issues within the framework of habitats that are multiuse human dominated environments. Although the species addressed in any propagation or restoration effort are the driver, the ability of the habitats to sustain imperiled species and biodiversity must be evaluated. Restoration efforts will also affect the surrounding ecosystems and are generally not curtailed by a set boundary or property fences. For example, the reintroduction of buffalo (*Bison bison*) into prairie habitats results in ecological changes that affect a variety of other species that historically or currently use those habitats. Prairie dogs, black-footed ferrets, swift foxes, Mexican and Northern wolves, bighorn sheep, aplomado falcons, red cockaded woodpeckers, California Condors, plant pollinators and bison all must interact in these human-influenced and altered habitats. Reintroductions or relocations within these environments require more than moving “healthy” species into historic ranges. The habitats that these species will occupy must be assessed for present and future compatibility with the understanding that a balanced, healthy sustainable ecosystem must remain the goal of all of these efforts. This must also fit into the mandate of the Turner Enterprises, Inc. as properties are “dedicated to managing Turner lands in an economically sustainable and ecologically sensitive manner while promoting conservation of native species.” This presentation will address the importance of wildlife veterinary perspectives in building a team of biologists, range managers, agronomists, environmentalists, livestock managers and landowners and creating strong core values that allow both economic and species sustainability.

### **Introduction**

All of the properties purchased by Turner Enterprises, Inc., were managed for one purpose - producing domestic livestock. These habitats underwent many alterations geared at maximizing the production of one species. These changes were accomplished without regard for native flora

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and fauna. A multidisciplinary team approached guidelines to be addressed before native species reintroductions on these human-altered habitats.

## **Discussion**

The process of ecosystem health will be discussed from a wildlife veterinary perspective. Input from veterinarians working on complicated wildlife issues is critical to long term monitors used to evaluate the success of a reintroduction.

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## NEARTIC OTTER TRANSLOCATION AND POPULATION RESTORATION IN NEW YORK STATE: A NINE-YEAR RETROSPECTIVE

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### Abstract

By the early 1940's environmental pollution of aquatic ecosystems, persecution as a predator and unregulated trapping collectively resulted in extirpation of the Nearctic otter (*Lontra canadensis*) from western New York state.<sup>7</sup> Since abundant numbers of otters were present in the northeastern and southeastern parts of the state, and dispersal of these populations to the western parts of the state was projected to take up to 20 yr, the New York State Department of Environmental Conservation (NYSDEC) elected to develop partnerships to re-establish the otters in western New York. Thus, a melding of private, public and state organizations became the New York River Otter Project, Inc. (NYROP). Although many individuals and groups made significant contributions, the principles included the Division of Wildlife, NYSDEC, the Board of Directors of the NYROP, and the Section of Wildlife Health, College of Veterinary Medicine, Cornell University.

In all 306 otters were captured, 279 of which were released at 16 sites deemed acceptable by NYSDEC Division of Wildlife Biologists. Health assessment, medical problems, captive management, and pre-release conditioning of the otters was intensive and has been previously reported.<sup>1,3-6</sup> Radiotransmitters were surgically placed in 37 otters which were released at three different sites, with the most (28) being released at a single large protected site.<sup>2,4</sup>

Between 1996 and 2004 the methods used to assess post-release survival and reproductive success have included observational techniques (e.g., specific identification of adult otter and offspring, signs [tracks], slides, scats, dens, and toilets) and the recovery of otter carcasses for detailed post-mortem evaluation. Reproduction has been documented at more than 90% (14 of

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the 16) of the sites via observation of females with young and by recovery and identification of immature and juvenile (based on cementum annuli tooth analysis) otters in and around these sites.

From 1996-2003, 77 otters were recovered and presented for necropsy. Of these, 36 were identified as released individuals, with 41 presumed to be offspring of released females. This was based on their age and lack of an implanted identification chip. Thirty (39%) of these were vehicular casualties, which occurred during the breeding season of March-April. The remaining 47 (61%) died as a result of non-target trapping accidents (open water beaver traps) occurring during the month of December. All ROP and non-ROP otters examined at necropsy were found to be in excellent body condition and exhibited minimal gastrointestinal and respiratory system parasitism. Some of these individuals had been released 6 yr prior to recovery.

In summary the procedures and protocols used, and contributing to the success of this project, have direct relevance and application to other *in situ* nearctic otter conservation efforts.

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## FROM SPECIES TO ECOSYSTEM CONSERVATION: TEN YEARS OF PRZEWALSKI'S HORSE REINTRODUCTION TO SOUTHWEST MONGOLIA<sub>[DJB4]</sub>

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### Abstract

The Przewalski's horse (*Equus ferus przewalskii*), or takhi in Mongolian, became extinct in the wild by the mid 1960's. The last recorded sightings of Przewalski's horses occurred in the Dzungarian Gobi desert in Southwest Mongolia. The species has only survived due to captive breeding based on 13 founder animals. A private fund and the Mongolian Society for the Conservation of Rare Animals of the Ministry of Environment initiated the Takhin Tal Project with the support of various international sponsors. In 1999 the International Takhi Group (ITG) was established to continue and extend this project in accordance with the IUCN reintroduction guidelines. In 1992 the first group of captive born Przewalski's horses were airlifted to the Takhin Tal site (45.53.80 N, 93.65.22 E) at the edge of the 9000 km<sup>2</sup> Greater- Gobi-B Strictly Protected Area (SPA) and International Biosphere Reserve. Subsequent transports were carried out in the following years and to date a total of 73 horses have been transported. In 1997 the first harem group was released into the wild from the adaptation enclosures and 1999 the first foals were successfully raised in the wild. At present 63 Przewalski's horses live at the Takhin Tal site with 50 horses belonging to four harems and one bachelor group ranging freely in the Gobi-B National Park. Due to its important symbolic value in Mongolian culture the Przewalski's horse has become an important vehicle for national park development. The Gobi-B is also a cultural landscape and management aims to conserve it as a biosphere reserve in the sense of the IUCN. The vision is the integral protection of the Gobi habitat and the life style of the semi-nomadic herders.

Establishing a permanent field station at the edge of the national park with the necessary infrastructure (solar power, laboratory, office, vehicles and petrol) and communication abilities (satellite based email and phone) has proven crucial to the development of the project. Initiating training possibilities for young Mongolian biologists and creating employment has resulted in a well-trained and motivated local staff and essential project advocates. Starting out initially as a single-species reintroduction project, the magnitude of the activities has greatly expanded in recent years. Seen from a species perspective, research projects dealing with the Mongolian wild ass (*E. h. hemionus*), grey wolf (*Canis lupus*) and various rodent species have been implemented. Whereas the initial reintroduction efforts were by and large driven by veterinarians and biologists, the disciplinary scope has also been significantly broadened with botanists and remote

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sensing experts involved with habitat mapping and assessment, community development experts establishing a socio-economic framework for future project development. Away from the field an important prerequisite for project advancement has proven to be lobbying activities both in Ulaanbaatar and to the international community. Lobbying activities not only enhance information flow and political understanding for the project but also create collaborative opportunities and necessary alliances.

Comprehensive interdisciplinary monitoring and research are the foundation for management decisions at the present, but training and empowerment of local scientists and residents will constitute the future of this program.

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## LANDSCAPE CONSERVATION INITIATIVES: THROUGH THE EYES OF WILDLIFE OR THROUGH THE EYES OF PEOPLE?

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### **Abstract**

A number of conservation NGOs have adopted a broad based landscape approach to protected area management but some of their initiatives are clearly being developed “through the eyes of wildlife” rather than “through the eyes of people”. This is a disturbing trend and may reflect a move towards a more protectionist approach to conserving biodiversity and wild lands in the developing world.

Protectionist approaches to the conservation of biodiversity that deprive indigenous people of the ability to support themselves and to sustainably utilize wildlife have a history of long term failure. They are unworkable and unsustainable in the developing world where issues of poverty are prominent and where subsistence livelihoods are the key to sustainable conservation practices and environmental stewardship. Health is a key area where holistic, integrated multi-disciplinary approaches across landscapes need to be adopted to ensure long term conservation success. Wildlife veterinarians and the agencies that support them can facilitate successful landscape conservation by developing holistic health programs that incorporate and integrate human, livestock and wildlife health.

With the development of large Transfrontier Conservation areas that may straddle several countries, a wide diversity of areas including traditional game reserves, hunting lands and conservancies and intervening areas of communal lands under traditional tenure may be included. Examples of conservation initiatives that, in the authors opinion will work, and others that won't, will be provided. For these vast areas a landscape based approach is essential. But, this landscape approach has to be people based in order to sustain animal health (livestock and wildlife), human health and wellbeing, and to maintain ecosystem services.

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## FOOT AND MOUTH DISEASE (FMD) IN BISON: SUSCEPTIBILITY. LESIONS, SHEDDING AND TRANSMISSION

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### Abstract

Foot and mouth disease (FMD) has never been diagnosed in bison (*Bison bison*) in North America. The disease has been observed in European bison (*Bison bonasus*),<sup>1,3-5</sup> American bison,<sup>2</sup> and hybrids<sup>1</sup> in European zoos and preserves. In recent years the commercial bison industry in the USA has grown substantially along with the development of new public herds, and there are currently over 250,000 bison in North America. Virtually nothing is known about the disease in American bison. In this pilot study, we attempted to compare the susceptibility of bison to FMD with that of cattle, determine whether intraspecies and interspecies transmission could occur in bison and cattle, determine if standard laboratory tests detect FMD in bison, and determine if bison are efficient long term carriers or shedders of FMD.

After 1 wk of acclimation to containment at Plum Island Animal Disease Center, two yearling intact male bison and two 7- to 9-mo-old castrated male Holstein cattle were anesthetized and temperature radiotransmitters implanted intraperitoneally. One bison and one steer were each inoculated with 10,000 lesion forming units of O1 Manisa FMD virus by four intraepithelial tongue injections of 0.2 ml each. The inoculated animals were kept overnight with the other two radiotelemetered animals. The following day the inoculated and exposed cattle and bison were placed in two rooms with two naïve yearling intact male bison in each room. The two cattle and all bison developed clinical signs and lesions consistent with FMD in other ungulate species. The two bison with temperature transmitters developed transient fevers of 41.8°C. Bison developed lameness, inappetence, and ptyalism. Physical examination revealed numerous small vesicles and erosions affecting tongue, gingiva, muzzle, hard and soft palates, coronary bands, and interdigital skin. At necropsy during the acute phase, there were also erosions located on the rumenal pillars of one bison. At necropsy 5 wk post-infection, bison had evidence of healed oral lesions and hoof deformities.

All bison developed antibody to FMD virus and were positive for FMD virus by RT-PCR. An attempt to transmit the infection from bison 11 days post exposure to naïve cattle by direct

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exposure failed. Future studies will attempt to demonstrate FMD virus transmission from bison to cattle and evaluate immunologic protection in bison produced by vaccination.

#### **ACKNOWLEDGMENTS**

The authors would like to thank Heather Lomaga, Jeffrey Hayes, Karen Wickert, Brenda Donahue, Jessica Dio, Jessica Montez, Marylou Berninger and members of the Animal Care Group on Plum Island Animal Disease Center for their assistance in various phases of this work.

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## ATTEMPTS TO REPRODUCE VACUOLAR MYELINOPATHY IN CHICKENS AND SWINE

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### **Abstract**

Avian vacuolar myelinopathy (AVM) was first recognized as a cause of bald eagle mortality in 1994 in Arkansas and has since caused more than 90 bald eagle and numerous American coot mortalities in five southeastern states. The cause of AVM remains undetermined, but is suspected to be a natural toxicant. In this study, chickens and swine were evaluated as potential animal models for AVM research. Chickens that consumed a mixture of tissues from coots with AVM for 28 days developed brain lesions consistent with those of AVM, as did chickens that received only the gastrointestinal tracts of affected coots in a subsequent trial. Additionally, chickens that consumed submerged vegetation (hydrilla) collected from a lake during an AVM outbreak developed brain lesions. Chickens that consumed only coot liver, kidney, brain, muscle, or adipose tissue did not develop brain lesions, nor did chickens that consumed submerged vegetation (hydrilla) from a lake where AVM's absence has been documented for several years. Brain lesions were not apparent in young pigs that consumed a mixture of tissues from affected coots for 28 days. Results of these studies indicate that chickens can serve as useful animal models for AVM research and that the cause of AVM is associated with the gastrointestinal tracts of affected coots, apparently as a consequence of consuming submerged vegetation from lakes during AVM outbreaks.

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**PATHOLOGY OF DICLOFENAC POISONING IN FREE-FLYING AND EXPERIMENTALLY EXPOSED ORIENTAL WHITE-BACKED VULTURES (*Gyps bengalensis*) FROM PAKISTAN**

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**Abstract**

In response to recent precipitous declines of vulture populations in the Indian subcontinent, the Peregrine Fund established the Asian Vulture Crisis Project. This collaborative project with the Ornithological Society of Pakistan documented adult and subadult mortality as high as 86% and estimated population declines of 34-95% at breeding colonies in Punjab Province of Pakistan between 2000 and 2003<sup>1</sup> (and Gilbert, recent unpublished data). Visceral gout (uric acid precipitation on visceral surfaces) was the significant finding in 219 of 259 (85%) vultures necropsied.<sup>3</sup> Most of the vultures with visceral gout were in good body condition, suggesting the vultures were foraging well and the disease condition was of short duration prior to death. Microscopic examination confirmed urate deposition with associated necrosis in kidney, liver, spleen, lung, heart, adrenal gland, parathyroid gland, skin, fascia of skeletal muscle as well as urate deposition on visceral surfaces. Renal damage in vultures was severe, acute, and throughout the sections of kidney. There was minimal inflammation and no evidence of repair of renal tubules. When renal architecture could be identified in kidneys with less extensive lesions, the proximal renal tubular epithelium was preferentially damaged without urate deposition, and the collecting tubules and glomeruli were relatively spared. Kidneys with more “chronic” lesions had necrosis of almost all renal epithelium with extensive urate deposition both in dilated renal tubules and interstitium. With the exception of early gouty topi in the kidneys with more advanced stages of renal disease, there was no consistent inflammation. Because kidneys with early lesions had acute renal tubular necrosis without evidence of urate deposition, the renal failure was considered the primary problem and cause of visceral urate deposition, rather than urate deposition causing the renal failure. Because kidneys in early stages of disease had necrosis of epithelium of proximal tubules and not collecting or distal convoluted tubules, the likelihood of an ascending nephrosis due to disease and subsequent obstruction of the ureter was unlikely. Published reports of dehydration-induced renal pathology that would have been

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consistent with the severity and distribution of that seen in these vultures could not be found. The most likely cause of acute renal tubular necrosis of this magnitude and uniformity, without inflammation was considered to be a nephrotoxic compound.

Vultures dying with extensive visceral gout have been reported throughout much of the Indian subcontinent<sup>4</sup> suggesting that a nephrotoxic agent would need to be available over a large geographic area. Extensive tissue analysis for infectious agents, metals and contaminants did not identify a likely etiology.<sup>3</sup> The primary food source for Oriental white-backed vultures in Pakistan is discarded carcasses of domestic animals. A survey of veterinarians and pharmaceutical retailers in Pakistan conducted by Oaks and Gilbert<sup>3</sup> in the Fall of 2002 identified widespread use of diclofenac, a non-steroidal anti-inflammatory drug, in domestic livestock. Non-steroidal anti-inflammatory drugs are potentially nephrotoxic, although reports of adverse effects associated with their use in birds are not common.<sup>2,5</sup> Subsequent analysis of vulture tissues identified diclofenac residues in all of the Oriental white-backed vultures that had necropsy evidence of visceral urate deposition and severe renal damage microscopically. Diclofenac residues were not found in Oriental white-backed vultures dying from other causes.<sup>3</sup>

Captive non-releasable Oriental White-backed Vultures that were either directly treated with diclofenac, or fed livestock that were treated with therapeutic doses of diclofenac before euthanasia confirmed the acute toxicity of diclofenac in these birds and strengthened the hypothesis that Oriental white-backed vulture could be exposed to lethal levels of diclofenac by scavenging disposed domestic animals treated with diclofenac before death. Severe renal lesions, indistinguishable from those seen in the 26 Oriental white-backed vultures examined from the wild, was seen in the experimental vultures both directly and indirectly exposed to diclofenac. The impact of this non-steroidal anti-inflammatory drug on the population of Oriental white-backed vulture in Pakistan makes diclofenac an important consideration when looking for the cause of mortality in other populations of vultures on the Indian subcontinent with identifiable visceral gout or renal failure. Risk to populations of scavenging raptors in other parts of the world would depend on species sensitivity and availability to scavengers of exposed carcasses treated with diclofenac.

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## **PATHOLOGIC EFFECTS OF DIETARY METHYL MERCURY IN AMERICAN KESTRELS (*Falco sparverius*)**

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### **Abstract**

Methyl mercury in the aquatic food web poses significant health risks to both wildlife and humans.<sup>1</sup> One primary source of mercury contamination for both the aquatic and terrestrial systems is atmospheric deposition of inorganic mercury from industrial emissions.<sup>2</sup> Once in the environment, inorganic mercury is converted to methyl mercury that enters the food web and bioaccumulates in prey species. Top level predators including piscivorous birds and humans are at greatest risk. Methyl mercury is the most toxic form of mercury, and is associated with behavioral changes, neurologic impairment, reproductive failure and death. There is concern that methyl mercury may threaten populations of many wildlife species.

In this study the American kestrel was exposed to dietary methyl mercury in a controlled setting at the United States Geological Survey (USGS) Patuxent Wildlife Research Center (PWRC). The purpose of this pilot study was to determine sensitivity and pathologic change of this species to the toxicant and gather information about methyl mercury absorption and distribution. Results from this study will help 1) design a reproductive study and 2) develop a physiologically based toxicokinetic model of bioaccumulation of mercury in kestrels.

Though the concern for methyl mercury in avian species is primarily in piscivores, the American kestrel was chosen as the animal model for study because it can be kept and bred in captivity and is a top level carnivore. Information from the kestrel studies will also provide needed information to better understand risk from methyl mercury to wild piscivorous birds.

Dosage of birds included control, 3,6, and 12 ppm methyl mercury chloride (dry weight) mixed with Nebraska Brand Bird of Prey Diet (Central Nebraska Packing, Inc., North Platte, NE) for 1, 2, 4 or 8 wk. Tissues examined histopathologically were kidney, liver, spinal cord and brain.

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Lesions were found only in the cerebellum of 12 ppm birds; all birds at this dose had similar lesions. Changes included neuronal degeneration, loss of myelin and mild inflammation.

#### **ACKNOWLEDGMENTS**

This project is a collaboration between the United States Environmental Protection Agency and the USGS.

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**A NEW STRAIN OF *Chlamydiophila psittaci*, STRAIN G, ISOLATED FROM RED TAILED HAWKS (*Buteo jamaicensis*): IDENTIFICATION, PREVALENCE, DIAGNOSTIC TESTING, AND PATHOLOGY**

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**Abstract**

Chlamydiosis is a global, zoonotic, bacterial disease caused by a heterogenous group of obligate intracellular, gram-negative bacteria with various clinical manifestations in birds and mammals, including humans. It has been reported in several species of wild birds with suspected or documented transmission to pet birds, captive raptors (falconer owned), dogs, cats, and humans.<sup>2-4</sup> Relatively little is known about the epidemiology and pathogenesis in wild bird populations, partly because mortality events rarely exceed predator or scavenger removal. Consequently, little is known about the implications of wild bird infections to wildlife populations, domestic animals, and humans.

From November 2002 to March 2003, there was a cluster event of chlamydiosis in red-tailed hawks (RTHs - *Buteo jamaicensis*) in the vicinity of Davis, CA, affecting seven of the 20 RTHs submitted for necropsy to the Anatomic Pathology Service, Veterinary Medical Teaching Hospital (VMTH), Davis, CA. All affected RTHs were juveniles, in poor body condition, and with variable concurrent disease processes. Cell culture and molecular characterization of the isolate from one RTH suggested it was a new strain of *Chlamydiophila psittaci*, most closely related to strain M56. Trauma with subsequent debilitation is the most common presentation of RTHs to the VMTH for treatment and rehabilitation,<sup>8</sup> and review of archived VMTH pathology cases over the last 20 yr yielded only three cases of chlamydiosis out of 215 RTH necropsies. Thus, either this was an unusual morbidity/mortality event, possibly related to a new strain of *C. psittaci*, or chlamydiosis has been under-diagnosed.

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Under the current taxonomic classification, the family Chlamydiaceae is subdivided into two genera, *Chlamydia*, which incorporates three species, and the newly designated *Chlamydiophila*, which incorporates six species including *psittaci*.<sup>1</sup> Assignment to species is based on less than 0.8% difference in the 16S rRNA segment of genome. Within *C. psittaci*, eight strains are recognized based on monoclonal antibody tests, the polymerase chain reaction (PCR) of the 16S rRNA sequence, and PCR sequence analysis of the major outer membrane protein (MOMP). Six of the strains (A through F) are considered endemic in birds, including most pet birds, domestic fowl and poultry, and wild birds.<sup>1,2</sup> In wild birds, however, too few isolates have been typed to know whether additional strains exist. The two remaining strains (WC and M56) have been isolated each once during mammalian epizootics, but are believed to have crossed from an avian host.<sup>10,11</sup> Transmission may be horizontal or, infrequently, vertical, and may occur from live birds and fresh and chilled carcasses.<sup>2</sup> The rate and success of transmission and pathogenicity is based on susceptibility of the host (with consideration of age, immune status, concurrent disease, and, possibly, genetic predisposition), the virulence of the strain for that host, the dose and route of infection, the persistence of infection and duration of shedding, and environmental factors.<sup>2,12</sup>

Diagnosis of avian chlamydiosis is relatively difficult, especially in ante-mortem cases. Ante-mortem confirmation requires a fourfold increase between acute and convalescent serum titers taken 2 wk apart or positive culture from respiratory secretions. Trends in antibody production are variable among species and individuals. Some have undetectable antibodies, others never seroconvert with acute onset of disease, and others have persistent titers despite complete resolution of disease without further shedding.<sup>5-7</sup> Consequently, it is difficult to correlate serum antibodies with active infection and potential for zoonotic transmission. Cell culture requires special handling procedures, is performed by only a few diagnostic laboratories due to zoonotic potential, technical demands, and expense, and requires 2-3 wk to ensure a negative result. Post-mortem confirmation requires demonstration of the organism by cell culture, immunohistochemistry (IHC) or immunofluorescent antibody testing against the genus-specific LPS antigen of *Chlamydia trachomatis*, or a serum titer change with compatible lesions. Bacterial isolation in cell culture is still considered the gold standard in veterinary medicine, because rapid, reliable tests available in human medicine, namely a PCR and antigen capture enzyme-linked immunosorbent assay (ELISA), have not been standardized in domestic and free-ranging species.<sup>2,7,9</sup>

This study was conducted 1) to determine whether other cases that occurred during the epizootic were caused by the new strain; 2) to determine the prevalence of chlamydiosis and this strain in RTHs in northern California; 3) to characterize gross, histologic, and immunohistochemical findings and thus identify associated lesions in RTHs; 4) to validate commercially available species-specific multiplex PCR (m-PCR, Gibco®) and genus-specific antigen-capture ELISA (Clearview™) and to develop a species- and strain-specific Taq-Man PCR (Lucy Whittier Molecular Core and Diagnostic Facility, Davis, CA). To address the first question, available samples from three additional RTHs from the epizootic were submitted for cell culture and strain identification. To determine prevalence, the sampling effort was increased to include not only all

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RTHs admitted to the VMTH, but also those admitted to the Lindsay Wildlife Hospital in the vicinity of San Francisco (Walnut Creek, CA). To characterize gross and histologic findings, a defined tissue set from each animal was submitted for routine histology and immunohistochemistry (IHC). To answer questions related to prevalence, diagnostic testing, and pathology, the ELISA, m-PCR, and species-specific Taq-Man PCR were run on fresh or frozen (-70°F) ante-mortem choana and cloacal swabs and post-mortem liver, spleen, and air sac samples. The results were compared to findings from histology and IHC. Additionally, at necropsy, liver, spleen, air sac, and coelomic swab were submitted for aerobic culture. Data was entered into the Epi Info™ program and statistical analysis run using chi-square, odds ratio, and sensitivity and specificity analyses.

To date, molecular testing confirmed that isolates from three additional cases from the cluster event had 100% homology with the index isolate. Consequently, this new strain of *C. psittaci* was designated strain G. Chlamydiosis was diagnosed in 22.8% (13/57) of RTHs examined from November 2002 to April 2004, of which 11 cases occurred during the epizootic. Strain identification of the two cases from the non-epizootic RTHs is pending. All chlamydiosis cases were observed in juvenile birds (36.1%, 13/36). Infection was associated with season, as all cases occurred between December and April, constituting 48% of the cases submitted during this timeframe. There was no significant association found between *Chlamydia* infection and sex, chronic disease, or location; however, interpretation of the later variable is confounded by the relatively small sample size from the San Francisco area.

By gross, histologic, and immunohistochemical examination, several features had statistically significant association with chlamydiosis in juvenile RTHs. RTHs with chlamydiosis were 12.25 (95% CI, 1.6-138.48) times more likely to have hepatomegaly and 22.29 (95% CI, 2.63-988.14) times more likely to have hepatitis of increased severity. Hepatitis was defined as periportal to random, predominantly mononuclear inflammation. RTHs with chlamydiosis were also 7.2 (95% CI, 1.24-45.27) times more likely to have moderate to severe splenic hemosiderosis and increased renal mesangioproliferative glomerulopathy (chi square,  $P < 0.001$ ). Splenomegaly (27.8 %, 10/36) with reticular sheath reticuloendothelial hyperplasia and air sacculitis (50%, 18/36) also were common lesions. A mild to moderate, mononuclear meningitis with predominantly intravascular and perivascular organisms (by immunohistochemistry) in the meninges and choroid plexus was identified in 23.1% (3/13) of the chlamydiosis cases. Finally, despite the lack of histologic lesions, the predominance of organisms identified by IHC in the ileocecal region, compared to low numbers in the colon, cloaca, and bursa, and few to none in the duodenum and jejunum, suggests that the ileocecal region is an excellent screening site for *Chlamydia* by IHC.

Thus far, there has been 100% concurrence between our gold standard test (histology supplemented by IHC) and Taq-Man PCR run on available ante-mortem choanal and cloacal swabs (n = 33 total, n = 2 *Chlamydia* birds; swabs were not available from the remaining 11 RTHs with chlamydiosis) and post-mortem liver, spleen, and air sac samples (n = 24 total, n = 7

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*Chlamydia* birds; tissue was not available from the remaining six RTHs with chlamydiosis). Comparing these results with those from ELISA and m-PCR, which were run on the same sample set, the data indicate that the later two tests are equally specific, but less sensitive, as there were four false negative results. Regarding the ELISA, the absence of false positive results despite the culture of gram-negative microbes from several cases, indicates that cross-reaction with the LPS antigen of other gram-negative microbes is not a real concern. Regarding the Taq-Man PCR, its sensitivity necessitates that molecular testing be interpreted in light of histology to accurately assess the contribution of chlamydiosis to morbidity and mortality of the bird. In summary, recommended samples to screen for chlamydiosis ante-mortem are pooled choana and cloacal swabs submitted for Taq-Man PCR. Recommended samples for post-mortem diagnosis are pooled liver, spleen, and, potentially, ileum/ceca for Taq-Man PCR and histology supplemented by IHC.

Work currently is being performed to complete the testing and statistical analysis on the current tissue set. Also, a strain-specific Taq-Man PCR is being designed in order to identify the strain of *C. psittaci* that caused disease in the non-epizootic RTHs (2003-2004), as well as to provide a strain-specific, sensitive and specific diagnostic test for *C. psittaci* on ante- and post-mortem specimens. Finally, continued ante- and post-mortem sample submission for Taq-Man PCR will be used to increase the sample size to validate this test and to determine if molecular methods can replace cell culture as the gold standard for diagnosis of chlamydiosis.

#### ACKNOWLEDGMENTS

The authors thank the Center for Companion Animal Health for funding this research, as well as Dr. Leslie W. Woods, Lydia L. Gan, Loren K. Jones, and the residents at the Companion Avian and Exotic Pet and Anatomic Pathology Services for their assistance.

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## FATAL WEST NILE VIRUS INFECTION IN FREE-RANGING GREATER SAGE GROUSE (*Centrocercus urophasianus*) IN WYOMING, MONTANA, AND ALBERTA

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### Abstract

Greater sage grouse (*Centrocercus urophasianus*) is a declining species native to sagebrush habitats of western North America. Historically widespread, the species has disappeared from much of its original range, with an estimated total population decline of 45-80% and local declines of 17-92%.<sup>1,2</sup> Loss and degradation of nesting and brood-rearing habitat from human change is thought to be the single most important factor leading to fragmentation, reduction, and extirpation of populations. These changes also increase the risks to sage grouse populations from other factors, including diseases like West Nile virus (WNV).

In the summer of 2003, WNV was diagnosed as the cause of mortality for 24 free-ranging sage grouse from Wyoming and Montana and five free-ranging grouse from Alberta. At necropsy, significant gross lesions were not observed in most birds. Consistent microscopic lesions included acute necrosis in many organs, including spleen, kidney, heart, and adrenal gland, without significant inflammation. West Nile virus infection was confirmed by real time PCR and immunohistochemistry in all birds, and by virus isolation in select birds. In contrast to most other species in the order Galliformes, sage grouse appear to be quite susceptible to fatal infection with WNV.<sup>3</sup>

Data collected from three marked populations of sage grouse in Wyoming and Montana indicate that WNV infection was responsible for a 25% decrease in annual survivorship in each of these populations. Serologic surveys performed on birds from two marked populations of sage grouse in Wyoming and Montana and on marked birds from Alberta and hunter-killed birds from areas in Wyoming that experienced WNV sage grouse mortalities demonstrated that 0/111 birds had serum-neutralizing antibodies against WNV. These findings are not conclusive, but at least suggest that few (if any) sage grouse survived WNV infection in the summer of 2003.

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In spring of 2004 an experimental trial will be performed at the University of Wyoming to determine the outcome of WNV infection in sage grouse. Level and duration of viremia, development of clinical signs, survivorship, and the potential for contact transmission will be investigated. Expanded field investigations into the epidemiology and pathogenesis of WNV in the field, including arthropod vector studies, also will be performed at several sites in Wyoming and Montana in 2004 and 2005, with participation by investigators from the University of Montana, the University of Wyoming, Montana State University, the Bureau of Land Management, and the USDA, ARS, Arthropod Borne Animal Disease Research Laboratory.

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## THE PATHOLOGY ASSOCIATED WITH AN OUTBREAK OF MAREK'S DISEASE IN GREEN JUNGLEFOWL (*Gallus varius*)

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### Abstract

The green junglefowl (*Gallus varius*) is a rare bird from Indonesia that is threatened by loss of habitat and hybridization with domestic fowl. Marek's disease virus (MDV) is a herpes virus often associated with neoplastic transformation of lymphoid cells in avian hosts (primarily chickens).<sup>1</sup> Over an 18-mo period, 13 captive-bred green junglefowl died with evidence of atypical or neoplastic lymphoid infiltrates in multiple organs. Clinical signs observed prior to death included lymphocytosis (>100,000 cells/ $\mu$ l in some cases), weakness, ataxia, lameness, and palor of the comb. Disease often progressed to severe ataxia and recumbancy. Most birds were euthanatized due to the progressive neurologic disease. Birds averaged 8mo of age at the time of disease/death.

Gross lesions were seen in multiple organs and included patchy pale tan infiltrates, organomegaly and palor, and distinct mass formation (Table 1). Gross lesions were most commonly seen in the skeletal muscle (7/13), spleen (5/13), and kidney (5/13). Enlargement of the sciatic nerves, a classic lesion of Marek's disease, was observed in four birds. Histopathology revealed lymphoid infiltrates in numerous sites (Table 1). Histopathologic lesions were most commonly seen in spleen (12/13), kidney (11/13), lung (11/13), skeletal muscle (11/13), peripheral nerve (10/13), and liver (10/13). Lymphoid infiltrates were in some cases interpreted to be inflammatory in nature and in other cases were obviously neoplastic. MDV infection was confirmed via PCR amplification of the oncogenic MEQ gene from abnormal sciatic nerve and muscle collected from four birds. Transmission electron microscopy confirmed the presence of viral particles in neoplastic lymphoid cells from one bird. Several birds had other diseases concurrent with Marek's disease including: intestinal coccidiosis (6/13), intestinal nematodes (4/13), systemic bacterial infection (3/13), and aspergillosis (1/13).

Following the diagnosis of Marek's disease in multiple birds, a decision was made to vaccinate current juvenile and future newly hatched junglefowl. Birds were vaccinated against MDV type 3 (Ft. Dodge, MD-VAC, 0.2 ml s.c.). The manufacturer's recommendation is to vaccinate birds at <24 hr of age. Three <24-hr-old chicks were vaccinated as well as four apparently healthy and serologically negative juvenile birds (2 mo old). Three of the four birds vaccinated as juveniles and two of the three birds vaccinated as chicks died of Marek's disease. Since MDV was first diagnosed in this flock, 12/14 (86%) of the on-site hatched juvenile junglefowl have died of this disease.

It is unknown whether green junglefowl are uniquely susceptible to MDV or if the MDV strain responsible for this outbreak is especially virulent. MDV has been isolated from plasma collected from affected birds, but the virus type has yet to be identified. Identification of the specific virus type may aid in future vaccination attempts for newly hatched birds. At this time, breeding of green junglefowl at this institution has been stopped until procedures to protect chicks from MDV can be developed.

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**Table 1.** Location of pathologic lesions noted in green junglefowl (*Gallus varius*) infected with Marek's disease virus.<sup>a</sup>

Bird#	1	2	3	4	5	6	7	8	9	10	11	12	13
Sex/age(mo)	M,9	M,10	F,10	F,8	F,9	F,10	F,3	F,5	M,8	M,5	M,6	M,10	M,11
Liver	H	G,H	-	-	H	H	G,H	-	H	H	G,H	H	H
Lung	H	H	-	H	H	H	-	H	-	H	H	H	H
Kidney	G,H	G,H	H	H	G,H	G,H	G,H	-	H	H	H	-	H
Spleen	G,H	G,H	-	H	H	G,H	G,H	H	G,H	H	H	H	H
Heart	H	H	-	H	-	H	-	-	H	H	H	-	H
Skel mus	G,H	G,H	G,H	G,H	G,H	G,H	G,H	H	-	H	H	H	-
Periph nv	H	-	H	H	H	G,H	G,H	G,H	H	G,H	H	-	-
Brain	-	-	-	-	H	-	-	-	H	H	-	-	H
Skin	H	H	-	-	-	H	H	H	-	H	H	-	-
Adrenal	G,H	-	-	-	G,H	H	H	G,H	H	H	H	-	-
Thyroid	-	-	-	-	-	H	-	-	-	-	-	-	-
Pancreas	-	H	-	-	H	H	H	H	-	H	H	H	-
Gonad	G,H	-	-	H	H	H	H	G,H	H	H	H	-	-
GI tract	-	-	-	H	-	G,H	-	H	G,H	H	H	H	-

<sup>a</sup>G = gross lesions present; H = histopathologic lesions present; - = no lesions present.

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## A REVIEW OF RED KANGAROO (*Macropus rufus*) NEOPLASMS AT THE KANSAS CITY ZOO, 1992-2002

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### Abstract

A perceived increase in the number of neoplastic cases in a captive population of red kangaroos (*Macropus rufus*) and paucity of reports of macropod neoplasms in general prompted a review of all kangaroo deaths from 1992-2002 at the Kansas City Zoo. Twenty-eight kangaroos died during this study period, and all animals were necropsied. Neoplasms were identified in six kangaroos, a prevalence rate of 21%. Neoplasms were the single leading cause of death in kangaroos during the study period. All six animals succumbed to or were euthanized because of their tumors.

The mean age of the animals at the time of death was 11 yr. All affected animals were female; however the collection was managed to exhibit only females. Two oral squamous cell carcinomas, two mixed mammary gland adenocarcinomas, one multicentric T cell lymphoma, and one pyloric submucosal lipoma were identified. This last mass led to gastric dilation and volvulus, and although decompression and surgical correction were successful, the kangaroo died 5 days later. Three of the six tumors were considered malignant and all three patients had developed metastases (Table 1).

A cause for the high frequency of neoplasms in red kangaroos in this population has not been identified, though neoplasia is generally more common in aged animals. Macropod neoplasia is uncommonly reported in the literature.<sup>1-4</sup> In one 14-yr review of pathology in macropodidae, neoplasia was identified in only one of 314 necropsies performed across all species.<sup>1</sup> Zoo veterinarians should consider neoplasia when presented with clinically ill older red kangaroos.

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**Table 1.** Summary of red kangaroo neoplasms identified at the Kansas City Zoo, 1992-2002.

Case	Age	Neoplasm	Location (s)
1	11 yr	Squamous cell carcinoma	Maxillary arcade
2	5 yr	Multicentric T cell lymphoma	Myocardium, liver, salivary gland, lymph nodes
3	13 yr	Lipoma	Pyloric submucosa
4	14 yr	Mixed mammary gland adenocarcinoma	Mammary glands, pulmonary tissue
5	10 yr	Squamous cell carcinoma	Mandibular arcade, regional lymph node
6	14 yr	Mixed mammary gland adenocarcinoma	Mammary glands, pulmonary tissue

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## AMYLOIDOSIS IN BLACK-FOOTED CATS (*Felis nigripes*)

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### Abstract

Black-footed cats (*Felis nigripes*) are native to arid grassy habitats in southern Africa and are currently listed in Appendix I of CITES. They are the smallest of the felid species weighing approximately 1-2 kg. In contrast to many species that tend to live longer in captive settings, black-footed cats tend to have shorter life spans in captivity than in the wild. The average life-span of black-footed cats in captivity is 3-5 yr while free-ranging cats have been estimated to live approximately 5-6 yr. A retrospective review of necropsy records identified amyloidosis as a significant cause of mortality within the captive population (N. Lamberski, unpublished data).

The amyloidoses are a group of diseases that are characterized by intracellular or extracellular deposition of insoluble fibrillar protein.<sup>2</sup> Over 20 different precursor proteins have been identified in the various forms of amyloidosis, with each disease having a specific amyloidogenic protein. Amyloidosis may be classified as systemic (involving multiple organs) or localized (affecting a single organ or tissue). The most severe clinical form of systemic amyloidosis in both mammals and birds is AA amyloidosis, which often results in hepatic or renal failure. In this condition, amyloid fibrils are derived from serum protein AA (SAA) that is produced predominantly in the liver. Production of this protein by the liver is increased in response to inflammation, and therefore AA amyloidosis is often seen secondary to chronic inflammatory processes. Among non-domestic species, cheetahs (*Acinonyx jubatus*),<sup>10</sup> Dorcas gazelles (*Gazella dorcas*),<sup>11</sup> and anseriformes<sup>3,6,8</sup> appear to be uniquely susceptible to develop AA amyloidosis secondary to chronic infections. Familial or breed specific forms of AA amyloidosis occur in dogs,<sup>4,7</sup> and cats,<sup>1</sup> and may be associated with chronic stress.<sup>5</sup> In the Abyssinian cat, amino acid sequence variations in the SAA protein may render it more amyloidogenic, but additional factors affecting production or processing of the precursor protein may also be involved in the pathogenesis of familial AA amyloidosis.

To better characterize the pathogenesis of amyloidosis in black-footed cats, necropsy samples from 33 black-footed cats representing the North American, European, and Southern African captive populations were reviewed. In addition, necropsy samples from one free-ranging juvenile were examined. Amyloidosis was present in 88% of animals, and was the cause of death in

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55.7% of all cases. Amyloid was present in the kidneys, gastrointestinal tract, spleen, lymph nodes, adrenal and/or thyroid glands. Within the kidneys, amyloid deposition occurred both in the glomeruli and the medullary interstitium. All eight facilities had at least one affected animal, and the free-ranging animal was also affected. Average age of affected animals was 4.67 yrs, while the average age of unaffected animals was 11.5 yrs. There was no apparent sex predilection. Concurrent inflammation was prevalent among affected animals, but inflammation was generally mild and in some cases only acute. Adrenal hyperplasia was also prevalent among affected animals. If the amyloid in black-footed cats is AA, then these concurrent conditions (chronic inflammation and chronic stress) may be the underlying cause. Ongoing studies are utilizing immunohistochemistry<sup>9,12,13</sup> to determine the type of amyloid in tissues of black-footed cats. Additionally, pedigree information from the International Studbook will be used to evaluate a familial basis for amyloidosis in black-footed cats. This information will be combined with the results of the necropsy survey to identify potential risk factors for the development of amyloid in black-footed cats. The results of these continuing studies will be critical for improved medical management of this species and may, if familial tendencies are determined, dramatically impact captive breeding recommendations.

#### ACKNOWLEDGMENTS

This study was funded through a grant from the American Association of Zoo Veterinarians Mazuri Fund. The authors wish to thank Dr. Alex Sliwa and Gea Olbricht for contributing samples from the European captive population and the samples from a free-ranging black-footed cat, as well as Dr. Lorna Bolton for providing samples and information from the South African captive population.

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## AMYLOIDOSIS IN STRANDED CALIFORNIA SEA LIONS (*Zalophus californianus*)

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### Abstract

Amyloidosis is a condition that affects humans and a large number of animal species; it is characterized by the abnormal deposition of extracellular fibrillar proteins in wide range of tissues. Amyloid can be classified based on its constituent chemical fibrils (e.g., AL, AA, A $\beta$ ), distribution pattern (systemic vs. localized), or based on association with other diseases (primary vs. secondary). Reactive systemic, or secondary, amyloidosis is associated with chronic inflammation.<sup>1</sup> Amyloidosis has been reported in captive and free-ranging wildlife and is often associated with severe inflammatory disease.<sup>6,8,9</sup> Among marine mammal species, amyloidosis has been reported in several stranded bottlenose dolphins.<sup>2</sup> Although severe inflammatory diseases are relatively common in stranded pinnipeds,<sup>3</sup> amyloidosis has not been previously reported. The purpose of this study was to describe the pathologic features of amyloidosis in stranded California sea lions (*Zalophus californianus*) and to identify any predisposing conditions associated with amyloid deposition.

Between 1983 and 2004 a total of 20 sea lions were diagnosed with amyloidosis via histopathologic examination of necropsy specimens by the Pathology Service of the Veterinary Medical Teaching Hospital, University of California, Davis, CA. All animals stranded live along the central California coast and were brought to The Marine Mammal Center, Sausalito, CA for rehabilitation. Sixteen of the twenty sea lions diagnosed with amyloidosis died or were euthanatized within 3 days of stranding. In the remaining four sea lions, time in rehabilitation ranged from 7 to 30 days. Antemortem blood work was available for five animals. Abnormal blood values in these sea lions included leukocytosis (5/5), and hyperglobulinemia (4/5). All animals were determined to be sexually mature adults, based on standard length, weight, tooth development, and presence of a sagittal crest in males.<sup>7</sup> Eighteen animals were females and two were males.

The most common organs affected were, in order of decreasing frequency, kidney (18/20), blood vessels (19/20), thyroid gland (13/20), gastric and intestinal mucosa (3/20), and liver (2/20). In all affected kidneys amyloid deposition was found in the peritubular interstitium, particularly in a

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distinct band along the outer medulla adjacent to the corticomedullary junction. Glomerular amyloid deposits were found in 16 of 18 affected kidneys and deposits were present in the walls of small and medium muscular arteries or arterioles in 17 of 18 affected kidneys. Amyloid deposits within glomeruli consisted of segmental or nodular deposits expanding the mesangium and capillary basement membrane. Glomerular and interstitial amyloid deposits were often seen in conjunction in affected kidneys. In affected thyroid glands, large deposits of amyloid were present in the interstitium separating thyroid follicles. Deposition of amyloid in extrarenal blood vessels occurred in 15 of 20 sea lions. Blood vessels most commonly affected were arterioles in the spleen, pancreas, heart, and adrenal gland. In five of these animals, amyloid deposits were also detected in arterioles within the brain, meninges, and choroid plexus. In the two affected livers, amyloid deposits were within the space of Disse lining hepatic cords. Localized amyloid deposits were restricted to blood vessels of the penis and prepuce of one adult male sea lion with severe balanopostitis.

Confirmation of amyloid deposition in affected organs was accomplished through examination of Congo red stained sections. Amyloid stained pale red-orange with Congo red and exhibited characteristic apple green birefringence under polarized light. Pretreatment with potassium permanganate abolished Congo red staining, suggesting that the amyloid fibrils were type AA.<sup>10</sup>

Underlying inflammatory conditions were common in most of the sea lions diagnosed with amyloidosis. Ten of twenty sea lions with amyloidosis had concurrent metastatic carcinoma of presumed urogenital origin.<sup>5</sup> In many affected tissues, the neoplastic masses contained large central regions of necrosis with widespread areas of inflammation. Moderate to severe interstitial nephritis was observed in 4 of 20 seal lions with amyloidosis. Two sea lions had severe bronchopneumonia associated with metastrongyle lungworm and bacterial infection. Two animals had lesions in the hippocampus and amygdala consistent with domoic acid toxicosis. One animal had chronic osteomyelitis and myositis, two animals had chronic abscesses, and one animal had severe granulomatous hepatitis associated with trematode infection. Other inflammatory diseases diagnosed concurrently with amyloidosis included enterocolitis (9/20), gastritis or gastric ulceration (8/20), and cholecystitis (7/20). Stress has been proposed as a primary factor in amyloidosis in other species.<sup>4,6</sup> Ten of the twenty animals with amyloidosis had adrenal cortical hyperplasia identified histologically. The significance of this finding is unknown, however, as normal weight ranges for adrenal glands and normal values for plasma cortisol have not been established for California sea lions.

The pattern of amyloid distribution in the sea lions in this study is similar to that described in other species with reactive secondary amyloidosis. The predilection for amyloid deposition in the outer cortex and corticomedullary junction was an unexpected finding, however, a similar pattern was also noted in bottlenose dolphins with renal amyloidosis.<sup>2</sup> The results of this study indicate that amyloidosis can be a significant cause of morbidity and mortality in stranded adult California sea lions and is often secondary to underlying inflammatory disease.

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## ACKNOWLEDGMENTS

We thank the staff of The Marine Mammal Center for submission of the cases and the anatomic pathology residents for initial review of cases.

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## NEW TECHNOLOGY AND *SORTA SITU*: CONSERVATION MEDICINE LINKING CAPTIVE AND WILDLIFE POPULATIONS

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### Abstract

Conservation medicine is defined as the study of the relationship between human ecological disturbance and the biologic health of populations and ecosystems, and the practice of applying this knowledge to biodiversity conservation and attempting to achieve ecological health. The applied goal of conservation medicine is both to improve the health of all living organisms and to conserve biodiversity. Through this discipline, veterinarians, physicians, wildlife ecologists and other conservation professionals are working together to provide an ecological context for health management in relation to many complex environmental issues facing the world today. Conservation Medicine places an emphasis on system thinking and discovering linkages, and consequently, is transdisciplinary.<sup>1,2</sup>

Human impact on the environment and ecological processes is well documented. Habitat destruction and species loss have led to ecosystem disruptions that include, the alteration of disease transmission patterns (i.e., emerging diseases), the accumulation of environmental contaminants and the invasion of alien species and pathogens. The health implications of these disturbing events require novel strategies for disease prevention, health management and conservation. Complex environmental problems increasingly require transdisciplinary solutions, new technologies that can be facilitated through interinstitutional collaborations. These changes call for a *sorta situ* approach to conservation, a fusion of *ex-situ* developed skills including small population management, hands-on care and special skills (veterinary, molecular, reproductive physiology) linked to field skills that include habitat restoration, community-based conservation and behavioral ecology (Table 1).

The presence of disease in individuals and populations can be an indicator of environmental health including local and global environmental impacts and ecosystem changes. All over the world, previously contiguous expanses of wild lands are being fragmented by encroachment of agriculture and other human activities. Habitat fragmentation and destruction are having many serious effects on threatened species. Using science, wildlife management, veterinary care, training and education, we are working toward mitigating the impacts of fragmentation on species whose survival will necessarily be within small, often isolated, habitat patches. A key area for this work is the Atlantic Forest of Brazil, the most endangered rainforest on the planet and only 2% of its original extent remains. Within these forest fragments are some of world's most endangered wildlife and planet species including the black lion tamarin (*Leontopithecus chrysopygus*). This ecosystem creates opportunities for disease transmission among species of

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wildlife, livestock, and humans. However, the species of wildlife, the diseases, the climate, and the forest structure and composition are all different, as are the economics and sociology of managing these issues. Wildlife Trust is developing a buffer zone research effort and examining the health, the risk of disease transmission among fragments, and the viability of black lion tamarins inhabiting this rainforest.

Human population expansion and unsustainable rural development are serious problems for much of the developing world, and climatic and environmental change has exacerbated the situation. The environmental consequences of these two issues are vast including loss of species and genetic diversity, and the spread of disease. In much of the developing world, these issues are reflected in an overall drop in the quality of life, with an increased proportion of the people living in abject poverty, and the ever-increasing unsustainable use of what should be renewable natural resources. In Southeast Asia these pressures have led the fragmentation or loss of much of elephant habitat. India has experienced extensive loss of most of the major wildlife populations over the years, leading to vegetative imbalances and a general deterioration in ecosystem health. Wildlife Trust is working with several local institutions to reverse these trends, and to stabilize or even restore elephant critical ecosystems. This endeavor will require a truly integrated *sorta situ* approach, and the collaborative efforts of many partners.

At the present time, the importance of wildlife diseases is recognized by private and governmental agencies in few countries. Wildlife Trust has ongoing collaboration with Mexican institutions regarding efforts to diagnose and control disease in migratory Neotropical bird populations during their wintering migration. Increasing data on disease agents in a greater number of species and scattered locations raise questions regarding the possibilities of disease introduction and exchange between geographic areas. There is supported evidence of annual reintroduction of pathogens from areas south of the US by migratory birds such as West Nile encephalitis, avian influenza, equine encephalitis, Newcastle disease and avian cholera. Surveillance for currently known diseases and isolation of new etiologic agents can be the initial attempt to establish the status of these diseases in Mexico. We are coordinating the effort to form a wildlife health cooperative in Mexico.

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**Table 1.** The changing nature of wildlife management and conservation.

	20th Century	21st Century
Wildlife Protection	Tactical, within protected areas	Strategic: populations in regional context
Wildlife Management	Passive (build a fence)	Active intervention
Management Skills	General	Specialized technologists working in transdisciplinary teams

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**THE USE OF SATELLITE TELEMETRY TO STUDY THE DISTRIBUTION AND LONG-RANGE MOVEMENT OF FLYING FOXES (*Pteropus* spp.) IN AUSTRALIA AND MALAYSIA: IMPLICATIONS FOR THE ECOLOGY OF EMERGING *Henipaviruses* (PRELIMINARY RESULTS)**

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**Abstract**

Over the past 10 yr, flying foxes (family Pteropodidae, genus *Pteropus*) have been identified as reservoirs for several emerging zoonotic diseases in South and Southeast Asia and the Western Pacific.<sup>1,2</sup> Hendra virus (HeV) and Nipah virus (NiV) are associated with a high case fatality rate, with HeV killing two of the three human cases in Australia and Nipah virus causing the death of nearly 40% of its cases in Malaysia (n = 265) as well as being associated with several recurrent and late-onset infections in survivors.<sup>3-5</sup> These two pathogens have been described as members of a new genus of paramyxovirus: *Henipavirus*. The Nipah virus outbreak in Malaysia also had very significant economic effects, with the destruction of over 1 million pigs leading to the loss of over US\$400 million in swine export.<sup>6</sup> Outbreaks of Nipah-like viruses in Bangladesh may have also stemmed from a flying-fox reservoir.<sup>3</sup> Preliminary data from this study suggests that flying foxes are capable of long-distance movement (>200 km) across geopolitical boundaries. Understanding the long-distance movements of flying foxes is particularly important for understanding the geographic distribution of *Henipaviruses*, as well as inter-population and interspecies transmission dynamics. Human activities such as deforestation, agricultural expansion, urbanization, global travel and trade have been linked to the emergence of zoonotic pathogens from wildlife by altering the ecology of wild animal species, creating more opportunities for spillover of zoonotic pathogens into humans via increased contact either between humans and wildlife, or wildlife and domestic animals.<sup>7</sup> From a conservation perspective, flying foxes play a critical role in rainforest propagation by dispersing seeds and pollinating flowers, and therefore efforts must be made to promote their conservation rather than vilifying them as hosts for deadly disease. The *Henipavirus* Collaborative Research Group, funded through the NIH Fogarty International Center, is studying the distribution and long-range movement patterns of pteropid bats where NiV and HeV have emerged, as a part of a large-scale ecological study designed to test the hypothesis that human environmental pressures such as agricultural expansion, deforestation, and hunting have altered flying fox behavior, which has

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acted as a driver for *Henipavirus* emergence. This report discusses preliminary findings from our initial cohort of satellite-collared flying foxes.

We placed Platform Terminal Transmitters (PTTs) (Microwave Telemetry, Maryland, USA) on four flying foxes of species known to carry Hendra virus (*Pteropus alecto*) (n = 3) or Nipah virus (*Pteropus vampyrus*) (n = 1). The movements of three black flying foxes (Bat A, B and C) in Australia and one Malaysian flying fox (Bat D) in Peninsula Malaysia were followed using Argos satellite telemetry systems (Collecte Localisation Satellites, French Space Agency, France). Location data was import into ArcView GIS 3.3 (ESRI, USA). Home range analysis was performed using the Kernal analysis method available in the Home Range Extension for ArcView GIS. Over a period of 120 days Bat A traveled 382 km to roost at four colonies, occupying a home range of 35,000 km<sup>2</sup> and was located foraging an average of 6.78 km from his roosting colony at night. Bats B and C remained in the same colony over a period of 143 and 128 days, occupying a home range of 11,000 and 1,000 km<sup>2</sup> and were located foraging an average of 5.3 and 2.7 km from their roosting colony at night, respectively. Bat D traveled 745 km over a period of 63 days roosting at four colonies, occupying a home range of 150,000 km<sup>2</sup> and was located foraging an average of 18.08 km from his roosting colony at night. The accuracy of the Argos Service Location Class (3, 2, 1, 0, A and B) errors were also compared with a known location to provide actual average errors (0.48, 0.82, 1.32, 8.13, 11.21 and 26.23 km respectively). The number of bats with transmitters will be augmented in order to have a more scientifically representative data set. With an improved understanding of the long-range seasonal movements of pteropid bats, coupled with disease distribution data, we hope to be able to better describe the geographic distribution of *Henipaviruses* in wildlife.

#### ACKNOWLEDGMENTS

This study is funded by an NIH/NSF "Ecology of Infectious Diseases" award via the John E. Fogarty International Center of NIH, TW05869 and by core funding to the Consortium for Conservation Medicine from the V. Kann Rasmussen Foundation. We would like to thank the consultants to our group, Tom Ksiazek (CDC, Atlanta) and Sai Kit Lam (Univ. Malaya).

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## THERMAL IMAGING: A NEW TECHNIQUE FOR MONITORING LIVE HISTORY OF NEWBORN EUROPEAN BROWN HARES

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### Abstract

During the last 25 yr the number of European brown hares (EBH) has been declined in Germany. In general, population dynamic is mainly influenced by reproductive success due to the number of neonates and postnatal mortality rate. The results of a reproductive assessment performed in 297 wild-caught EBH (160 males, 137 females) did not indicate any kind of reproductive disorders which could be responsible for the dramatic population decline.<sup>1,2</sup> There are no reliable data available regarding the postnatal situation in new-born EBH and the mean survival rate. Information about the exact impact of environmental factors like predators, diseases, climate changes or type of agriculture of the survival rate of new-born EBH are very difficult to receive for field researchers. It is very laborious to find new-born hares by classic field observation technique at daytime or by searchlight observation at night. These two techniques are characterized by a high potential of disturbance for hares observed. A newly developed thermal imaging system was applied to find and monitor neonates over a time period of at least 14 days. The study was carried out at night in different agricultural areas of the state Northrhine-Westfalia, Germany. The portable infrared thermography camera (Inc. Emerge Vision) equipped with a colour screen (5.4 × 4.0 cm) and connected to a portable VCR was successfully applied to detect hares based on their body surface temperature that differed clearly from the environmental temperature. Due to the high resolution imaging system and the special telephoto lens even small or hidden animals could be identified with high precision over a distance of 1.0 to 100 m. Each detected individual was categorized according its body size in three different age groups (neonate, juvenile, adult). A number of 13 young were detected at different field sites and their activities were closely monitored up to 14 days before they disappeared. The preliminary results of this study indicate a great potential for further application of this system. It offers new opportunities for monitoring life history of new-born hares and other small mammals.

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## OVARIAN STIMULATION, OOCYTE RETRIEVAL, AND ICSI FOLLOWED BY EMBRYO TRANSFER IN A WESTERN LOWLAND GORILLA (*Gorilla gorilla gorilla*)

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### Abstract

Assisted reproductive techniques are becoming more important as an alternative strategy for the breeding management of zoo animals. In this case, semen collected from a currently unrepresented male western lowland gorilla was used to fertilize oocytes collected non-surgically from a parous female gorilla. Pregnancy was not established. Nevertheless, this investigation demonstrates that sophisticated assisted reproductive techniques can help to better manage the captive gorilla population.

### Introduction

As management of current captive populations becomes more intense, it stands to reason that reproductive management would become increasingly sophisticated. As a consequence, assisted reproductive techniques are becoming more important as an alternative strategy for the breeding management of zoo animals. Although the AZA Gorilla SSP has one of the largest founder populations of any management program, there are still 21 (12 males, 9 females) potential founder animals that have no living offspring. One such male founder, SB# 268, was housed alone at the Gladys Porter Zoo and was treated chronically with steroids for an apparent immune-mediated disease. Due to ill health and social problems, this gorilla male had never reproduced in 40 yr and was therefore considered a good candidate for assisted reproductive techniques. Semen was collected from this male on three occasions using rectal probe electrostimulation and the samples were cryopreserved using standard techniques. Probably due to the long term lack of sexual activity, the sperm count and motility were extremely poor making in vitro fertilization by intracytoplasmic sperm injection (ICSI) the only option for embryo production since this requires only a single viable sperm per oocyte.

### Methods

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A 17-yr-old, parous female gorilla (SB # 376) housed at the Gladys Porter Zoo was given an oral monophasic estrogen/progesterone pill (OvCon 35, Bristol-Myers Squibb Co.) daily for 4 mo to control her menstrual cycle. Daily urine was collected to monitor urinary estrogen and progesterone and to synchronize her ovarian cycle. Immediately following pill withdrawal, her urine was tested daily for occult blood (Hemastix, Bayer Corporation, Elkhart, IN 46515) to detect the onset of menses (day 1). The ovarian stimulation protocol was as follows: on days 3 and 4, 225 IU FSH (Follistim, Organon Inc., West Orange, NJ 07052) and 75 IU hFSH + 75 IU hLH (Pergonal, Serono, Inc., Rockland, MA 02370) were administered; on days 5-10, she received 300 IU FSH (Follistim); on days 8-11, 25 mg GnRH (Antagon, Organon Inc., West Orange, NJ 07052) was included, and on day 11 she received 150 IU FSH (Follistim) and 150 hFSH + 150 IU hLH (Pergonal). Each of these treatments was administered i.m. by hand injection. Final oocyte maturation was stimulated on day 11, 12 hr following the last stimulation injection, by administering 10,000 IU hCG (Pregnyl, Organon) i.m.

Thirty-six hours post-hCG administration, the female was immobilized and the oocytes were retrieved using transvaginal, ultrasound-guided aspiration. Approximately 10-12 maturing follicles (10-15 mm diameter) were aspirated and six oocytes were recovered. The oocytes were transported in Hepes-buffered transport media in a portable incubator at 37°C by airline immediately following retrieval from Brownsville to Dallas, Texas. Less than 6 hr post-retrieval, the oocytes arrived at the Center for Reproductive Medicine in Dallas, Texas, and were fertilized by intracytoplasmic sperm injection (ICSI) using the cryopreserved sperm from the male SB #268. Fertilized oocytes were cultured in Gardner's Sequential Media at 37°C in 6% CO<sub>2</sub>. Three cleaved and by 115 hr post-ICSI, two of these developed into quality blastocysts five days post-retrieval. Both blastocysts were then transported back to the Gladys Porter Zoo and transferred transcervically using ultrasound guidance into the oocyte donor. At the time of embryo transfer, she was given 500 mg progesterone i.m. and then oral progesterone 400 mg b.i.d., p.o. (Prometrium, Solvay Pharmaceuticals, Inc., Marietta, GA 30062). Urine was monitored weekly using Ovuquik test strips for pregnancy detection. Unfortunately, pregnancy was not established.

## **Results and Discussion**

In spite of the fact that pregnancy was not established, this investigation demonstrates that ICSI is a viable option for assisted reproduction in the western lowland gorilla, a species that commonly is found to have poor semen quality. Further investigation into this and other techniques for assisted reproduction (including the use of sex-sorted sperm to produce female embryos) are currently in development as an alternative means to manage the captive gorilla population. In the future, such sophisticated techniques may offer population management programs a tool for genetic enhancement of captive populations. Sex-sorting sperm prior to fertilization of oocytes offers yet another opportunity for SSPs to help create a more manageable population. For instance, those species where there is currently a surplus of males that creates a

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housing problem, such as gorillas, elephants, and chimpanzees. Zoo veterinary staff are the key and must have an open mind towards such alternative means of animal management.

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## CRYOPRESERVATION OF BENNET'S WALLABY SPERM USING STANDARD AND DIRECTIONAL CRYOPRESERVATION TECHNIQUES: PRELIMINARY RESULTS

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### **Abstract**

A split ejaculate comparison was made between a traditional method of cryopreservation using a standard bovine freezing curve (IMV Technologies) and a novel method of cryopreservation using a directional cooling technique.<sup>1</sup> The objective was to test the hypothesis that directional cooling controls the rate of ice crystal propagation and ice crystal morphology more efficiently thus reducing mechanical and osmotic stress exerted on spermatozoa by random seeding and slow cooling. Additionally, the effects of centrifugation and long versus short equilibration of spermatozoa at 4 °C on post thaw sperm viability was evaluated. In conclusion, neither of the treatments applied produced acceptable post thaw sperm viability; therefore successful freezing of Bennetts wallaby semen remains an enigma. Follow up work on ultrastructure of both fresh and frozen sperm membranes is being undertaken.

### **Introduction**

Macropod sperm viability has historically been extremely poor post freeze-thaw using conventional methods of cryopreservation.<sup>3,7</sup> Increasing sperm viability over time will help maximise success of future artificial breeding in macropod species that are not widely held and/or are endangered. Ways of increasing viability while minimising the amounts of toxic cryoprotectants used to protect the sperm from rupture due to the freezing process need to be investigated. A directional freeze technique has recently been perfected by harmony cryocare ([www.harmonycryocare.com](http://www.harmonycryocare.com)) with apparent improvements in sperm viability in bulls and stallions. We aim to test this technique against standard cryopreservation practices, in the Bennett's Wallaby.

### **Methods**

Epididymides were collected from seven Bennett's Wallabies. The samples were processed based on previously published methods for marsupials.<sup>2-4</sup> HEPES buffered MEM (Minimum Essential Medium) was warmed in a waterbath to 35°C. Each epididymis was separated from the testicle and placed in the MEM. The head of each epididymis was removed. Surface connective tissue, including blood vessels was gently peeled back from the head using dissection scissors and forceps, while the epididymis was held in an MEM soaked gauze swab. The head was then removed from the rest of the epididymis and placed into 100mm diameter Petri dishes, lined with dental wax and holding 40 ml MEM. Each head was then scored in a cross hatch pattern with a

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scalpel blade to free the spermatozoa and dishes placed in an incubator set to 35°C to allow swim up for 45 min. The samples were then split, half being centrifuged and the resultant pellet re-extended with TRIS-citrate Buffer to be chilled at 4°C for 2 hr, the other half not centrifuged, and extended in a 1:1 mix of MEM: Tris buffer. Half the non centrifuged samples were held at 4°C for 2 hr, as per the centrifuged samples. The other half were cooled to 4°C and frozen immediately. Glycerol (at concentrations of 0%, 5%, 15% and 20% ) was added with the buffer at 4°C. Samples were frozen routinely for each freezing process, and thawed and analysed 2 wk post freeze.

## Results and Discussion

Our results thus far are preliminary, but we have found that centrifugation of wallaby sperm pre freezing (a common method of concentrating in domestic livestock) dramatically reduces sperm motility (from 75% to 5%). 6% of sperm were alive after centrifugation, compared to 78% (5% glycerol) and 40% (20% glycerol) in samples that weren't centrifuged.

Conversely, a short equilibration of sperm at 4°C of 10-15 min, did not appear to affect motility, when compared to keeping them chilled at 4°C for over 1 hr. There was minimal motility of all samples post thaw, and close to 100% membrane rupture was confirmed using 1 mg/ml propidium iodide and 5mg/kg Hoechst 33342 (bisbenzimidazole stain).

The spermatotoxic effects of glycerol have been well documented, however, some cryopreserver is required to protect the sperm membranes from cryoinjury. Results from Cooper, et al. (1995) indicate that the effect of 2% glycerol on the percentage of sperm showing forward motility is minimal when compared to motility without glycerol.<sup>2</sup> However, viability of sperm post freeze with glycerol any lower than 15% has been unsuccessful (Holt, personal communication). Prefreeze results the present experiment indicate that a glycerol percentage of 2.5% to 5% yielded best pre-freeze motility.

Freezing and recovery of electroejaculated sperm in Tammar wallabies was conducted by Molina and Roger (1996).<sup>7</sup> The spermatozoa were screened for toxicity in diluents containing a range of cryo-protectants. The most promising was 7.5% glycerol + 10% DMSO. Pellets were thawed to 35°C and spermatozoa were washed by centrifugation (200g for 5 min) and resuspended in diluent to minimize cryoprotectant toxicity. Progressive motility was high (3 on a 5 scale) but only 10% of motile sperm were recovered (the best post thaw motility found for any macropod species). This was considered by the researchers as possibly adequate for in vitro fertilization and AI. This has yet to be tested.

Diluent selection is critical for successful preservation of sperm in any species. Johnston, et al. (2000) found that TRIS- citrate buffer was superior to PBS as a preservation diluent at all temperatures for koala spermatozoa.<sup>6</sup> This indicates that methods developed for the preservation of eutherian spermatozoa (in this case, the widespread use of PBS) may not necessarily be

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suitable for marsupial semen. The present experiment indicates that TRIS-Citrate and MEM combination maintains a high motility percentage in wallaby sperm.

Antibiotic use in extended semen, to control the growth of bacterial contaminants is standard practice for domestic species, but was not included in the current experiment. When koala spermatozoa were extended in diluents containing 4000 IU/ml penicillin and 400 µg/ml of gentamicin, the rate of sperm motility declined significantly after 4 hr storage.<sup>5</sup> Also, in the current experiment, the sperm were harvested directly from the epididymus, thus eliminating contamination of preputial sperm, but contamination while in storage is a potential issue.

Marsupial spermatozoa can survive for about 1 wk at 4°C if left in the reproductive tract.<sup>10</sup> Koala spermatozoa remained motile even after 42 days storage at 5°C in TRIS-citrate buffer diluent.<sup>6</sup> This has implications for artificial insemination in macropod species not widely held (for example the tree kangaroos, allowing selective outbreeding of an inbred population), and needs to be investigated further. Processing for non frozen sperm from recently deceased individuals can be conducted as outlined in the present experiment.

The largest issue for post freeze viability in marsupials appears to be damage to lipid membranes sustained on thawing. Electron microscopy work is currently being undertaken to examine the ultrastructure of fresh sperm and those that have undergone the freezing process to investigate specific freezing injury. Until this issue can be resolved, successful cryopreservation of macropod sperm will remain problematic.

#### ACKNOWLEDGMENTS

Thanks to staff and Management of Whipsnade Wild Animal Park for their assistance in conducting this experiment, and Professor Michael McGowan for technical assistance.

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## TAIL-MOUNTED RADIO TRANSMITTERS IN BEAVERS (*Castor canadensis*): AN ALTERNATIVE TO ABDOMINAL IMPLANTATION?

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### Abstract

### Introduction

In addition to being a Canadian icon, the beaver (*Castor canadensis*) still has important economic and social value for the First nations in Quebec. Hydroelectric projects in the James Bay basin can locally alter the beaver's habitat. Translocation of beavers from sites of future hydroelectric development to non-altered habitats has been advocated as a management strategy to preserve local populations of this fur animal. However, these translocations should be closely monitored to assess successes and failures. One of the objectives of our pilot study was to evaluate if ear-tag transmitters designed to be used in wild ungulates could be successfully attached to the tail of beavers, and therefore used to assess dispersal and survival of translocated animals. If reliable, these external devices would represent a less invasive alternative to the surgically implanted abdominal transmitters currently used in this semi-aquatic rodent species.

### Methods

Twenty beavers (15 adults, 4 subadults and 1 juvenile) ranging from 4-19 kg were live-captured during summer 2003 near Eastmain River, James Bay, Quebec, Canada. With the exception of one juvenile male, all the other juvenile beavers captured (10) were judged to be too small to accommodate the transmitters available. Each animal was anesthetized with an intra-muscular injection of a combination of ketamine and xylazine (either 0.32 mg/kcal of ketamine + 0.03 mg/kcal of xylazine OR 0.23 mg/kcal of ketamine + 0.07 mg/kcal of xylazine; for an average of 9.7 mg/kg of ketamine + 2.1 mg/kg of xylazine). When the beaver was sufficiently immobilized for handling, the attachment site on the tail (approximately 12 mm thick) was disinfected with several passages of 2% chlorhexidine. These attachment sites were located between 2 and 4 cm laterally to the midline of the tail and 5-10 cm caudally from the base of the tail depending on the size of the animal. The surgical site was then transmurally infiltrated with 0.3 ml of bupivacaine 0.5%. A transmural anchor hole was made using a sterile 5.5-mm surgical drill bit powered by a cordless drill. The transmitters used in this study were manufactured by ATS (model M3530, 470 First Avenue North, Isanti, MN 55040). Each transmitter (35 g; 47 mm X 16 mm X 43 mm; 11

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cm antenna) was affixed by ATS to an AllFlex "global female" ear tag (Allflex USA, Inc., 2805 East 12th Street, Dallas Ft. Worth Airport, Texas 75261-2266). The transmitters were fixed on the dorsal aspect of the tail using a sterile attachment button, composed of a 12-mm long plastic post perpendicularly linked to a 25-mm in diameter circular flat disk. This button was inserted ventrally in the pre-drilled hole and secured to the "global female" ear tag using a Universal Total Tagger (Allflex USA). The radio tags were set up with an activity switch that monitors the motion of the animal and increases the pulse rate after 24 hr of inactivity. After the completion of the procedure the anesthesia was partially reversed with 0.2 mg/kg of atipamezole i.m. and the animals were transferred by helicopter to the relocation sites. The beavers were then housed in retention pens and released either the same day or up to 5 days post-capture. Telemetric surveys started a few days after the translocation of the tagged beavers. The relocation sites were surveyed by helicopter at seven occasions during the 3-mo period following the translocation.

## **Results and Discussion**

All the beavers survived the procedure and recovered well. The level of anesthesia obtained was sufficient for the procedure, and the recoveries were fast and uneventful. The broadcast distance of transmitters was evaluated to be 1.5 km at an altitude of 100 m. Increased pulse rates indicative of immobility was recorded for eight of the 20 transmitters within the first 3 mo. Two of these beavers died: one was preyed upon by a black bear while still in its retention pen and the other was shot by a hunter. Four of the six other immobile tags were found on the ground without any sign of predation. Examination of the fallen tags suggested that they were either pulled by the beavers or dislodged from their tail after being entangled in branches or bushes. The two last transmitters were located, but couldn't be retrieved since they were underground on edges of lakes, most likely in underwater tunnels. This study shows that attachment of transmitters on the tail of beavers is a relatively non-invasive procedure that can be easily performed in the field. Nevertheless this procedure, which involved the surgical drilling of a well innervated structure, should only be performed under general anesthesia. The anesthetic protocol used in this study was sufficient for the level of pain induced by the procedure and well tolerated by the animals. However, this level would have been insufficient for abdominal implantation of transmitters. In this study, the working life of these ear tags mounted on beaver's tails was not optimal. Indeed, when removing mortalities (2), at least 25% (4 out of 16) of the tags were lost within 3 mo. It is likely that this figure will increase over the winter. This will be assessed by telemetric surveys planned for the spring of 2004. Some modifications of the attachment buttons, such as increasing the diameter and rigidity of the disk, might increase the working life of these tags. In addition, posts of adjustable lengths (up to 30 mm) would enable the fixation of the tags in the thickest area of the tail (closer to the base and the center of the tail). This should increase the strength of the anchorage and would therefore likely decrease the rate of tag lost encountered in our pilot study. Further work will have to be conducted to evaluate if the working life of these tail-mounted tags would reach an acceptable level with these modifications.

## **ACKNOWLEDGMENT**

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We would like to thank George Coonishish, and Matthew Wapachee (Cree trappers) and Joël Poirier (Wildlife Technician) for their help with the field work. This study was financially supported by the *Société d'énergie de la Baie James* (Eastmain-1 project).

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## LINKING ECOLOGY, EPIDEMIOLOGY AND PATHOLOGY OF SOUTHERN SEA OTTERS: A BRIDGE OVER TROUBLED WATERS

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### **Abstract**

The southern sea otter (*Enhydra lutris nereis*) has been listed as “threatened” under the Endangered Species Act (ESA) since 1977.<sup>1</sup> Despite periods of slow growth and range expansion since the early 20<sup>th</sup> century, the population has recently ceased growing appreciably and mortality events have occurred. A collaborative and multidisciplinary effort is underway to determine what forces are limiting the recovery of these animals

### **Introduction**

The limited ability of the southern sea otter population to recover to levels that would allow delisting appears to be the result of adult, particularly prime age female, mortality, a large proportion of which apparently results from diseases, parasites and intoxications. At this time it is critical to link what we know about pathology and epidemiology of these sea otters with what is known about their ecology. This is being done by collaborative examination of extensive databases, risk analysis, and by observation of instrumented living animals which are periodically captured and sampled for markers of health and often eventually recovered and examined by pathologists when they die.

### **Methods**

Approximately 80 southern sea otters have been surgically implanted with temperature sensitive VHF radio transmitters and time depth recorders (TDR’s) at three study sites along the California central coast. Animals are tested for general health, IgG levels, genetic diversity, disease and contaminants exposure, and other factors. Many of these animals have been intensively observed and extensive ecological data including time activity budgets, habitat utilization, food habits and prey preferences, variations in core body temperature, social interactions and

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movement, have been recorded. The TDR's are recovered from many otters that die or those that are recaptured and the core body temperature, exact time and depth to which animals dive is retrieved from the archival tags. This information is compared to ecological observations and correlated with measures of health, disease and contaminant exposure. For animals that die and their bodies are recovered, pathologic observations as to cause of death and additional laboratory data can be added into the dataset. Certain behaviors and activities, locations and diet types appear to place animals at different levels of risk of exposure and/or at risk of dying from various causes.<sup>2,3</sup>

## Results and Discussion

We will present findings on several animals whose lives and deaths are typical and instructive. By using implanted physiologic monitoring devices we have been able to link behavioral and ecological observations with epidemiologic risk factors and verify outcomes at post mortem examination. This integrated program is allowing us to gather unprecedented information about the life, health and death of an otherwise hard to observe free-ranging marine mammal. Some causes of significant mortality have apparent links to terrestrial sources of pollution<sup>3</sup> and may represent outstanding examples of "pathogen pollution."<sup>4</sup> Large scale, multi-year, transdisciplinary studies like those described have the likelihood of providing sufficient biologic and biomedical data to support the potentially difficult regulatory and expensive policy decisions that may be required.

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## UNDERSTANDING IMPACTS OF SOUND ON DOLPHINS AND WHITE WHALES

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### **Abstract**

The first detailed audiogram of the bottlenose dolphin showed good underwater hearing with a threshold of around 42 dB re 1  $\mu$ Pa ( $10^{-14}$  W m<sup>2</sup>) at 60 kHz. Studies also showed dolphin and white whale sensitivity to sound frequencies from about 60 Hz to 150 kHz, almost 8 times the frequency span of human hearing (humans are slightly more sensitive to sound pressure in air but human frequency range is limited to about 20 kHz).

The audiogram of both species has been replicated many times and it is clear that sensitive ears connected to a massive auditory central nervous system are fundamental to the dolphin's echolocation and communication. It is reasonable to ask how the animal, with such excellent hearing, avoids damaging its own ears with the loud sounds it produces during echolocation. The dolphin ear, anatomically only a few centimeters away from its sound production mechanism, processes high-frequency echolocation pulses up to 230 dB re 1  $\mu$ Pa in peak-to-peak amplitude. Using intense pulses and sensitive ears, dolphins can detect echoes (as quiet as a human whisper) from small objects at 100 m and more. Because the dolphin's pulses are very brief, on the order of 40  $\mu$ s, and 25,000 of these would equal 1 sec of sound, therefore the total energy within each pulse is miniscule. Anatomic structures, including highly reflective air sinuses that attenuate sound, probably help the animal avoid damaging its own ears. Humans introduce loud sound in the sea for purposes of improved sonar, construction, oil exploration, and acoustic communication. So how do we determine if human generated sound in the sea poses a serious threat to marine life?

My presentation will cover experience with simulated sonar pings and impulsive devices that have been employed in open water hearing tests with trained dolphins and white whales. Simulated 1-sec pings of 140 to 202 dB re 1  $\mu$ Pa at frequencies of 0.4, 3.0, 10.0, 20.0 and 75.0 kHz were presented on many different days to seven different cetaceans without ill effect. These pings did produce a temporary shift in hearing threshold at an average level of 195 db re 1  $\mu$ Pa in the mid-frequency range.<sup>1</sup> Single impulses from an underwater seismic water gun up to peak pressures of 160 kPa (226 dB re 1  $\mu$ Pa) were tested with a white whale and 207 kPa (228 dB re 1  $\mu$ Pa) with a bottlenose dolphin.<sup>2</sup> No short or long term injury was observed although some hormonal indicators of mild immune system stress were observed in blood specimens collected after exposure.<sup>3</sup>

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## MARINE MAMMAL MORTALITIES ASSOCIATED WITH ALGAL TOXINS

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### Abstract

Marine mammal mortality events associated with harmful algal blooms appear to have increased in recent years, in concert with the increased frequency and expanded geographic distribution of harmful algal blooms and improved diagnostics for the identification of these toxins. These events typically present as large numbers of animals presenting ill or dead on beaches over a short time period. Because of the significance of these events, veterinarians should possess knowledge of the potential toxic agents, clinical and post-mortem findings, and appropriate sampling methods to assure that proper diagnostics can be performed.

To date, domoic acid and brevetoxin have been definitively linked to episodic marine mammal mortality events. Two other algal toxins, saxitoxin and ciguatoxins are highly suspect for mortalities. Beginning in 1998, domoic acid, produced by the diatom, *Pseudo-nitzschia australis*, has been associated with extensive mortalities of California sea lions, dolphins, and Southern sea otters on the California coast. A second class of algal toxin with definitive links to manatee and bottlenose dolphin mortalities is brevetoxin, produced by the dinoflagellate *Karenia brevis*.<sup>3,4</sup> Saxitoxin has been implicated in the unusual stranding and mortality of humpback whales in Cape Cod Bay in 1989, and the mortality of more than 100 Mediterranean monk seals on the coast of Mauritania in 1997. Although not currently implicated in mortalities, ciguatoxins have long been suspected to be involved in the poor survival of the Hawaiian monk seal.<sup>1</sup>

Although the mechanisms of action vary, all of these toxins are neurotoxins and both clinical and post-mortem findings reflect this action. Routes of exposure also vary based on the toxin and species affected. Exposure occurs either directly through respiratory exposure or indirectly via food-web transfer. The susceptibility of marine mammals to algal toxins is therefore dependent not only upon the occurrence of toxin-producing algae within the habitat but often the co-occurrence of appropriate prey species at the time of an algal bloom to serve as vectors. For example, for domoic acid in the same geographic area, the primary vector for sea lion exposure is anchovies as compared to sea otters where invertebrates, specifically the spiny mole crab, are identified as the vectors of concern.

Because of the neurotoxic method of action of these toxins, history and gross findings are often suggestive but not diagnostic for toxicity. Clinical presentation can vary from individual affected

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animals to mass mortalities. History and environmental assessments may include associated die-offs of birds or fish, and detection of pathogenic algal blooms within the area. Because of variations in vectors, it is possible for a single geographic area to have temporal variation in affected marine mammals associated with changes in the levels of toxic algae within the food chain.

Clinical signs associated with domoic acid intoxication vary by species affected and include head weaving, scratching, tremors, convulsions, vomiting, blindness, abortion and sudden death. Gross examination findings are limited and include animals in good nutritional condition with empty stomachs from vomiting. Occasionally, areas of patchy cardiac pallor can be seen. Histologic review demonstrates hippocampal necrosis often with associated nonsuppurative multifocal encephalitis. Heart often demonstrates patchy multifocal myocardial degeneration and fibrosis.<sup>2</sup>

Brevetoxicosis clinical signs have been documented in manatees and include disorientation, inability to properly submerge, listing, listlessness, back flexing, lip flaring, and labored breathing. In manatees, gross findings reveal animals in good body condition typically with sea grass-filled stomachs. Histologic review reveals severe congestion of respiratory tissues, kidney, and brain.<sup>2</sup>

Clinical and postmortem sample collection from animals suspected to be affected by biotoxins should be based on available diagnostic modalities and biologic behavior of these toxins. Domoic acid can be detected in stomach content, serum, feces, and urine. Pregnant animals have had detectable levels in amniotic fluid. Collection of appropriate diagnostic samples is often impeded by the rapid clearance of the toxins by urination, defecation, and vomition. Because of this, collection of samples early in affected animals, and collection of a variety of fresh (non-fixed) post-mortem samples is critical for proper diagnosis. Highest levels of toxin have been identified in urine and feces. Scanning electron microscopy of gastric content and fecal samples can identify the presence of *P. australis* frustules.

Brevetoxin can be found in stomach content, liver, kidney and lung tissues of affected animals via receptor assay, ELISA, and HPLC-mass spectrometry. Additionally, immunohistochemistry utilizing anti-brevetoxin antibody performed on fixed tissues has identified positive staining in lymphocytes and macrophages in respiratory, renal, and nervous tissue.

Toxin level determinations are the key to both diagnosis and ecological assessment of marine biotoxin impacts. Because there are multiple biotoxins implicated and potentially affecting marine mammals, sample collection for analysis of unknown toxins should include multiple fresh or frozen samples. These should include gastric intestinal and colonic content, urine, bile, serum, CSF, brain, lung, liver, and kidney. As more biotoxins are identified and as the frequency and distribution of blooms increases, it is likely that new biotoxins will be identified as the causes of marine mammal strandings and mortalities. Further investigations remain needed in

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determining dose response and toxicokinetics, the impacts of multiple biotoxins, as well as chronic, low-level exposure impacts.

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## PARALYTIC SHELLFISH POISONING IN GENTOO PENGUINS (*Pygoscelis papua*) FROM THE FALKLAND (MALVINAS) ISLANDS

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### Abstract

From December 2002 through January 2003 a large scale seabird mortality was recorded off the shores of the Falkland (Malvinas) Islands, in the southwestern Atlantic Ocean. Affected species were gentoo (*Pygoscelis papua*), rockhopper (*Eudyptes chrysocome*) and Magellanic penguins (*Spheniscus magellanicus*), as well as albatrosses, petrels and prions (*Procellariidae*). Best estimates suggest that 100,000-200,000 seabirds may have died during this event. Clinical signs in affected birds led us to investigate for the presence of toxins. Results showed the presence of neosaxitoxin (NeoSTX) and gonyautoxin 4 (GTX4), which are components of the paralytic shellfish poisons. Histopathology of affected birds showed minor microscopic changes. Infectious disease serology on albatross and gentoos (affected and apparently healthy animals), included paramyxovirus (Newcastle's, Type 2 and Type 3), avian adenovirus, avian reovirus, avian influenza virus, avian laryngotracheitis virus, infectious bronchitis virus, infectious bursal disease, avian encephalomyelitis, *Salmonella pullorum*, *Chlamydophila psittaci* and Marek's disease. Results were negative for all of the above diseases except avian adenovirus. This is the first report of seabird mass mortality in the Falklands to affect such a wide range of species simultaneously and the first report of PSP affecting/killing seabirds in the South Atlantic. Because of the increasing occurrence of toxic algal blooms in this area, we encourage the establishment of a regular monitoring system and response team to investigate wildlife mortalities as they are reported. An organized, joint multi-agency effort, with governmental support is key for successful investigation and preventive action.

### Introduction

From December 2002 through January 2003 a large scale seabird mortality was recorded off the shores of the Falkland (Malvinas) Islands, in the southwestern Atlantic Ocean (51 45 S, 59 00 W). Affected animals were mostly adult penguins, including gentoo (*Pygoscelis papua*), rockhopper (*Eudyptes chrysocome*) and Magellanic penguins (*Spheniscus magellanicus*).

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However, several other species were reported ill and/or dying, such as albatrosses, petrels and prions (*Procellariidae*). Mortalities occurred at several seabird colonies located on different islands within the archipelago, mostly in islands located to the northwest and south. In some areas, breeding pairs were down to 10% of the usual size of the specific colony and in others, entire breeding sites were abandoned even though eggs had been laid.

## Methods

In order to evaluate possible causes of death, an array of samples were collected from healthy, clinically ill and dead seabirds. Blood samples were collected from clinically “normal” black browed albatross (*Diomedea melanophrys*) (n = 26), rockhopper (n = 55), Magellanic (n = 11) and gentoo penguins (n = 68) and from nine gentoo penguins in bad physical condition or showing severe clinical signs. After blood collection, four of the severely affected gentoo penguins were euthanatized and complete necropsies were performed. Additionally, tissue samples were collected from freshly dead animals that were found on the beach, four Magellanic and six gentoo penguins. Blood samples were centrifuged and harvested plasma was stored in liquid nitrogen. Tissue samples were collected for histopathology and stored in 10% buffered formalin. A duplicate set of tissues (liver, spleen, kidneys, GI tract and brain) and gastrointestinal contents when present, were frozen in liquid nitrogen.

Analyses for biotoxins were conducted at the Instituto de Fomento Pesquero (IFOP, P.O. Box 101, Punta Arenas, Chile). Samples were analyzed by high-performance liquid chromatography (HPLC) on a Shimadzu spectrofluorometric analyzer in search of red tide toxins from the paralytic shellfish poisoning group (PSP). Histopathology and bacteriology analyses were performed at the National Veterinary Institute in Uppsala, Sweden.

## Results and Discussion

Results showed the presence of neosaxitoxin (NeoSTX) and gonyautoxin 4 (GTX4), which are components of the PSPs. Five of eleven individual penguins submitted tested positive (45%). Of these positive individuals, one or more tissue samples had high levels of toxins (levels above those considered unsafe for humans: 80 ug STXeq 100<sup>-1</sup> g shellfish meat). Additionally, low or trace levels of other PSP toxins were detected, particularly GTX1, GTX2, GTX3 and STX. Tissues from which high toxin levels were recovered were intestine or intestinal content (80% of tested samples were positive), followed by liver (45%) and stomach content or stomach wall (33%). No toxins of this group were found in fat tissue or aqueous humor of the eye.

Positive animals included two gentoo penguins collected after death (livers positive to NeoSTX). Both of these animals and a third gentoo also had low levels of STX and GTX 2 & 3. On the other hand, three of the four ill gentoos euthanatized were positive to NeoSTX & GTX4 and also had trace levels of STX, GTX1, 2 & 3. In these animals, tissues with detectable toxins were intestinal content, liver, kidney and stomach content in two of them, and liver, brain, spleen, intestine and intestinal content in the other. Assuming that dead birds had died from toxin

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ingestion, samples collected from euthanatized or dying animals proved to be better for toxin isolation, compared to those from animals found dead, even if they were fresh.

Histopathology of birds found ill (n = 4) or dead (n = 6, five gentoos and one Magellanic) did not show microscopic changes indicative of acute intoxication. For those birds which were euthanatized, there were no gross necropsy findings of significance, most were in good body condition and no parasites were found in their gastrointestinal tract. On histology, most birds showed older inflammatory reactions in bile ducts with periportal infiltrates of eosinophils and heterophils and some other leukocytes. Areas of fibrosis were found in one bird. Hemosiderosis was present in the liver of seven penguins, but no hepato-splenomegaly was observed. Liver cells and parenchyma appeared normal with no signs of necrosis or reaction. No inclusion bodies (neither IC nor ICP) were observed. Older inflammatory reactions were found in intestines with degeneration of crypts and epithelium and infiltration of WBC. No lesions were present in the brains, meninges, hearts, kidneys or lungs (except for congestion in the lungs). Spleens were found to be active, some with hyperplasia, but no necrosis. No signs of adenovirus (inclusion bodies) or plasmodium infection were detected. Bacteriology was performed on lung, kidney, brain, muscle and heart tissue of one Magellanic and one gentoo penguin with negative results.

Paralytic shellfish poisoning (PSP) is caused by several dinoflagellates including *Alexandrium* spp., *Gymnodinium catenatum*, *Pyrodinium bahamense* that produce toxins known as saxitoxins and derivatives such as neosaxitoxin and gonyautoxins I to IV. PSP results from ingestion of shellfish, crustaceans, fish and other organisms that have accumulated those potent toxins as a result of consuming dinoflagellates.<sup>9</sup> PSP manifestations in humans are acute paresthesias and other neurologic manifestations which may progress rapidly to respiratory distress, muscular paralysis and death.<sup>13</sup> It is believed that birds are more susceptible to PSPs than other warm blooded animals. For seabirds, symptoms such as loss of equilibrium, incoordination, convulsions, paralysis, vomiting, abnormal green-brown feces and congestion of organs including lung have been previously described.<sup>13</sup>

Organisms of genus *Alexandrium* have been reported in Argentina (*A. tamarensis*)<sup>2,8,9</sup> and Chile (*A. catenella*).<sup>5</sup> Geographically, positive animals in our investigation were collected at different locations in the archipelago which lies on the Argentina continental shelf. Gentoo penguins are opportunistic feeders, and around the Falklands are known to take fish (56% of diet, mainly from the cod family *Patagonotothen* sp., but also *Micromesistius australis* and other species), crustaceans (36%, *Munida gregaria*) and squid (11% *Loligo gahi*, *Gonatus antarcticus*, *Moroteuthis ingens*).<sup>3,10</sup> PSP toxins have been detected in sardines (plankton feeders) but also in higher trophic level feeders such as mackerel (*Scomber japonicus*)<sup>1,8</sup> and anchovy (*Engraulis anchoita*)<sup>9</sup> in the South Atlantic. Montoya, et al. (1997) report a mackerel mortality event off the shores of Buenos Aires province in 1993, probably associated with penguin and other seabird deaths. It has been suggested that herbivorous zooplankton is the main biologic PSP vector for these fish,<sup>8,9</sup> and that mackerel could have the ability of accumulating toxins in their liver. However, no information on toxin detections on the above mentioned gentoo prey items has been reported.

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This is the first report of seabird mass mortality in the Falklands (Malvinas) to affect such a wide range of species simultaneously. Producing a realistic figure for the number of birds that died as a result of this event is very hard to do at the islands' scale. In the few colonies of gentoo penguins that were again monitored in November 2003, after the event, numbers were down to one third, while colonies not affected in the north east of the islands were either stable or increasing. This leads to a rough estimate of a minimum of 30,000 to 50,000 gentoo penguins missing. Estimating the number of rockhopper penguins is more complicated as fewer colonies that were affected were monitored the following season. Some affected colonies had their numbers drop by one third only, while the main colony of Steeple Jason, lost 60,000 pairs out of 89,000 counted in the 2000 census. Again, a rough estimate of birds actually dying from poisoning, island wide, might be between 30,000 to 70,000 for rockhopper penguins. Census of albatross after the event was only conducted on Steeple Jason where numbers dropped from 158,000 in 2000 to 112,000 in November 2003. Albatross also suffer from mortality associated with fishing activities, so not all the 45,000 missing pairs might be due to intoxication and a reasonable estimate would be between 10,000 and 30,000 albatross may have died from poisoning. Due to lack of effective census no numbers can be produced for Magellanic penguins and burrowing petrels, but considering the number of reports of affected birds, numbers could be in the tens of thousands (Huin, unpublished data). Even though seabird numbers suggest that up to date these events have had a very low frequency of occurrence, it seems like they have been getting more important in the last decade, reflecting global features of harmful algal blooms (HABs), exhibiting a higher frequency, a higher geographic cover and a higher intensity.

In addition, serology for infectious diseases was performed on the euthanatized penguins and on a sample of apparently healthy animals at various locations with evidence of mortalities. Serology included paramyxovirus (Newcastle's, Type 2 and Type 3), avian adenovirus, avian reovirus, avian influenza virus, avian laryngotracheitis virus, infectious bronchitis virus, infectious bursal disease, avian encephalomyelitis, *Salmonella pullorum*, *Chlamydomytila psittaci* and Marek's disease. The albatross and gentoos were antibody negative for all of the above diseases except avian adenovirus. Results for Magellanics and rockhoppers are still pending. For the gentoos tested, of the ones showing neurologic symptoms 7 of 9 tested positive (78%), and of the ones that appeared clinically normal 15 of 27 tested positive (55%).

There are no published references for adenovirus causing disease in penguins or seabirds. Nonetheless, avian adenoviruses are distributed worldwide and many avian species are known to be susceptible. This agent causes disease in galliformes and has also been reported as a possible pathogen in pigeons, raptors, psittaciformes, and waterfowl.<sup>4</sup> Positive antibody titers have been found by Karesh, et al. (1999) in rockhopper penguins, in southern giant petrels (*Macronectes giganteus*),<sup>15</sup> and in other seabirds such as Magellanic penguins, imperial cormorants (*Phalacrocorax albiventer*) and rock shags (*P. magellanicus*) from Argentina (Uhart, et al., unpublished data). However, the test used for avian adenovirus antibody detection presents moderate specificity and has not been validated for these species, and could result in false positives.<sup>6,12</sup>

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An unexpected result was the relative absence of evidence of exposure to the other infectious diseases in the birds sampled. We have found positive titers to many of these in seabirds in Argentina and Peru using the exact same sampling and test methods<sup>7</sup> (Karesh and Uhart, unpublished data). A possible explanation would be that other migratory birds do not come in contact with the albatross or the gentoo colonies which, due to their relative isolation, have not been exposed to infectious diseases that are endemic in populations elsewhere. The lack of positive antibody titers also suggests that these birds may suffer heavily if or when these diseases are introduced to the colonies. Good sanitation practices to prevent contamination or cross-contamination among colonies and islands would be prudent to prevent the spread of infectious agents introduced by tourists/visitors, researchers, land owners or officials.

This is the first report of PSP affecting/killing seabirds in the South Atlantic. Though it is possible that events such as the one reported here have occurred before<sup>8,11,14</sup> in this area, it has not been possible to identify specific toxins in tissues of affected/dead animals. The spatial and temporal distribution of toxic dinoflagellate blooms seems to have expanded over the last 2 decades in the Argentinian Sea.<sup>2</sup> Therefore, we encourage the establishment of a regular monitoring system of plankton in coastal waters as well as an immediate response team to investigate wildlife mortalities as they are reported. The experience gained has shown that there must be an organized joint multi-agency effort with governmental support for successful investigation and preventive action.

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## THE ROLE OF OILED WILDLIFE CARE IN SEABIRD POPULATION HEALTH AND SPILL INVESTIGATION: THE S.S. JACOB LUCKENBACH CASE STUDY

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### Abstract

In response to the devastation wreaked by oil spills such as the *Exxon Valdez*, California legislation passed in 1990 required the state's Department of Fish and Game to establish the Office of Spill Prevention and Response (OSPR) to prevent and protect California's coastline from the impact of such catastrophes. The OSPR was also charged with the establishment of rescue and rehabilitation stations to care for seabirds, sea otters and other marine mammals that might be impacted by such events. The Oiled Wildlife Care Network (OWCN) was established by the OSPR in 1994 to ensure that wildlife exposed to petroleum products in the environment receive the best achievable treatment through access to permanent wildlife facilities and trained personnel that are maintained in a constant state of preparedness for oil spill response. During response, the OWCN receives assistance from many or all of its twenty five participating organizations trained in state-of-the-art skills for wildlife care, and uses one or more of twelve regional facilities either built specifically for, or modified to accommodate, oiled wildlife. Since 1997, the OWCN has been administered by the Wildlife Health Center at the School of Veterinary Medicine, UC Davis, and has since become recognized as the world leader in oil spill response, rescue, and rehabilitation.

In addition to individual animal care during oil spills, the OWCN has spent considerable effort focusing on other issues critical to understanding and preventing the impact of oil on wildlife. Since 1996, the OWCN has led a competitive grants program focused on better understanding the effects of oil on wildlife. This program has funded more than sixty applied and hypothesis-driven research projects that have allocated over \$1.5 million to increase knowledge of the consequences of oil exposure to wildlife (both at an individual as well as a population level), and to improve the quality of response technology for oil spill response. In addition to fostering research, the OWCN has been a key player in California (as well as internationally) for the development of protocols and procedures aimed at the collection of evidentiary and baseline information both before and during spills – information necessary for the better understanding of

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the “true” effects of spills at a population level and for any investigations necessary to determine the party responsible for such releases in the marine environment.

An example of the multifaceted impact of oiled wildlife care to overall spill response efforts was seen starting in the winter of 2001. In November, the OWCN was notified that a large number of oiled birds were being observed on the San Mateo County coastline, with no apparent fouling of the coastline. Search and collection teams were rapidly deployed to collect these animals, document specific location information on where animals were found, and identify information on the search effort. All affected animals were recovered as rapidly as possible, and transported to the OWCN-managed San Francisco Bay Oiled Wildlife Care and Education Center so that the rehabilitation process could begin. This process includes comprehensive medical examination and detailed evidence of the collection process, including the collection of feather samples for gas chromatographic (GC-FID) “fingerprinting” to link the product with the oil in the environment.

While oiled animals continue to be collected throughout the central coast area, no apparent source of the oil has been identified even after extensive over-flights using fixed-wing aircraft. Oiled animals were reported on the Farralon Islands and have been collected as far north as the Point Reyes seashore and as far south as Monterey Bay, indicating that the source of the oil might be further offshore than previously expected. Additionally, greater numbers of affected animals follow significant storm events, lending evidence to a single point-source cause. The OSPR, in conjunction with the US Coast Guard and other governmental agencies, quickly developed a joint task force to investigate the cause(s) of the event(s). Animal location and species data (collected by search and collection teams) and oceanographic data were used to “hindcast” the probable areas where the release(s) were occurring – an area immediately southeast of the Gulf of the Farallones National Marine Sanctuary. Feather samples collected during the animal intake process were analyzed using GC-FID, and were determined to largely come from a single source. This information, combined with the evaluation of other available data (such as vehicular transit through the proposed area and the accessing of selected satellite images taken of the area), quickly pointed to a sunken vessel as the most likely cause. By combing thorough maritime data, the list of possible sources (over 1,600 wrecks off of the California coast alone) was quickly narrowed to four possibilities. A remote-operated vessel (ROV) was chartered to investigate the most likely source and, through comparisons of oil found on the ROV’s tether to that on feather samples, the source was confirmed as the *S.S. Jacob Luckenbach*, a 148-meter freighter which collided on 14 July 1953 with its sister ship and sank in 55 m of water about 27 km west-southwest of San Francisco. Over the ensuing 8 mo, the USCG, in collaboration with OSPR and other agencies and salvage companies, successfully embarked on an unprecedented effort to recover as much of the over 10,000 barrels of fuel oil that might be within the ship to reduce future impacts on the environment. However, the direct impact of the spill on wildlife was significant with over 2,100 live and dead birds collected over 9 mo.

In addition to this acute impact during 2001-2002, further work by the OSPR has linked the *Luckenbach* as the cause of multiple “mystery spills” in and around the San Francisco Bay area,

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dating at least back to 1992 (the inception of the OWCN's feather collection protocols) and possibly as far back as 1973. Through evaluating animal intake data, collected samples, beach search efforts (providing more precise animal impact estimates) and historic records from rehabilitation and ornithologic organizations, it appears total bird mortalities may be in excess of 90,000 animals since 1973 and, were the ship leaking at the same rate since its sinking, might have exceeded 150,000 animals in total.<sup>1</sup> These numbers may help to clarify the significant population decline that has been observed in the common murre (*Uria aalge*), the species most frequently collected during all northern California "mystery" spill events. This population impact further exemplifies the role of the OWCN, not only in caring for and returning to the environment healthy individuals, but for participating and playing an active role in the overall spill response effort.

#### **ACKNOWLEDGMENTS**

The authors wish to thank Steve Hampton, Kim McCleneghan and Julie Yamamoto (DFG-OSPR), Jay Holcomb and the staff of International Bird Rescue Research Center, and the staff, members and volunteers of the Oiled Wildlife Care Network for their tremendous help and support during this spill event.

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## COMPARING THE INFLUENCE OF AGE, SEX, AND LOCATION ON DISEASE AND PERSISTENT ORGANIC POLLUTANT EXPOSURE OF LIVE FREE-RANGING SOUTHERN AND NORTHERN SEA OTTERS

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### Abstract

The southern sea otter (*Enhydra lutris nereis*) was listed as “threatened” under the Endangered Species Act (ESA) in 1977 and, despite 25 yr of protection and efforts to foster recovery, they remain well below the threshold for delisting. Some populations of northern sea otters (*Enhydra lutris kenyoni*) are in the process of being listed under ESA following recent sharp and poorly understood population declines. Persistent organic pollutant (POP), disease exposure and overall health data was derived from apparently healthy free-ranging sea otters captured in Monterey Bay California from 1998-2000. Similar data was derived from several populations of Alaskan sea otters in 1997. Age, sex and location appear to exert significant influence on POP and disease exposure. In general southern sea otters have higher levels of accumulated POP’s, with the exception being some specific locations in Alaska where military dumping is known to have occurred. POPs appear to accumulate with age in both males and females and reach highest levels in adult males. Pregnancy followed by pupping results in sharp drops in POP burdens of female southern sea otters, probably as a result of lactational transfer. Patterns of disease exposure are very different for Alaskan and California sea otters, and in some locations differences in disease and POP exposure may influence the health of sea otters, patterns of mortality and population demographics. Conservation strategies for northern and southern sea otter populations need to reflect these findings.

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## MERCURY CONTINUES TO BE A THREAT TO THE CONSERVATION OF MARINE MAMMALS

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### Abstract

Heavy metal pollution tends to be persistent, have long lasting effects in the environment, and bioaccumulates up the food chain to affect the top predators. Relevant to wildlife conservation efforts, there are conflicting reports as to the effects of pollution by heavy metals such as mercury on species particularly pertinent to zoological parks and aquaria. Information on the total amount of mercury ( $\mu\text{g/g}$  wet weight) in livers of various marine mammals such as pinnipeds (pinnipeds: harp *Phoca groenlandica*; harbour *Phoca vitulina*; northern fur *Callorhinus ursinus*; ringed *Phoca hispida*; Antarctic fur *Arctocephalus gazella*), cetaceans (dolphins: striped *Stenella coeruleoalba*; Risso's *Grampus griseus*; bottlenose *Tursiops truncatus*; white beaked *Lagenorhynchus albirostris*), and polar bears (*Ursus maritimus*) from different geographic locations around the world spanning over 35 yr are reviewed.

Depending on the geographic location, species, age or sex, mercury levels vary from very low (0.05  $\mu\text{g/g}$  seals in Greenland) to very high values (700  $\mu\text{g/g}$  dolphins in the Mediterranean). However, across all areas, the mercury levels within species has either increased or at best remained unchanged over time. For example, in Greenland seals, mercury increased from 0.05  $\mu\text{g/g}$  (1978-87) to 7  $\mu\text{g/g}$  (1994-95). Mercury levels in seal pups from the east coast of Canada (1972-78) increased three-fold over a 6-yr period. Mean mercury levels in seals world-wide showed a dramatic increase to 25  $\mu\text{g/g}$  by 1994. Dolphins in the waters around the United Kingdom have values which rose 12-fold from 1989 to 1998 (120  $\mu\text{g/g}$ ). In the Mediterranean, even in 1972, dolphins had high mercury levels (up to 700  $\mu\text{g/g}$ ) and these continued to increase over time so that by 1996 levels reached up to 1000  $\mu\text{g/g}$ . Many are showing levels which are well above the toxic level reported in dolphins of 50  $\mu\text{g/g}$ . Mean mercury levels in polar bears from Greenland have risen 2.5 times from 1983 to 1994, with some regions showing values as high as 26  $\mu\text{g/g}$ .

In summary, since 1972 the amount of mercury in the livers of seals found in the Canadian Arctic, east coast of Canada and from Greenland has either remained stable or dramatically increased. Values in dolphins are high and have continued to increase, and levels in both seals and dolphins show geographic variability. Mercury levels in polar bears from Greenland have doubled. Geographically, levels of pollutants appear to vary considerably depending upon

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several factors: animal species, feeding behaviors, and bioavailability. Symptoms of mercury toxicity include renal failure, toxic hepatitis, changes in gonadal and adrenal steroid synthesis and severe neurologic dysfunction. Additionally, stress is known to release stored mercury, and procedures such as tagging, sampling and handling could potentially exacerbate symptoms. High levels of mercury could negatively impact the well being of both captive and wild animals, and, in pregnant females, produce offspring that are neurologically impaired or developmentally challenged who cannot survive under natural conditions. This could adversely affect breeding programs in aquaria and zoos, leading to unsuccessful reintroduction of many threatened species and even the extinction of populations of marine animals.

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## A HEALTH ASSESSMENT APPROACH TO STELLER SEA LION RESEARCH IN ALASKA

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### Abstract

Since 2000, the Alaska Department of Fish and Game has incorporated a health assessment approach to the study of the population decline and failure of stock recovery for the western stock of the Steller sea lion (*Eumetopias jubatus*). The declining western stock of the Steller sea lion (SSL), listed as “endangered”, ranges from the central Gulf of Alaska westward through the Aleutian Islands and has declined by 80% since the last 1970s. The eastern stock, which is listed as “threatened” and ranges from the eastern Gulf of Alaska southward to California, is steadily increasing.

The main question is: Has a single or combination of endemic or new epidemic diseases or organic or inorganic contaminants resulted in decreased survival or births of sea lions through direct mortality or reduction of individual fitness? The main objectives of the health assessment approach in attempting to answer this question are to 1) determine if there have been any new disease agents introduced into the populations, 2) determine if there are differences in exposure to select disease agents between stocks, 3) identify and describe the endemic agents, 4) determine whether these agents are pathogenic 5) determine what levels of select contaminants are present in the different stocks and 6) determine whether any of these factors affect the health of individuals by relating this data to parameters of growth, condition and potentially survival.

Utilizing collaborative research opportunities with several agencies, data has been collected on over 400 SSL pups and juveniles during live-capture/release. Morphometrics and foraging studies examine differences between sexes, ages, regions, stocks, etc. Health and disease information is collected concurrently on the same individuals as well as pathologic examinations on dead animals. Samples on each live-captured animal are collected for hematology, serology, clinical chemistries, organochlorine and heavy metal analysis, histopathology of lesions, bacteriology (culture and PCR), virology (culture and PCR), mycology, parasitology, and selected immune function studies. Disease agents currently under surveillance by culture, PCR, or serology include: *Chlamydophila psittaci*, poxviruses, caliciviruses, phocid herpesvirus-1, *Toxoplasma gondii*, morbilliviruses, *Leptospira interrogans*, *Salmonella* sp., pathogenic *E coli*,

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influenza A, *Brucella* spp., canine parvovirus, canine adenovirus 1 + 2, *Sarcocystis neurona*, and *Uncinaria* sp.

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## IMMUNE CHALLENGES FOR CALIFORNIA SEA LION PUPS: INBREEDING-INFLUENCED RESPONSES?

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### Abstract

This study investigates the influence of inbreeding on California sea lion (*Zalophus californianus*) pup survival at San Miguel Island, California, between June 2002 and January 2003. Of the 349 dead California sea lion pups examined, full necropsies and histopathologic examinations were conducted only on 187 fresh carcasses. Hookworm (*Uncinaria* spp.) load was determined by visual examination of the intestinal tract. For molecular analyses we genotyped all pups at 13 highly polymorphic (3 to 15 alleles) microsatellite loci and calculated internal relatedness (IR) as an estimator of inbreeding. Contrary to our initial hypothesis, relatively inbred pups do not die earlier in the season than less in-bred pups. Instead, mean IR was highest in October (0.10; ANOVA,  $F_{6, 180} = 2.08$ ,  $p = 0.05$ ), when 100% of pup mortality was attributed to hookworm infection. A generalized linear model showed both pups' IR and age as key predictors of *Uncinaria* load ( $P < 0.0001$ ). Testing each main cause of death (hookworm-associated haemorrhagic enteritis, starvation, and trauma) separately revealed that although all examined pups showed varying numbers of *Uncinaria* spp, the association between IR and *Uncinaria* load was only significant for those pups that died due to hookworm enteritis ( $r^2 = 0.09$ ,  $P < 0.001$ ,  $n = 138$ ) in contrast to pups that died due to starvation ( $r^2 = 0.04$ ,  $P > 0.1$ ,  $n = 18$ ) or trauma ( $r^2 = 0.11$ ,  $P > 0.1$ ,  $n = 13$ ). These results suggest that more inbred pups may be less successful at mounting an immune response against hookworms and are thus more likely to die due to related lesions, particularly when the prevalence of *Uncinaria* is highest in the population.

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## SUMMARY OF MARINE TURTLE CONSERVATION EFFORTS BY THE WILDLIFE CONSERVATION SOCIETY'S ST. CATHERINE'S ISLAND WILDLIFE SURVIVAL CENTER AND PARTNERS IN GEORGIA

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### Abstract

Along the coast of Georgia, eight clusters of barrier islands are separated from the mainland by an extensive system of salt marshes and sounds. Unlike many of the developed barrier islands of the east coast, the Georgia barrier islands still retain much of their native wilderness. Approximately two-thirds of the islands are designated as parks, wildlife refuges, research reserves, and heritage preserves, with limited or no public access.

Five species of sea turtles can be found in Georgia's coastal waters, but the loggerhead (*Caretta caretta*) is the only one to nest in Georgia in abundance. Approximately 1000 loggerhead sea turtle nests are found on the Georgia barrier islands each year. Green (*Chelonia mydas*) and leatherback (*Dermochelys coriacea*), turtles nest occasionally in Georgia and use the coastal waters as foraging habitat and as a migratory pathway.

All five species of sea turtles found in Georgia are protected by state and federal law, principally by the Endangered Species Act (ESA). Loggerhead turtles are currently listed as threatened under the ESA.

Sea turtles face several hazardous obstacles in their daily lives, such as drowning and entanglement in nets and fishing line, ingestion of plastics, hooks, and other human debris, propeller wounds, environmental pollutants, infectious and parasitic diseases, and injury from natural predators such as sharks.

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The Georgia Department of Natural Resources (Georgia DNR) coordinates all sea turtle conservation efforts in the state. The mandate of Georgia's Sea Turtle Conservation Program (GSTCP) is to maintain the long-term viability of sea turtle populations in Georgia. The GSTCP has 3 primary components including research, management, and education. The primary activities of the conservation program include loggerhead turtle nest protection and monitoring, monitoring of live and dead stranded sea turtles, working with fishermen to reduce sea turtle mortality, and educational programs. The Georgia DNR currently coordinates 12 loggerhead nest protection programs on all barrier island beaches.

St. Catherine's Island (SCI) is one of the barrier islands off the coast of Georgia. The island is managed by the SCI Foundation (SCIF). Conservation efforts and wildlife research have been a major focus for the island's activities for decades. The Wildlife Conservation Society (WCS) has managed a breeding program for endangered species, the Wildlife Survival Center (WSC), on the island since 1974. More recently, the WSC has become involved with health issues pertaining to free-ranging wildlife in Georgia. These programs tie in nicely with other ongoing conservation efforts on SCI and in the state of Georgia.

The SCI Sea Turtle Conservation Program was instituted in 1990 to conserve loggerhead sea turtles nesting on the beaches of SCI, Georgia. The holistic program integrates conservation of threatened and endangered sea turtles with applied research and conservation education. Fourteen teachers per year are trained in conservation of loggerhead sea turtles and practice while in residence on the island for 7 days. These teachers take the learned content and real-world experiences back to their classrooms to teach school children about sea turtle conservation. The program has impacted over 138 teachers and 120,000 school children. Active role modeling of integrated science is provided as teacher-interns learn conservation skills, processing skills, field triage, and apply critical thinking in the field in an exceptional hands-on, real-world conservation project.

The quality of nesting habitat on SCI, Georgia, has significantly declined since 1995 due to erosional effects thought to be driven by rising sea level, attenuation of long-shore migration of sand caused by dredging of the Savannah harbor, and lack of local fluvial sand systems. A quantitative assessment tool was established to score the habitat in terms of back beach geomorphology including presence of inlets, bluffs, and scarps; berms, terraces and dunes; wash-over and wash-in fans; and the presence of relict marsh mud or skeletal trees. Rapid Habitat Assessment is performed annually during the nesting season using point data based upon a beach grid or GPS data. The assessments indicate SCI currently hosts approximately 15% adequate nesting habitat. The Rapid Assessment Tool was modified by Georgia DNR and has been used since 1999 for temporal study of potentially deteriorating habitat and for longitudinal assessment of Georgia sea turtle habitat.

St. Catherine's Island is a sentinel island for assessing health of sea turtle nesting habitat on the Atlantic Coast of the USA, predicting future history of successively more distant barrier islands

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in Georgia, and in Florida and the Carolinas: a model potentially transferable to other areas of the world's oceans and to other coastal rookeries.

Management plans for conserving sea turtles must accommodate geologic factors and processes that can rapidly modify nesting habitat on a world-wide basis as global warming continues to cause rising sea levels and as humans continue to modify sand movement in the coastal environment. More details on this program are available at: <http://www2.gasou.edu/cturtle/001welc.html>.

As sea turtle populations continue to dwindle, it becomes more critical for scientists to ascertain their health status in the wild and to address the health-related problems that could decimate already fragile populations. In 1999, the Field Veterinary Program (FVP) of the WCS began a sea turtle health assessment program in the Caribbean and Atlantic. The initial study sites included four key sea turtle nesting and/or foraging grounds in Congo, Costa Rica, Gabon, and Nicaragua. Turtles in the study were green, hawksbill, loggerhead, Kemp's ridley, olive ridley (*Lepidochelys olivacea*), and leatherbacks. In 2001, Georgia was selected as the North American site because of the infrastructure and expertise already in place at the WSC. The program is a collaborative effort between researchers from a variety of institutions. The objectives of this program are to establish baseline blood values, and to determine the prevalence of select parasitic and infectious agents, fibropapillomatosis, and contaminants in free-ranging sea turtles at the study sites. Additionally, the project has provided training to biologists, NGO staff, veterinarians and students in sea turtle health-monitoring techniques.

The Georgia portion of the study is still ongoing. We are collaborating with several ongoing studies to obtain samples from the various sea turtle life stages and sexes (eggs, hatchlings, subadults and adult males and females). Diagnostic tests performed on live sea turtles include complete blood counts, plasma biochemical panels, plasma protein electrophoresis, pesticide and heavy metal screens, reproductive hormone levels, infectious disease serology, epibiota and internal parasite identification.

Biomedical samples for health assessments are collected from nesting loggerhead sea turtles on Blackbeard Island National Wildlife Refuge in GA (40 nesting females evaluated from 2001 to 2003).

Biomedical samples are collected from various age classes and sexes of free ranging sea turtles, using facilities on the Georgia Bulldog, a University of Georgia Marine Extension research vessel (25 sub-adults, 5 adult females and 8 males have been evaluated over 3 yr).

Unhatched eggs from approximately 50 loggerhead sea turtle nests on SCI have been necropsied each year for the past 3 yr. The eggs are evaluated for fertility, stage of embryonic death, and deformities. Some embryos are selected for histopathology, and contaminant analysis is performed on representative yolk samples.

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Biomedical samples are collected from freshly dead or euthanatized stranded sea turtles for histopathology, parasitology, microbiology, and contaminant analysis following standardized necropsy protocols.

A complete health assessment, similar to that performed on the free-ranging turtle population, is used to assess live stranded sea turtles in Georgia (15 loggerheads, 3 greens, and 3 Kemps ridleys have been evaluated to date).

Sea turtles are often found stranded dead and less commonly alive on the beaches and other coastal areas in Georgia. Approximately 10 live turtles are found injured or ill on Georgia beaches each year. Over the past decade, there has been a general trend of a steady increase in stranded turtles along the southeastern US Atlantic coastline. Currently, sea turtles that strand alive in Georgia are evaluated and treated by one of the authors (TMN). Since there are no facilities in Georgia to rehabilitate the turtles, after the initial evaluation, they must be transported long distances to reach a suitable facility. There have been occasions when all the facilities were filled to capacity and the turtles had to be prematurely released or housed in sub-optimal conditions.

For the past 2.5 yr the WSC staff members have been doing intensive fund raising to build a sea turtle rehabilitation center on SCI. It was recently decided that Jekyll Island would be a more suitable site for the facility for several reasons. Jekyll Island is centrally located along the Georgia coast. There is a bridge to access the island allowing for interaction with the general public. In addition, this direct access will allow injured or sick turtles found along the Georgia coast to be moved quickly to the center. Sea turtle conservation and education programs are already in place on Jekyll Island. The Jekyll Island Sea Turtle Project conducts nightly beach walks on Jekyll Island during the sea turtle nesting season. There were over 200 loggerhead sea turtle nests laid on Jekyll Island during the 2003 nesting season.

A 5500 square foot historic power plant will be renovated and serve as the Jekyll Island Sea Turtle Rehabilitation and Education Center. Most of the funds necessary to build a suitable veterinary clinic, turtle tanks and filtration, and an education facility are already in place. These funds have been obtained through the Woodruff Foundation, National Fish and Wildlife Foundation, The Environmental Regional Network (T.E.R.N.), a fundraiser called the "Turtle Crawl", private donations, Jekyll Island Authority, and the Jekyll Island Foundation. Fundraising efforts are still ongoing. The WSC will provide the veterinary care for the turtles presented to the facility and will continue long-term health assessment on stranded and free-ranging sea turtles in Georgia. The center will rehabilitate ill and injured sea turtles stranded in Georgia and will also receive turtles from surrounding states as needed and if space is available. The center will also provide care to cold stunned turtles from more northern states.

Over the past few years, the WSC staff has had the opportunity to spread the word about our programs with sea turtle conservation and other conservation efforts involving native and non-native wildlife. Numerous presentations on sea turtle conservation have been given to the

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general public, local middle and high schools, rotary clubs, undergraduate and veterinary students, zoological parks, and veterinary associations. Several workshops have been held for schoolteachers and the scientific community on SCI and other barrier islands. The Jekyll Island Sea Turtle Rehabilitation and Education Center will provide an excellent opportunity to expand our sea turtle conservation education programs.

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## TEN YEARS REMOVING HOOKS FROM INCIDENTALLY CAUGHT WILD LOGGERHEAD TURTLES (*Caretta caretta*)

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### Abstract

Loggerhead sea turtles (*Caretta caretta*) are migratory animals that come to the Northwestern Mediterranean Sea mainly in spring and summer, at the time when long-line fisheries have their highest activity. Therefore, interaction with long-line fisheries is considered the main threat to loggerhead sea turtles in this area. It is estimated that around 15,000 turtles are incidentally caught every year in the Spanish Mediterranean Sea.<sup>1,4</sup> One of the aims of the Foundation for the Conservation and Rescue of Marine Animals (CRAM) is to rescue, treat and release all types of marine wildlife, including marine turtles. During its 10 yr of existence, approximately 470 turtles have been registered at the rescue centre, of which 291 arrived with hooks (286 alive), with 272 successfully treated and released back into the wild.

Most of the hooks seen during these 10 yr are located in mid-esophagus, just cranial to the s-bend. Very few references about hook removal in sea turtles exist.<sup>2,3</sup> However, the ideal technique should be minimally invasive, causing no further damage to the animal and allowing for a quick recovery and release back into the wild. Surgery and opening of the skin of the neck should be avoided, since healing in these animals takes very long.

At CRAM we have developed a simple technique to remove these hooks from the turtles' esophagus orally, without having to resort to surgery in most cases. The animal is first heavily sedated with an intravenous injection of 10-15 mg/kg ketamine (Imalgene 1000; Merial Laboratorios S.A., Tarragona 161, 08014, Barcelona, Spain) and 0,1 – 0,2 mg / kg Diazepam (Diazepan-Prodes; Prodes S.A., Pont Reixat 5, 08960 Sant Just Desvern, Barcelona, Spain). The mouth is then opened using a canine mouth-gag, allowing visualization of the hook attached to the wall of the esophagus. Using straight, long, forceps, and with the fishing-line still attached to the shank, the hook and the wall of the esophagus are slightly prolapsed for a better view of the affected area. This can be performed very easily in these species, since the esophagus is quite mobile. The point of the hook is then pushed back into the lumen of the esophagus making a new small hole in the wall. A piece of string is attached to the barb of the hook, so it can be held from the mouth. The shank of the hook is cut with tongs, while the barb is held by the string. Using forceps the remainder of the hook is then completely passed through the wall of the esophagus, causing no damage to the area.

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This non-invasive technique is only possible in uncomplicated cases where there is no secondary damage caused by the hook and when the hook is located cranial to the s-bend. Fortunately, over the past 10 yr the vast majority of turtles seen at CRAM present in this manner. This ensures release of turtles 5-7 days following treatment, thus allowing successful migration to warmer waters by autumn.

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## EQUIPMENT FOR USE IN MONITORING ANESTHETIZED ANIMALS IN REMOTE GEOGRAPHIC LOCATIONS

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### **Abstract**

Monitoring anesthetized animals in remote geographic locations with no electrical power source can be accomplished with the use of commercially available equipment or with modifications of available equipment. The use of portable solar panels to recharge batteries can supply adequate power to operate most equipment. Equipment for monitoring oxygenation, ventilation, cardiac rhythm and rate, blood pressure and core temperature have been successfully used in areas without an electrical grid or electrical generators.

### **Criteria for Choice of Equipment for Field Use**

Size, weight, power requirements, durability and the ability to operate in harsh environmental conditions should be considered when choosing monitoring equipment for field use. Of concern are the power requirement and the source of the power, particularly in areas where there is no power grid or generator available.

### **Power Source**

There are a number of types of rechargeable batteries on the market.<sup>1</sup> Nickel metal halide batteries (NiMH) were chosen for use in monitoring equipment in this study (MAHA Powerx 2100mAh, Thomas Distributing, 128 East Wood, Paris, IL 61944). NiMH batteries have several features that make them attractive for remote use.<sup>1</sup> They can be recharged 500 to 1000 times, have no memory, have a fairly steady discharge curve and have the least negative environmental impact when disposed of than other available batteries.<sup>1</sup> One disadvantage of NiMH batteries is that they have a self discharge rate of 2-3% per day when not in use. AA NiMH batteries produce 1.2 volts.

Battery energy output is measured in milliamp hours (mAh)<sup>2</sup>. A battery rated at 1700 mAh will produce 1700 mA for 1 hr. Different manufacturers produce batteries with different power outputs. AA NiMH batteries are rated at up to 2400 mAh. The higher the mAh, the greater the output of the battery.

Batteries are charged using fast, smart chargers attached to portable solar panels (iPowerUS fast smart charger, iPower corporation, CA, USA). A fast charger delivers the amount of current

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necessary to recharge the battery in 1 hr or less. In general, a slower charge rate will extend the overall life of the battery.<sup>3</sup> To overcome the deleterious effects of rapidly charging a battery, a smart charger has a current-limiter built into it that reduce the current as the battery is charged, thereby preventing most of the deterioration.<sup>3</sup> The fast smart charger is attached to a portable solar panel (Sun Catcher Expedition solar charger, PowerQwest, Inc. 3400 Corporate Way, Suite C Duluth, GA 30096 USA) via a 12 volt “cigarette lighter” type plug.

The panel produces 25 watts of power, which is more than enough power to charge 8 AA NiMH batteries at a time.

Equipment that uses AA or AAA batteries is preferred so that a large number of different sized rechargeable batteries are not required in the field.

### **Monitoring Equipment**

Oxygenation is measured with a pulse oximeter or by arterial blood gas determination using a portable clinical analyzer. Several brands of pulse oximeters have been successfully used and recharged in the field. An Invacare model 3402NV (Sims BCI, Inc., Waukesha, WI 53186) is relatively small, light weight and operates on 6 AA batteries. This oximeter is durable and operates well on rechargeable AA NiMH batteries.

An I-Stat portable clinical analyzer (Heska Corp. 1613 Prospect Parkway, Fort Collins, CO 80525 USA) has been successfully used in the field using rechargeable 9-volt NiMH batteries. A challenge of using the I-Stat in the field is the analyzer’s normal operating temperature of 16-30°C (61-86°F). The I-Stat has been kept in the proper operating temperature range by placing it in a 12-volt thermoelectric cooler (Coleman, Spirit Lake, IA 51360, USA). The thermoelectric cooler runs directly off of the solar panel.

Ventilation is measured using capnography or arterial blood gas determination. The criteria for choice of a capnograph include a waveform display, mainstream and sidestream capabilities and powered by rechargeable AA batteries. The Novamatrix Tidal Wave model 615 (Novamatrix Medical Systems, INC., Wallingford, and CT USA 06492) meets these criteria. The Tidal Wave comes standard with a rechargeable computer-type battery, but can be ordered with a battery tray, which holds 7 AA batteries. This instrument is durable and operates well on rechargeable NiMH batteries. The sidestream capability allows a large gauge needle to be placed in the lumen of a large endotracheal tube for sampling.

Cardiac rate and rhythm are monitored by use of an electrocardiograph (ECG). A compact ECG unit (Heska Vet/ECG 2000, Heska Corp., 1613 Prospect Parkway, Fort Collins, CO 80525 USA) that operates on 3 AAA rechargeable NiMH batteries is durable and dependable in the field.

Blood pressure is measured by a direct arterial line or by indirect methods. Of the indirect methods, automated oscillometry has been successfully used in the field. No automated

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oscillometric blood pressure machine that runs on replaceable batteries could be found. A compact, durable instrument, Oscillomate 9300 (CAS Medical Systems, Inc., 44 East Industrial Blvd., Branford, CT 06405), was modified for field use. A transformer was manufactured which is inserted between the internal battery of the blood pressure monitor and the solar panel. This allows the internal battery of the blood pressure monitor to be recharged directly from the solar panel.

All monitoring equipment, battery chargers and rechargeable NiMH batteries are transported into the field in a backpack that is designed for photographic equipment (Lowepro Supertrecker AW II, Lowepro USA, P.O. Box 6189, Santa Rosa, CA 95406).

All of the above equipment has been dependably used to monitor immobilized elephants in a variety of remote habitats in Cameroon, including dry, hot habitat,<sup>2</sup> hot humid habitat.

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## FIELD INHALATION ANESTHESIA IN FREE-RANGING JUVENILE STELLER SEA LIONS (*Eumetopias jubatus*)

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### Abstract

The Alaska Department of Fish and Game has caught and anesthetized 511 free-ranging Steller sea lions (*Eumetopias jubatus*) over a 10-yr period (1993-2003). Captures occurred at different haulouts and rookeries throughout Alaska as part of scientific investigations into the Steller sea lion decline. Animals were captured using one of three methods: chemical darting (n = 48), beach netting (n = 60), or underwater noose (n = 403), in order to perform physiologic, morphometric, and biologic sampling. Since 1998, all captures were achieved using either the underwater or beach capture method due to the difficulties and high risks associated with dart capture as previously described by Heath, et al.<sup>1</sup> Beach captures have the drawbacks of emptying the entire haulout with each capture thereby limiting the pool of sea lion candidates for that day and sometimes longer. Working on slick rock surfaces during beach captures can also be hazardous for the capturer as well as the sea lions.

In order to successfully capture juvenile Steller sea lions from the same site over a short span of time with minimal disturbance, a technique was developed using underwater divers to safely place a noose with attached floating buoy around a sea lion thus allowing a capture team to load the animal into a capture box with a small skiff.<sup>2</sup> Animals are then loaded onto a research vessel for inhalation isoflurane anesthesia and processing. This method of capture and anesthesia has been very successful and safe for the animals. No mortalities have occurred during the underwater captures, but potential risks to the animals include loss of the animal with noose around neck, asphyxiation from overly tight noose, and boat strike while chasing after the floating buoy. To counter these risks, the noose is equipped with a corroding pin that will release the noose over time, the capture team in the skiff is skilled in boat handling and trained to recognize and alleviate possible asphyxiation caused by noose. Emergency drugs are also carried on the skiff, accompanied by a veterinarian or technician. The underwater diver method has greatly enhanced the number of juvenile sea lions that can be captured from a single site causing minimal disturbance to the haulouts and rookeries.

A total of 403 juvenile Steller sea lions (2-41 mo of age) with a mean mass of  $92.8 \pm 33.5$  kg (mean  $\pm$  SD, range 32-230 kg) were anesthetized after capture by the underwater noose method. Following capture and rest, anesthesia was administered using a portable field anesthesia machine (Seven Seven Anesthesia, Fort Collins, CO, USA) delivering isoflurane via mask for

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short procedures or for induction for intubation for longer procedures. A comparison of sevoflurane to isoflurane in Steller sea lions was performed in 17 of the underwater captured animals.<sup>3</sup> In animals anesthetized using sevoflurane, there was a significant improvement (decrease) in the time from anesthesia off to extubation and from the time of extubation to safely swimming. Sevoflurane recoveries were subjectively characterized by the authors as producing a more alert and stronger animal at extubation. Even though the time benefit was significant with sevoflurane, it was outweighed by the greater cost of the newer anesthetic. The anesthesia parameters reported in this study include only animals anesthetized with isoflurane.

Post-capture rest was found to be important to allow the animals to stabilize from their capture. In those animals taken directly from capture to anesthesia, body temperature dropped precipitously to  $32.9 \pm 0.89^{\circ}\text{C}$  ( $n = 9$ ), and vigorous temperature correction with artificial heat sources was necessary. Most animals allowed 45-60 min of rest before anesthesia maintained body temperature throughout subsequent anesthesia with no external heat measures necessary. Sea lions were restrained in the capture box to allow masking with isoflurane at 4-5% vaporizer setting with 5-10 L/min oxygen. During maintenance of anesthesia, isoflurane concentrations were reduced to 1-2% with 2-3 L oxygen flow. Induction time from mask application to intubation was  $14 \pm 6$  min (range 4-45,  $n = 154$ ). The induction time data does not accurately estimate the time needed before intubation could be performed because some animals were intentionally kept on the mask in order to complete a procedure (i.e., blood draw) before intubation was attempted. Time of anesthesia was  $52 \pm 17$  min (range 14-139,  $n = 234$ ) and time from anesthesia vaporizer off to extubation was  $4 \pm 3$  min (range 0-16,  $n = 236$ ). For procedures that did not require extended periods of time or for animals that could not be intubated, anesthesia was maintained by mask for  $22 \pm 10$  min (range 9-83,  $n = 252$ ). Physiologic parameters monitored throughout the anesthetic procedure included temperature, respiration and heart rate, mucous membrane color and refill, oxygen saturations, and some end-tidal  $\text{CO}_2$  measurements. The anesthetic procedure was very safe; resulting in one mortality from a total of 463 animals anesthetized using the capture box/mask induction method. Apnea occasionally occurred at the time of intubation or at the finish of the procedure after turning off the vaporizer but before extubation. During this time, some animals needed mechanical assistance with respirations. Additionally, doxapram was used to stimulate respirations in 18 animals (i.m. or sublingual) during these times. Diazepam was given to seven animals that were extremely aggressive in order to sedate them in the capture box prior to masking. Other emergency drugs administered included atropine (i.m.) to one animal and epinephrine to another that went into respiratory arrest following extubation. This animal was revived after being reintubated, oxygenated mechanically, and administered emergency medications. The one mortality occurred in a 7-mo-old female during the recovery period following a short isoflurane mask-only procedure. The animal was found dead in the box with its head twisted and nose pressed into a corner. Necropsy revealed no abnormalities except asphyxiation.

The combination of the underwater noose capture technique, capture box restraint, and inhalation isoflurane anesthesia has proven to be a safe and effective method for capturing and sampling large numbers of juvenile Steller sea lions.

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## EVALUATION OF ISOFLURANE, SEVOFLURANE AND NITROUS OXIDE ANESTHESIA IN DUMERIL'S MONITOR (*Varanus dumerili*)

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### Abstract

Isoflurane is the most commonly used inhalant anesthetic in reptiles,<sup>3</sup> but sevoflurane has shown promise as a rapid and safe alternative.<sup>4,7</sup> Despite their popularity only limited information on their performance in reptiles is available.

The induction and maintenance of anesthesia in ten Dumeril's monitor (*Varanus dumerili*) with isoflurane (I), sevoflurane (S) and sevoflurane with 66% nitrous oxide (N<sub>2</sub>O), and the effects of the inspired oxygen (O<sub>2</sub>) concentration on anesthetic induction were investigated in a randomized prospective study.

During anesthetic induction with the desired gas mixture delivered through a facemask, the tone and reactivity of the tail, hind limbs, front limbs and neck were evaluated every 60 sec, as was the righting reflex. The tone was judged subjectively on a scale from 0 to 3 with 3 being full reaction and strength upon touch and zero being no tone or reaction. The righting reflex was evaluated by placing the animal on its back and observing the reaction. No reaction was scored as zero and full ability to flip itself over scored a 3. Induction was considered complete, when all parameters evaluated scored zero. The overall sequence of complete muscle relaxation was consistent: The front limbs always lost tone first, followed by the hind limbs and the neck nearly simultaneously; then the righting reflex would be lost, and finally the tail tone. The pattern was not significantly different among the four treatments.

The mean time to induction for I in 100% O<sub>2</sub>, S in 100% O<sub>2</sub>, S in 21% O<sub>2</sub> : 79% nitrogen, and S in 66% N<sub>2</sub>O : 34% O<sub>2</sub> was 13.0 ± 4.55 min, 11.2 ± 3.77 min, 10.4 ± 2.5 min, and 9.4 ± 2.8 min respectively at 26°C. Mask induction with sevoflurane was faster than with isoflurane. There was no significant difference between the induction time for sevoflurane in 100% O<sub>2</sub> and in room air, but sevoflurane combined with N<sub>2</sub>O resulted in significantly faster inductions than sevoflurane alone.

The minimum alveolar concentrations (MAC) of I, S and S in 66% N<sub>2</sub>O: 34% O<sub>2</sub> were determined by a bracketing technique.<sup>6</sup> Anesthesia was induced with the desired gas mixture

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delivered through a facemask. Animals were then endotracheally intubated end-tidal and inspired anesthetic concentration was continuously measured. Animals were mechanically ventilated with a ventilator set to deliver a tidal volume of 25ml/kg at a rate of 4 breaths/min. After equilibration at an end-tidal-to-inspired agent concentration ratio of >0.9 for 20 min an electrical stimulus of 50Hz, 50 V was delivered to subcutaneous electrodes on the ventral aspect of the tail at 6.5 msec pulses with 6.5 msec intervals for 1 min or until purposeful movement was observed. The vaporizer setting was then decreased to effect a 10% decrease in end-tidal agent concentration, and equilibration and stimulation were repeated. The MAC was calculated as the mean of the lowest end-tidal concentration that prevented a positive response and the highest concentration that did not. A blood sample for blood-gas analysis was collected from the tail vein at the beginning and end of the anesthetic period.

MAC  $\pm$  SD of I and S were  $1.54 \pm 0.17\%$  and  $2.51 \pm 0.46\%$  respectively at 32°C. A significant reduction ( $26.4 \pm 11.4\%$ ) in sevoflurane requirement was found when delivered with 66% N<sub>2</sub>O : 34% O<sub>2</sub>, and the MAC of N<sub>2</sub>O was estimated to be 244%.

Mean heart rate at the upper and lower MAC bracket was  $32.4 \pm 3.1$ ,  $30.7 \pm 4.5$  and  $34.4 \pm 4.9$  and  $34 \pm 4.5$ ,  $34.5 \pm 5.2$ ,  $36 \pm 3.6$  beats/min, respectively during anesthesia with I, S and S in 66% N<sub>2</sub>O : 34% O<sub>2</sub>. Over the course of the experiment, there was a significant decrease in PaCO<sub>2</sub> a significant increase in blood pH and HCO<sub>3</sub>. For example, for I PaCO<sub>2</sub> decreased from 43.1 to 27.9 mmHg and blood pH and HCO<sub>3</sub> increased from 7.33 to 7.64 and from 25.3 to 32.9 mmol/L, respectively.

The pattern of complete muscle relaxation described confirms observations made in several species of lizards induced with halothane<sup>2</sup> and in turtles anesthetized with ether.<sup>1</sup> This pattern may be consistent regardless of the inhalation anaesthetic agent used and perhaps even among species.

The MAC of isoflurane in Dumeril's monitors was similar to that reported in mammals<sup>6</sup> but lower than values reported in other reptiles,<sup>4,5</sup> possibly reflecting the more mammalian cardiovascular physiology of monitor lizards.

In Dumeril's monitor anesthetic induction with both isoflurane and sevoflurane was feasible and safe. Sevoflurane offers a slightly faster anesthetic induction than isoflurane, and the use of nitrous oxide at a 2:1-ratio with oxygen further reduces induction time. In both cases, however, it is questionable whether the limited reductions in induction time alone warrants the cost of new vaporizers and additional equipment.

## ACKNOWLEDGMENTS

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The work was supported by the Toronto Zoological Society and the Ontario Veterinary College Pet Trust. We thank Abbott Canada for supplying anesthetics and vaporizers, and are grateful to S. Lee for invaluable technical assistance, to W. Sears and S. S. Nielsen for statistical advice, and to the staff at the Toronto Zoo and the Central Animal Facility, University of Guelph for expert care for the animals.

Some of these results were presented at the Meeting of the European Association of Zoo and Wildlife Veterinarians in Ebeltoft, Denmark, May 2004.

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## EVALUATION OF THE POTENTIAL FOR INJURY WITH HIGH VELOCITY REMOTE DRUG DELIVERY SYSTEMS

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### Abstract

We investigated different high velocity remote drug delivery systems (HVRDDS), characterized by dart mass and mechanism of drug expulsion, to identify factors contributing to tissue injury and to evaluate the potential for HVRDDS to cause significant long-term injury. Flight velocity and kinetic energy were determined by analyzing trajectories of darts of known mass using a Doppler radar chronograph. Identification of factors contributing to tissue injury was determined by using high-speed video to record impact behavior of dye-filled darts fired into hide-covered ballistics gelatin, and by measuring the dimensions of dye tracts. For some systems, dart velocities were highly variable within replicate tests resulting in low precision at target. Instability of darts in flight (yaw) also contributed to low precision. Heavy mass darts decelerated slower than lighter darts although dart length also affected this relationship. Large bore, end-ported needles consistently pushed hair or tissue into dye tracts. Rapid injection darts (explosive charge) caused deeper dye tracts than slow injection (air pressurized) darts. Further, the repulsion of rapid injection darts following impact caused separation of hide and gelatin and partial injection into the resulting space. Findings with ballistics gelatin were comparable to results obtained using animal carcasses. Overall, injury potential with HVRDDS can be reduced through various design modifications and appropriate selection of needle and charge or power setting.

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## RAPID IMMOBILIZATION OF HOOFSTOCK IN LARGE HERDS

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### Abstract

Field immobilization of exotic hoofstock in large herds presents significant challenges for animal and human safety. Animals may experience heat stress and/or capture myopathy during immobilization. Aggression toward darted animals or team personnel from other animals and danger from environmental hazards (e.g., waterways) are further concerns. Precise anesthetic dosing that minimizes induction time is essential. Strategic coordination of personnel for efficient darting, capture, and removal of the animal from the field are paramount to success. Animals chemically immobilized in open environments amid numerous other hoofstock may respond differently than those in confined exhibits. Species variation in tendencies to sprint for significant distances following darting with various drug combinations, relative species sensitivity to  $\alpha$ -2 agonists, and herd interactions affect immobilization outcomes. Anesthetic regimens were designed for reliable, rapid induction of exotic hoofstock in expansive field settings at a park exhibiting hundreds of African and Indian species. Opioid/xylazine combinations were used in impala (*Aepyceros melampus*), greater kudu (*Tragelaphus strepsiceros*), waterbuck (*Kobus ellipsiprymnus*), wildebeest (*Connochaetes taurinus*), and zebra (*Equus burchelli*). Telazol combined with ketamine and xylazine was used in blackbuck (*Antilope cervicapra*), mouflon (*Ovis musimon*), and nilgai (*Boselaphus tragocamelus*).

### Introduction

The need for population management of several herds of nondomestic ungulates afforded a unique opportunity to refine anesthetic dosages and field strategies for immobilization. Lion Country Safari is a “drive-through” preserve exhibiting several bovid and equid species. Geographic features of the park, including numerous water bodies, provided a major impetus for refining immobilization protocols to minimize induction times. The large mixed herds also offer the potential for aggression toward darted animals. Many of these procedures are performed within public view underscoring the need for safe, efficient animal immobilization.

### Materials and Methods

All animals reported here were darted in the field except for six of eight nilgai anesthetized (darted) in a barn. All animals were apparently healthy and weighed soon after immobilization. Prior to darting, a field team met to discuss approach strategies, vehicle positioning during induction, and animal support for veterinary procedures. Communication between field team

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members was done by two-way radio. Environmental temperatures were frequently between 28 to 35°C during immobilizations. Anesthetic dosages may be higher for animals darted in hot weather.<sup>2</sup>

## Results and Discussion

### Opioid/Xylazine Combinations (See Table 1 for drug dosages)

Waterbuck: Approximately 130 waterbuck resided with 44 wildebeest, 31 eland (*Taurotragus oryx*), 8 mouflon and 14 ostrich (*Struthio camelus*) in a large preserve bordered by canals and sloughs. They were generally easy to approach and had the potential to sprint long distances when administered opioids. Adult males exhibited aggression to darted waterbuck. The ostriches would occasionally block the darting vehicle, eat syringes and peck the field team. These dosages were used to reduce sprinting after darting. Five young animals, 41-58 kg, included in data had higher mean dosages of carfentanil (ZooPharm, Fort Collins, CO 80522, USA; 0.019 mg/kg) and xylazine (Phoenix Scientific, St. Joseph, MO 64503, USA; 0.9 mg/kg) compared to the group. Younger animals may require higher anesthetic dosages than older animals.<sup>2</sup> An adult male (155 kg) was immobilized in field and barn settings > 12 times for treatment of interdigital dermatitis. Supplemental oxygen was used to reduce hypoxemia. Respiratory depression was treated with doxapram (Dopram, Fort Dodge Animal Health, Fort Dodge, IA, 50501, USA; 0.4 mg/kg i.v.) or 25% of the total tolazine (Congaree Veterinary Pharmacy, Cayce, SC 29033, USA) dosage early in the procedure. Improved muscle relaxation was seldom needed, but could be achieved with low dosage diazepam (Valium, Abbott Laboratories, North Chicago, IL 60064, USA; 0.1 mg/kg, i.v.). The primary author believes that an adverse reaction to intravenous tolazine caused fatal or nearly fatal pulmonary edema in two waterbuck and one Nile lechwe (*Kobus megaceros*) not reported here. Intramuscular administration of is recommended.

Wildebeest: Wildebeest were fairly easy to approach although they sometimes required herding toward the darting vehicle. They did not sprint after darting. Aggression from other wildebeest and male waterbuck toward the darted animal was not observed. Induction was very smooth and quick.

Zebra: Approximately 56 zebra and 10 wildebeest resided together in a preserve immediately adjacent to canals and sloughs. The zebra were wary of field vehicles and the entire herd could panic if any animal was startled. The darting vehicle slowly approached the individual to be darted while the field team hid behind brush until the dart was fired. Field vehicles then emerged to block waterways. This long established herd has strong social bonds and females sometimes guarded an individual selected for immobilization, blocking darting attempts. Aggressive posturing from mares toward the field team occurred when their young offspring were darted. Zebra would sprint and “high-step” after darting with opioids. The dosages used reduced induction time and high-stepping as compared to similar etorphine dosages combined with lower dosage xylazine. Muscle relaxation was generally good and was sometimes improved with a single bolus of detomidine (Dormosedan, Pfizer Animal Health, Exton, PA 19341, USA; 5 mg

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i.v.) and/or butorphanol (Torbugesic, Fort Dodge Animal Health, Fort Dodge, IA 50501, USA; 5 mg i.v.) Low dosage diazepam(0.05-.1 mg/kg) was also used to improve relaxation. We have used similar dosages of etorphine and xylazine in more than 20 immobilizations of zebra weighing up to 309 kg, including an individual immobilized multiple times for laceration repair. Procedure times have ranged from 20-75 min. Recoveries using naltrexone (ZooPharm, Fort Collins, CO 80522, USA) were very quick (1-2 min.). Dosage and route of administration of naltrexone (1/4 iv, 3/4 s.c.) may warrant revision given previous investigations with this drug.<sup>1,4</sup> Xylazine is not reversed. Zebra appear to tolerate and benefit from higher xylazine dosages. The domestic horse is more tolerant of  $\alpha$ -2 agonists than the bovid.<sup>6</sup>

Greater kudu: Field dosage for an adult male kudu (204 kg) was carfentanil (0.027 mg/kg ) + xylazine (0.9 mg/kg) reversed with naltrexone (2.8 mg/kg 1/4 i.v. 3/4 s.c.) and tolazine (2.45 mg/kg i.m.) Significant sprinting occurred if dosages were lowered. Multiple immobilizations for lameness and joint fusion were performed with similar dosages.

Mouflon: Mouflon were often difficult to approach in field vehicles. They quickly caught on to changes in routine indicative of darting attempts. Staff often attempted to disguise the darting vehicle by using cars or vans not field vehicles. Wildebeest postured aggressively toward darted mouflon and were warded off with field vehicles or by waving a towel. Anesthesia Data: 3 apparently healthy male mouflon, at least 8 yo to geriatric, 49-57 kg, were anesthetized for treatment of lameness, horn repair with telazol (Fort Dodge Animal Health, Fort Dodge, IA 50501, USA; 8.4 mg/kg) + ketamine (Fort Dodge Animal Health, Fort Dodge, IA 50501, USA; 4.2 mg/kg) + xylazine (1.0 mg/kg). The mixture was prepared by mixing 2.5 ml ketamine (100 mg/ml) with 500 mg of powdered telazol. An appropriate volume (2.3-2.5 ml) of this mixture was then placed in the dart to which the xylazine dosage was added. Time elapsed from darting until sternal was 3-6 min. Working time was 40-75 min. A single bolus of diazepam (5 mg i.v.) was sometimes used to prolong anesthesia time. Reversal was with yohimbine (Yobine, Lloyd Laboratories, Shenandoah, Iowa, USA; .1mg/kg i.v.). Time elapsed from injection of yohimbine until the animal stood was 2-60 min, the latter value was for mouflon that received diazepam late in the course of anesthesia. Propofol (PropoFlo, Abbott Laboratories, N. Chicago, IL 60064 USA; 0.5 mg/kg i.v.) was also used to lengthen anesthesia and can be considered as an alternative to diazepam. This telazol, ketamine, xylazine dosage has been reliable for 4 other mouflon immobilizations. Time elapsed from darting until sternal may be up to 14 min if mouflon have been excited and attempt to evade darting vehicle. Lower field dosages were not effective.

Nilgai: Approximately 45 nilgai, 180 blackbuck, 22 Asiatic water buffalo (*Bubalus bubalis*) reside in a preserve having thick vegetation, numerous canals and sloughs. Nilgai are very skittish if they became aware of a darting attempt. They initially sprint hard after dart impact and tremendous herd excitement is exhibited. The darted individual and entire herd will effectively hide in brush after darting. Nilgai frequently cross waterways and will enter water after being darted. Aggression from other hoofstock has not been noted. Anesthesia Data: 8 adult male nilgai (205-209 kg) were anesthetized for castration (n = 6) or vasectomy (n = 2) with average dosages

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of telazol (4.1 mg/kg) and xylazine (2.5 mg/kg.) Two were darted in the field, others were conditioned with food to come into a holding area, then darted in a barn to avoid risk posed by waterways. Time elapsed from darting until sternal was 2-6 min. Bouts of hyperpnea and presumed hypoxia sometimes occurred. Partial reversal of xylazine with 30-60% of a total tolazine dosage (2.5 mg/kg i.m.) was frequently given early in the procedure to improve ventilation. Anesthesia was sometimes lengthened with ketamine (2 mg/kg i.m.) or propofol (0.6 mg/kg iv). An additional 6 nilgai that were not weighed were successfully neutered with similar dosages. This regimen provided smooth anesthesia for neutering and did not result in the excessive thrashing and bouts of apnea that occurred with opioid/xylazine combinations.<sup>3</sup> Nilgai often did not stand until 40-60 min after reversal. Refinement of this anesthetic regimen may be possible given recent work with other alpha-2 agonists.<sup>5,7</sup> Reduction of the xylazine dosage would be prudent in captive situations where maximum reduction of induction time is not critical. Nilgai may have rough recoveries when tolazine is given to reverse xylazine if ketamine has been administered within the previous 30-45 min.

**Blackbuck:** Nearly 80 blackbuck (27 – 39 kg) were darted with a standard 1.2 ml dose of a telazol (500 mg) + ketamine (400 mg) + xylazine (100 mg) mixture for castration, vasectomy, or preshipment testing. Reliable induction times of 4-6 min from darting until sternal occurred. No reversal was given due to thrashing that occurred if tolazine was given. Animals did not stand for 3 hr post-dart. This regimen may be revised to preserve advantageous induction times and improve recovery times.

Supplemental oxygen is recommended for chemically immobilized hoofstock.

#### **ACKNOWLEDGMENTS**

Thanks to Brian Dowling, Jen Robertson, Marsha Abrams, RonCameron, Terry Wolf, Sherri Garz, the animal capture team and hospital staff of Lion Country Safari

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**Table 1.** Drug dosages.

Bovidae:										
Species	n	Body weight (kg)	Carfentanil <sup>a</sup>	Xylazine <sup>a</sup>	Naltrexone <sup>a</sup>	Tolazine <sup>a</sup>	DT	PT	RT	Procedure
Waterbuck <sup>b</sup>	40	41-186	0.014	0.5	1.5	1.7	4	13	4	Exam
Wildebeest <sup>b</sup>	7	112-146	0.012	0.4	1.2	1.8	2	24	6	Castration
Equidae:										
Species	n	Body weight (kg)	Etorphine <sup>a</sup>	Xylazine <sup>a</sup>	Naltrexone <sup>a</sup>	Tolazine <sup>a</sup>	DT	PT	RT	Procedure
Zebra <sup>b</sup>	6	173-250	0.024	1.1	2.4	0.0	5	24	1	Castration

<sup>a</sup>All drug dosages average in mg/kg; route of administration=carfentanil i.m., xylazine i.m., naltrexone 1/4 i.v., 3/4 s.c., etorphine i.m., tol 1/2 i.v., 1/2 i.m. (i.m. recommended for tolazine).

<sup>b</sup>Waterbuck (*Kobus ellipsiprymnus*), wildebeest (*Connochaetes taurinus*), zebra (*Equus burchellii*).

n = number; DT = Dart time (average minutes from darting until sternal); PT = Procedure time (average minutes from sternal until reversal); RT = Reversal time (average minutes from injection until standing).

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**INCREASED PRE-ANESTHETIC STRESS REDUCED THE QUALITY OF MEDETOMIDINE-KETAMINE IMMOBILIZATION IN MARKHORS (*Capra falconeri heptneri*): PRELIMINARY RESULTS**

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**Abstract**

Twelve healthy markhorses (*Capra falconeri heptneri*) (4M; 8F) were immobilized for a total of 16 times for routine procedures in the Helsinki Zoo between March 2000 and October 2003 with a combination of medetomidine (MED: 120.8±37.7 µg/kg (mean±SD)) and ketamine (KET: 1.5±0.5 mg/kg). Prior to darting, the stress (SS) exhibited by the target animal(s) was scored. The animals were then assessed for either a sufficient or insufficient level of anesthesia (LA), depending on whether they required additional anesthetics (propofol at 1.2±0.5 mg/kg i.v. or half of the original dose of MED-KET i.m.) in order to achieve a satisfactory immobilization. Paired venous and arterial samples were taken at T<sub>1</sub>=23.7±6.6 and T<sub>2</sub>=50.2±7.4 min (from dart impact) for serum cortisol concentration (S-COR) and blood gas analysis, respectively.

Animals requiring additional anesthetics had higher stress scores prior to being darted ( $P < 0.01$ ). S-COR did not correlate with SS or arterial PaO<sub>2</sub>. In addition, a significant reduction in S-COR over time between paired samples was found ( $P < 0.01$ ). Preliminary results suggest that acute stress cannot be measured with S-COR in Markhorses, when a MED-KET combination is used for chemical immobilization. Propofol proved to be an efficient and safe method for inducing an adequate plane of anesthesia when the original anesthetic response was regarded as insufficient for a satisfactory immobilization. We conclude that in order to achieve an optimal anesthetic response in markhorses immobilized with MED-KET, acute pre-anesthetic stress should be avoided.

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## IMMOBILIZATION OF FREE-RANGING MOOSE (*Alces alces*) WITH ETORPHINE OR ETORPHINE-ACEPROMAZINE-XYLAZINE IN SCANDINAVIA 1984-2003: A REVIEW OF 2,754 CAPTURES

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### Abstract

A total of 2,754 chemical immobilizations of free-ranging moose (*Alces alces*) were carried out in Scandinavia from 1984 to 2003 as part of ecological studies or for management purposes. Standard procedures involved darting from a helicopter in winter (November to April).

In Norway, etorphine (M99® 9.0 mg/ml, Novartis Animal Health, Litlington, UK) at 6.0-9.0 mg/animal was used for immobilization of 1,373 individual moose on 1,491 occasions [820 adult cows (≥ 1 yr), 250 adult bulls, 244 female calves (< 1 yr), 177 male calves]. Diprenorphine (M5050® 12 mg/ml, Novartis Animal Health) at 12-15 mg per 9 mg etorphine was used for reversal. The overall mortality rate was 0.5%. Two cows (0.2%) and 5 calves (1.2%) died or were euthanatized during the capture process. One of the cows was hit high in the neck and died within 2 min of darting. This cow was pregnant and in good body condition. Necropsy showed possible intravascular drug injection and shock development. The other cow drowned during the induction phase when it tried to cross a deep river. Four of the calves died due to respiratory arrest shortly after darting. Necropsies showed poor body condition in all these animals. The fifth calf developed bilateral hind leg paresis and was euthanatized. Necropsy showed traumatic spinal lesions, caused by dart impact in the lumbar region, osteoporosis, cachexia and verminous pneumonia. Follow-up radio telemetry was done for at least 1,222 of the animals (97.6%). No mortalities caused by the capture (residual drug effects, stress, exertional myopathy, or predation) were seen. The number of darts used per captured animal (including missed darts, multiple darts in some animals) varied from 1.2 to 1.5 in various projects. Average helicopter time per captured moose (including weighing of the animals) was 26 min for an experienced pilot and 35 min for a pilot with less experience, involving several projects (with the same experienced shooter) and different topography, vegetation, and moose density.

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In Sweden, combinations of etorphine-acepromazine Large Animal Immobilon® (Novartis Animal Health) and xylazine (Rompun®, Bayer, Germany) were used for immobilization of 1,178 individual moose on 1,263 occasions (617 cows, 291 bulls, 173 female calves, 182 male calves). A drug mixture was made by adding 5 ml of Immobilon® (2.25 mg/ml of etorphine and 7.38 mg/ml of acepromazine) to one vial of Rompun® dry powder (500 mg of xylazine) and the following doses were applied: 1.0-1.5 ml for adults and 0.5-0.7 ml for calves. Diprenorphine (Large Animal Revivon® 3 mg/ml, Novartis Animal Health) was used to reverse the effects of etorphine at a dose ratio at 12-15 mg per 9 mg of etorphine. Initially, no reversal agent was used to counteract xylazine. However, since 1995 atipamezole (Antisedan® 5 mg/ml, Orion Pharma Animal Health, Turku Finland) at 7.5 (adults) or 5 (calves) mg was used for antagonism of xylazine (935 captures). The overall mortality rate was 1.0%. Mortalities included seven cows (1.2%), one bull (0.3%), and four calves (0.6%). In five of these animals, all before 1995, atipamezole was not administered. One cow and one calf died due to exertional myopathy. One calf died due to respiratory arrest during immobilization. Five cows, one bull, and two calves were found dead close to the marking place days or weeks after immobilization. In addition, one cow was killed by a brown bear shortly after immobilization. For conservative reasons, these deaths are included as capture related. Follow-up radio telemetry within 2 wk was done for all of animals. On average 1.3 darts were used and 30 min of helicopter time (including ferry and weighing of animals) were spent per captured animal. No animals have died since 1997 and the mortality has been reduced drastically since 1995 when atipamezole was included as a reversal agent.

We conclude that etorphine or etorphine-acepromazine-xylazine are very safe and effective drugs for immobilization of free-ranging moose from helicopter in winter. A review of the literature showed that mortality rates routinely range from 6 to 19% during capture and translocation of free-ranging moose (drugs including carfentanil, carfentanil-xylazine, xylazine, succinylcholine) in North America.<sup>1,3</sup> In Sweden, a mortality rate of 1.7% was found in 650 free-ranging moose immobilized with etorphine-acepromazine-xylazine from 1979 to 1984.<sup>4</sup> By using immobilizing drugs with proven safety and by refining the capture methods, we believe that such mortalities can be significantly reduced.<sup>2</sup> In our opinion, etorphine or etorphine-acepromazine-xylazine should be considered the drugs of choice for moose immobilization.

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**DETERMINATION AND EVALUATION OF AN OPTIMAL DOSAGE OF CARFENTANIL AND XYLAZINE FOR THE IMMOBILIZATION OF WHITE-TAILED DEER (*Odocoileus virginianus*)**

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**Abstract**

Optimal hand-injected immobilization dosages of carfentanil/xylozine (CAR/XYL) were individually determined for 13 adult white-tailed deer. Deer were temporarily restrained in a squeeze chute and were repeatedly immobilized one to four times at 2-5 wk intervals from December 2002-March 2003. A fixed ratio of 1 mg CAR : 10 mg XYL intramuscularly was used, increasing or decreasing the dosage until the optimal dosage (defined by an induction time < 3 min and PaCO<sub>2</sub> < 60 mm Hg) was reached for each animal. Inductions were videorecorded and reviewed by observers blinded to drugs and dosages, who rated qualitative aspects of each induction.

The median optimal dosage (mOD) was 0.03 (range, 0.015-0.06) mg/kg CAR + 0.3 (range, 0.15-0.6) mg/kg XYL. Initial effects that would likely decrease post-darting movement in a field immobilization situation were noted in ≤ 1.6 min for all deer using the mOD. Induction times using the mOD were rapid (median 3.0 min [range, 1.8-10.0]) but quality ratings were considered “undesirable” for 9 of 13 deer. There were significant (*P* < 0.05) dosage-dependent decreases in induction time, time to first effect (TE), PaO<sub>2</sub>, SaO<sub>2</sub>, and arterial pH, and significant dosage-dependent increases in PaCO<sub>2</sub> and quality ratings. Increased rectal body temperatures of 40.6 ± 0.5° C (mean ± SD) were noted in all deer and hyperthermia (T > 41° C) was noted in three. Heart rates significantly decreased from 5- to 15-min post-induction and remained decreased at the 20-min reading; there was occasional bradycardia. There was a significant increase in pH from 10- to 20-min post-induction, but metabolic acidemia (pH < 7.3) persisted throughout the immobilization periods for all deer. Hypoxemia (SaO<sub>2</sub> < 90 mm Hg) was present after induction but resolved by 20-min post-recumbency; hypercapnea (PaCO<sub>2</sub> > 60 mm Hg) did not occur. Reversals with naltrexone and yohimbine were rapid (mean 3.7 ± 1.5 min), complete, and uneventful, with no evidence of renarcotization.

This study successfully identified optimal dosages for all deer and demonstrated a CAR/XYL dosage-dependent linear relationship for both selected criteria (induction time and PaCO<sub>2</sub>),

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validating the use of the iteration method. Additional study is needed to determine whether the optimal CAR/XYL dosage identified in this study is applicable to and safe for field immobilization of white-tailed deer.

#### **ACKNOWLEDGMENTS**

This project was funded in part by The University of Tennessee, College of Veterinary Medicine, Hill's Research Fund and the University of Georgia McIntire-Stennis Project (GEO-0126-MS). Antler King Trophy Products, Inc., Moultrie Feeders, Inc., and Pennington Seed, Inc. made additional contributions. The authors gratefully acknowledge W. Lance of Wildlife Pharmaceuticals, Inc., and K. Kadidlo of Diametrics Medical, Inc., for contributions of drugs and materials used in this study. We thank students at University of Georgia for assistance with immobilizations; A. Saxton for performing statistical analyses; S. Dahmes, B. Harbison, and S. Harbison for recording and editing video footage; and R. Harvey and T. Doherty for rating of induction qualities.

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**PULMONARY GAS EXCHANGE AND ACID-BASE STATUS IN IMMOBILIZED BLACK RHINOCEROS (*Diceros bicornis*) AND WHITE RHINOCEROS (*Ceratotherium simum*) IN ZIMBABWE**

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**Abstract**

Few studies have described arterial blood gas values in black rhinoceros<sup>1</sup> (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*),<sup>2-5</sup> and only one involved free-ranging animals.<sup>3</sup> The aim of this study was to evaluate pulmonary gas exchange and acid-base status in immobilized black and white rhinoceros. Arterial blood samples were collected from 13 black and four white rhinoceros during 19 immobilization procedures, which included ear notching, snare removal, and translocation. Sixteen free-ranging rhinoceros were darted from a helicopter with a combination of an opioid, an alpha<sub>2</sub>-agonist, azaperone, and hyaluronidase (Table 1). Once immobilized, nalorphine was given i.v. to improve respiration by partial reversal of the opioid effect. One boma-held black rhinoceros (subadult) was immobilized three times due to a snare injury, using 1.7 mg etorphine and 30-45 mg azaperone. Pulse oximetry derived oxyhemoglobin saturation (SpO<sub>2</sub>), rectal temperature, heart and respiratory rates were recorded every 10 min. Thirty-nine samples were taken from auricular arteries 6-76 min after darting, and processed in the field using an i-STAT Portable Clinical Analyzer (Abbott Scandinavia AB, Box 509, SE-169 29 Solna, Sweden). The samples were analyzed for pH, PaCO<sub>2</sub>, PaO<sub>2</sub>, base excess, HCO<sub>3</sub><sup>-</sup>, SaO<sub>2</sub>, and lactate. Supplemental oxygen (10 L/min) was provided through a nasal tube to one black and one white rhinoceros.

All free-ranging rhinoceros developed acidemia (pH 7.13-7.34), hypercapnia (PaCO<sub>2</sub> 48-77 mm Hg) and hypoxemia (PaO<sub>2</sub> 40-79 mm Hg). Least physiologic changes were observed in the boma-held black rhinoceros. Metabolic acidosis was present in all free-ranging rhinoceros, and initially high lactic acid levels decreased during the course of immobilization. In 28 out of 35 readings SaO<sub>2</sub> were lower than SpO<sub>2</sub>. Oxygen supplementation markedly improved oxygenation (PaO<sub>2</sub> 108-194 mm Hg). In conclusion, hypercapnia and hypoxemia, indicative of impaired pulmonary gas exchange, and lactic acidemia were evident in both species of free-ranging rhinoceros with the capture method and drug combinations used in this study.

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## ACKNOWLEDGMENTS

Special thanks to the staff at the Wildlife Veterinary Unit, Parks and Wildlife Management Authority, World Wide Fund for Nature (Southern African Regional Program Office) and their Senior Ecologist Raoul du Toit, Bubiana Conservancy, and Malilangwe Trust, for valuable assistance during preparations and field operations in Zimbabwe. Also, many thanks to the helicopter pilot John McTaggart. We wish to acknowledge U.S. Fish and Wildlife Service, the Animal Welfare Association in Växjö, Sweden, and Abbott Scandinavia in Solna, Sweden, for their generous support to this study.

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**Table 1.** Age, sex, and range of drug doses used in two species of free-ranging rhinoceros.

Species	Black rhinoceros			White rhinoceros		
	Age	adult	subadult	calf	subadult	calf
Number of animals and sex		7M <sup>a</sup> , 2F <sup>b</sup>	1M, 1F	1M	2M, 1F	1M
Etorphine <sup>c</sup>	(mg) n <sup>d</sup> =14	3.2-4.3	2.8	1.5	2.8-3.5	2.0
A3080 <sup>e</sup>	(mg) n = 2	3.0	2.0	-	-	-
Detomidine <sup>f</sup>	(mg) n = 8	3.5-4.5	2.5	1.0	3.5-4.5	-
Xylazine <sup>g</sup>	(mg) n = 8	20-45	20	-	-	22
Azaperone <sup>h</sup>	(mg) n = 16	70-90	40-50	20	50-60	35
Hyaluronidase <sup>i</sup>	(IU) n = 16	2000-8000	4000-5000	4000	4000	1250
Nalorphine <sup>j</sup>	(mg) n = 16	1.0-8.0	2.0-2.5	3.0	20-26	6.0

<sup>a</sup>Male.

<sup>b</sup>Female.

<sup>c</sup>M99<sup>®</sup>, 9.8 mg/ml, Novartis South Africa (Pty) Ltd., 72/74 Steel Rd, Spartan, Kempton Park 1620, South Africa.

<sup>d</sup>Number of immobilization procedures the drug was used in.

<sup>e</sup>A3080, 10 mg/ml, Wildlife Pharmaceuticals Inc., Fort Collins, Colorado 80524, USA.

<sup>f</sup>Domosedan<sup>®</sup>, 10 mg/ml, Novartis South Africa (Pty) Ltd.

<sup>g</sup>Rompun<sup>®</sup>, 100 mg/ml, Bayer, Leverkusen, Germany.

<sup>h</sup>Stresnil<sup>®</sup>, 40 mg/ml, Janssen Animal Health, P O Box 651 Halfway House 1685, South Africa.

<sup>i</sup>Hyaluronidase, lyophilized powder, 5000 IU/vial, Kyron Laboratories (Pty) Ltd., 29 Barney Road, Benrose 2094, South Africa.

<sup>j</sup>Nalorphine<sup>®</sup>, 20 mg/ml, Kyron Laboratories (Pty) Ltd.

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## **SURVIVING THE WORST: THE LENINGRAD ZOO DURING THE BLOCKADE, 1941-1944**

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### **Abstract**

The Leningrad blockade during World War II is still alive in the collective Russian memory. Almost every Russian over 30 has heard about the Nazi siege from eyewitnesses. For 900 days and nights of cold, hunger, misery, and death, 3 million inhabitants struggled to survive by hard work, courage, and hope. Almost half died, many from starvation and exposure.

One symbol of peace and hope during these difficult times was the Leningrad Zoo. It survived due to the heroism of 18 people who lived and worked at the Zoo, making every possible effort to save the zoo animals. With no electricity, heat, or running water, and little food for the animals or the keepers, these 18 people managed to care for 237 animals. Unfortunately, less than half of these animals survived. Bombing and artillery assaults were the most common causes of animals' deaths. Two bombardments alone in September 1941 killed 70 animals. This poster commemorates those people who cared for and saved so many animals, including our hippopotamus named "Beauty," a griffon-vulture, antelope, and others. By saving these animals they saved the Leningrad Zoo, and demonstrated that people could remain human, even while struggling to survive in the most inhumane conditions of hunger, deprivation, and conflict. Today, with so many zoos around the world suffering the ravages of war, we can take inspiration from this epic story.

### **ACKNOWLEDGMENTS**

We acknowledge E. Popova, the Leningrad Zoo librarian, for providing all the archive materials to study and for allowing us to duplicate photos for this presentation.

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## PARASITIC HELMINTHS OF ALIEN OR ENDEMIC TERRESTRIAL VERTEBRATES IN JAPAN

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### Abstract

An understanding of ecology and/or zoogeography of the parasitic helminths that are present in Japan is an essential conservation tool for wildlife.<sup>1-3</sup> We have investigated the helminths obtained from Japanese endemic and alien terrestrial vertebrates including reptiles (*Trachemys scripta* and *Chelydra serpentina*), birds (*Garrulax canorus*, *Leiothrix lutea* and *Cairina moschata* var *domestica*) and mammals (*Callosciurus erythraeus*, *Myocastor coypus* and *Procyon lotor*) since 1983. Using cases obtained from our investigations, we aim to provide an overview of 3 types of the host-parasite relationships: 1) between alien vertebrates and alien helminths, 2) between alien vertebrates and endemic helminths, and 3) between endemic vertebrates and alien helminths. Implications for endemic vertebrates affected by alien parasitic helminths and potential strategies for risk reduction were considered.

### ACKNOWLEDGMENTS

The present survey was supported by a grant-in-aid (no. 14560271) and High Technological Research Center (Rakuno Gakuen University) from the Ministry of Education, Science and Culture of Japan, and Kankyo-Gijyutsu-Kaihatsu-Suishin-Jigyo from the Ministry of the Environment of Japan.

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## IN VITRO EVALUATION OF A PHENOLIC DISINFECTANT'S (ENVIRON LpH<sup>®</sup>) EFFECT ON CHRONIC WASTING DISEASE-ASSOCIATED PRION

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### Abstract

We used an in vitro system to evaluate the effects of a phenolic disinfectant (Environ LpH<sup>®</sup>) on chronic wasting disease-associated prion (PrP<sup>CWD</sup>) in brain tissue from naturally-infected mule deer (*Odocoileus hemionus*). A suspension of homogenized brain tissue from CWD-infected mule deer was exposed to a 5% LpH solution for 15 or 30 min, then centrifuged; suspensions of the same homogenized brain tissue without LpH exposure, as well as a suspension of CWD-negative brain tissue, served as controls. After centrifuging, we examined pellets of various exposure groups by Western blot (WB) for evidence of banding typically associated with PrP<sup>CWD</sup>. Phenolic disinfectant treatment did not affect WB performance. After 15 min exposure to 5% LpH, WB banding patterns were partially eliminated as compared to positive controls; after 30 min exposure, PrP<sup>CWD</sup>-associated bands were completely eliminated. Our findings are consistent with previous in vivo studies of LpH efficacy in inactivating scrapie-associated prion.<sup>1</sup> Use of WB may be an efficient alternative to more time-consuming in vivo approaches for evaluating candidate prion inactivating compounds.

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## ANESTHESIA, MORTALITIES, AND LOGISTICS OF CAPTURE WITH TRANSLOCATION OF LARGE NUMBERS OF BEARS IN THE ALASKAN BUSH

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### Abstract

During a 3-wk period in May 2003, 86 black bears (BB) and nine grizzly bears (GB) were immobilized with Telazol<sup>®</sup> (lypholyzed tiletamine HCl/zolazepam HCl reconstituted with sterile water to 200 mg/ml) at a dose range of 1-13 mg/kg (BB) and 1-3 mg/kg (GB) with a mean induction dose of 2.7 mg/kg. The majority (n = 74) were darted from an R-44 helicopter, and the remainder were darted from the ground while restrained in foot snares or attempting to escape by climbing trees (yearling BB only). All initial immobilizations (except hand captured GB cubs) were attained using Palmer Cap-Chur darts from a CO<sub>2</sub> pistol with target areas generally in the rump or shoulder musculature. Darted bears ranged in weight from 8-145 kg (BB) and 75-320 kg (GB). All but four bears (that were captured solely to retrieve GPS collars) were moved from the initial capture site to a pre-translocation processing station by one of three methods in order of frequency: in a sling under the helicopter, inside the helicopter, or by riverboat.

The purpose of the capture operation was to experimentally remove ursine predators of newborn moose calves in a 1368 square km area of the Kuskokwim River drainage near the Alaska native village of McGrath. Prior to translocation, bears were either maintained under anesthesia for the duration or were allowed to recover in cages and then induced with Telazol at 2-3 mg/kg via pole syringe. Induction doses of Telazol<sup>®</sup> generally gave full-restraint for 40 min before any additional drugs were required. For maintenance of anesthesia prior or during transport, bears were given one dose of 1 mg/kg of Telazol<sup>®</sup> (i.m. by hand injection) when purposeful movement was noted. This second dose usually gave an additional 40-60 min of adequate restraint. Thereafter, if additional restraint was required during transport, bears were given hand injections of 10-25 mg diazepam and 50 to 1500 mg of ketamine HCl i.m. Duration of the diazepam/ketamine restraint was 20-30 min before re-dosing was required. No additional anesthesia was given within 20 min of reaching release sites. Of the bears captured, 74 BB and all GB were translocated 290 to 400 km from the pre-translocation processing station in a DeHavilland Beaver (three to seven bears at a time with an attendant) or singly in other fixed wing aircraft. Six BB were transported to a captive facility and not released.

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Anesthetic complications included severe hypothermia, hyperthermia, prolonged recovery time (BB at Telazol doses over 7 mg/kg), hypersalivation, petit mal seizures (more frequent in GB), and mortalities. Direct capture mortalities occurred in only three of 95 captures. One BB died 1 hr after darting from myocardial hemorrhage secondary to blunt chest trauma during darting. One yearling BB was euthanatized because a dart wound penetrated the abdomen. Necropsy revealed a lacerated spleen, as well as a and fractured femur from a second dart administered when the bear was treed. Subsequently, capture of yearling BB was immediately suspended. In addition, an adult male BB died in captivity 3 days post-transport from aspiration pneumonia (one of only three bears that received xylazine at 0.22 mg/kg). In addition to the capture-related mortalities, an adult female BB was euthanatized 24 hr post-darting in captivity and was found to have significant perirenal hemorrhage that may have eventually resulted in morbidity or mortality. Many bears were able to lift their heads at the time of departure of the transport crew from the release site. Three bears were able to rise and walk away before crew departure. No bears that were released failed to recover from anesthesia, and all left the release area. Twenty-two BB received radio-collars for tracking purposes and of those, one BB died during the summer of unknown causes. All bears were marked with ear tags and colored flags indicating the withdrawal time for meat consumption. Only two BB were taken by hunters, both after the withdrawal time.

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## USE OF "ACELL VET™ SCAFFOLD," AN EXTRACELLULAR MATRIX INCORPORATING PIG URINARY BLADDER FOR WOUND HEALING IN ZOO SPECIES

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### Abstract

Successful management of large skin wounds that cannot heal through primary intention can be particularly challenging in zoo and wild animal practice. When the large size of a skin defect precludes surgical closure, therapeutic intervention often includes repetitive wound cleaning, debridement, and bandaging. Zoo and wild animal patients must often be repetitively anesthetized for wound treatments throughout the slow process of second intention healing.

The use of an extracellular matrix derived from pig urinary bladder and marketed as "ACell Vet™" (ACell, Inc., 10555 Guilford Road, Jessup, Maryland, 20794 USA) has shown promise for wound repair in horses, other domestic animals, and people. This acellular matrix promotes new blood vessel formation and constructive remodeling of tissue by serving as a biologic scaffold across which new cells populate and differentiate. When applied to a wound, host tissues grow into the matrix, degrade it, and replace it with site appropriate tissue.

The use of "ACell Vet™" for treatment of wounds sustained by a dall sheep (*Ovis dalli*) and a lion (*Panthera leo*) resulted in complete, uncomplicated healing of large skin defects. The sheep had sustained a severe horn injury for which complete cosmetic dehorning was necessary. Dehorning created an 8 cm × 7 cm open wound over the cornual sinus that could not be surgically closed. The lion had sustained a 7 cm × 7 cm bite wound over the dorsal and lateral aspects of its tail that was not amenable to surgical closure.

In each case, the "ACell Vet™" matrix was applied over the wound, moistened with saline, sutured to the wound edges, and lightly bandaged. Once this extracellular matrix material was applied, no further intervention was necessary, except for a single bandage removal in the sheep. (The lion removed the bandage on its own, leaving the "ACell Vet™" in place.)

Complete wound closure was achieved in both animals via natural host mechanisms and the matrix was absorbed without complication. Time until full wound closure with hair re-growth was approximately 4 mo in each case.

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## **ACKNOWLEDGMENTS**

Special thanks to Lion Country Safari lion keepers, hospital and petting zoo staff.

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## CAUSES OF MORTALITY IN STRANDED ALASKAN SEA OTTERS (*Enhydra lutris lutris*)

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### Abstract

During much of the 20<sup>th</sup> century, sea otter populations throughout their range were recovering from the effects of an international fur harvest that ended with the near extirpation of the species by 1900.<sup>8</sup> Recovery rates of geographically isolated sea otter populations varied, but causes of differences are poorly understood.<sup>1</sup> Despite large scale rapid declines in sea otter populations southwest of Cook Inlet in Alaska,<sup>5,6</sup> populations east of Cook Inlet appear generally stable or increasing.<sup>2,12</sup> The Southern sea otter (*Enhydra lutris nereis*) in California has demonstrated a consistently low population growth rate and a variety of factors have been identified as contributing to the delayed recovery, including starvation, predation, mortality related to fisheries, and infectious diseases.<sup>3,4,7,10,11</sup> In this initial pilot study, we began to establish a network for recovering fresh-dead animals from Southcentral Alaska and performing necropsies comparable to those in conducted in California. Our hypothesis was that the prevalence of major infectious diseases is lower in the Northern sea otter compared to the threatened Southern sea otter in California. Our objective was to identify causes of mortality and compare disease prevalence rates between Northern and Southern sea otters.

During 2002 and 2003, we recovered eight carcasses suitable for this protocol. Of the eight, two were rehabilitation animals from the Alaska SeaLife Center, and the rest were beach cast. Two were from Kodiak, two from Homer and four from the Seward area. Seven were male, and one female. Three (38%) died due to severe valvular vegetative endocarditis with associated extensive thrombotic disease. Organisms were isolated from the heart blood of two of the three and included a non-hemolytic *Streptococcus* sp. and *Aeromonas* sp. in one case and *Streptococcus bovis* in the other. One animal that had been suffering from seizures and was euthanatized had an encephalocoele. Two animals were emaciated and had massive colonic impactions; another died of massive trauma (boat strike); and the last was a prime condition female with a term fetus which died acutely with massive pulmonary edema, pleural effusion and myocarditis. All cases have been negative by serology and culture for *Sarcocystis neurona* and *Toxoplasma gondii*. One animal had a *T. gondii* titer of 1:80; however the significance of this

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finding is unknown since corresponding histologic and culture results were negative. Parasites that have been identified include the acanthocephalan *Corynosoma enhydri*, gastric nematodes (*Anisakis* sp.), a cestode (*Diplogonoporus tetrapterus*) and a hepatic trematode (*Orthospanchnus fraterculus*).

Although a very small number of cases have been reviewed to date, the emerging pattern is different from that described for the southern sea otters. In the CA animals, protozoal encephalitis, acanthocephalan-related disease, shark attack and cardiac disease (primarily a chronic non-suppurative myocarditis of unknown etiology) have been identified as the most common causes of death.<sup>9</sup> Valvular endocarditis has been reported in the southern sea otters but appears to be rare. Our findings will help determine causes of the patterns observed in population trends in Alaska sea otters and may help clarify the role of infectious disease in the California population.

#### ACKNOWLEDGMENTS

This project was supported by the US Geological Survey, Alaska Science Center, Alaska Veterinary Pathology Services, the US Fish and Wildlife Service and the Alaska SeaLife Center. The authors would like to thank Dr. Daniel Mulcahy (USGS) for performing some necropsies and proofing the abstract, Dr. Natalie Noll (ASLC), Dr. Shawn Johnson (ASLC), and John Haddix (USFWS) for their assistance in performing necropsies. Our thanks to the UCD protozoa laboratory including Woutrina Smith, Patricia Conrad, Andrea Packham, and Ann Melli, as well as to Spencer Jang at the UCD Bacteriology laboratory and to Murray Dailey at The Marine Mammal Center in Sausalito for parasite identifications. We would like to thank the staff of the ASLC for their work with the rehabilitation animals and to the people participating in the stranding network .

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## RANDOM AMPLIFICATION OF POLYMORPHIC DNA REVEALS CLONAL RELATIONSHIPS AMONG ENTEROPATHOGENIC *Escherichia coli* (EPEC) ISOLATED FROM NONHUMAN PRIMATES AND HUMANS

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### Abstract

Enteropathogenic *Escherichia coli* (EPEC) strains are important agents of infantile diarrhea all over the world. In contrast to other diarrheagenic *Escherichia*, EPEC does not produce any classic protein toxin but induces diarrhea by intimate binding to intestinal cells. Diarrhea is the result of a series of signals triggered by the pathogen-host membrane interaction, which in turn provokes reorganization of the cytoskeleton of the affected cell, with a consequent loss in microvillus structure and effacement of intestinal villi. This lesion is called attaching and effacing (A/E).<sup>3</sup>

EPEC infections undertake greater importance in developing countries since the rate of infection and consequent infantile mortality reaches higher level. With respect to animals, EPEC have been isolated from various species but most isolates belong to serotypes that differ from those recovered from man. Furthermore, human serotypes isolated from animals usually exhibit different phenotypic and/or genotypic characteristics when compared with human strains, receiving the denomination of atypical EPEC.

Recently we demonstrated that in the case of nonhuman primates, several isolates belong to serogroups and/or serotypes related to those implicated in human disease, such as the traditional EPEC serogroups O127, O128, O142 and O26. In addition, the nonhuman primates strains showed genotypic and phenotypic characteristics similar to those of human typical EPEC serotypes indicating that not only man, as has been postulated, but also those animals, may represent a natural reservoir and source of infection of these bacteria for both, human and nonhuman primates.<sup>2</sup>

The objective of this study was to evaluate genetic differences among strains isolated from nonhuman primates compared with human collection strains, through the analysis obtained by the random amplified polymorphic DNA (RAPD). This method has been considered as an appropriated molecular tool in epidemiologic analyses of *E. coli*. The study included a total of 18 EPEC strains isolated from healthy and sick nonhuman primates, being phenotypic and genotypic strain characteristics described elsewhere.<sup>2</sup> Thirteen human EPEC strains recovered from sick children were obtained from culture collections kept at Instituto Adolfo Lutz, São

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Paulo, Brazil, a reference center for *E. coli* serotyping. The assay was performed using three different primers as described before<sup>1</sup> and the analysis done at a “Numerical Taxonomy and Multivariate Analysis System”-pc program (1.7 version) generated a dendrogram.

Dendrogram analysis showed that there was not clustering between human and monkey strains. Two main groups were distinguished. The first one included all human and nonhuman primates strains belonging to serogroup O26, all of them recovered from sick individuals. In the second group, two sub-groups were defined, one mostly composed of strains isolated from healthy nonhuman primates besides human isolates, and the other composed of strains obtained from sick individuals. In this last cluster the nonhuman primate strains showed genotypic characteristics of typical human EPEC,<sup>2</sup> even when they did not belong to classic human serotypes. Human and nonhuman isolates of the EPEC serotypes O127:H40 and O128:H2 shared, respectively, 85% and 80% of their bands indicating strong genetic similarity between the strains, allowing for speculation that they have arisen from the same pathogenic clone.

The results of this study provide further evidence that Monkey EPEC (MEPEC) are correlated to human EPEC as pointed out in prior research.<sup>2</sup> Due to nonhuman primates phylogenetic proximity to man and their similar susceptibility to human pathogens, they could represent important experimental models for the study of EPEC infection. The potential risk of transmission between human and nonhuman primates should be considered in order to minimize the impact these bacteria may have on the health of colonies of animals held in captivity.

#### ACKNOWLEDGMENT

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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## SEROSURVEY FOR FELINE LEUKEMIA VIRUS AND LENTIVIRUSES IN CAPTIVE FELIDS AT FUNDAÇÃO PARQUE ZOOLOGICO DE SÃO PAULO, SÃO PAULO STATE, BRAZIL

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### Abstract

Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are recognized pathogens that cause persistent infections in domestic cats. Although these retroviruses have been found in domestic cats in Brazil, serosurveys carried out in captive small neotropical felids in São Paulo state were negative.<sup>1</sup> Retroviral infections in large cats have been recognized as “emerging diseases.” Lentiviruses have been shown to be endemic in free-ranging large cats and FeLV seems to infect wild cats that are exposed to infected domestic cats.<sup>2</sup>

The aim of this study was to investigate the prevalence of lentivirus and FeLV infections in 126 nondomestic felids kept in captivity at Fundação Parque Zoológico de São Paulo. Serum samples collected from 29 *Panthera leo*, 14 *Panthera tigris altaica*, 6 *Panthera onca palustris*, 2 *Puma concolor*, 2 *Uncia uncia*, 3 *Panthera pardus*, 1 *Panthera pardus mielas*, 2 *Acinonyx jubatus*, 33 *Leopardus tigrinus*, 9 *Leopardus wiedi*, 10 *Oncifelis geoffroyi*, 15 *Herpailurus yagouaroundi* were tested by immunoassay (“Snap<sup>TM</sup> Combo FeLV Antigen/ FIV Antibody Test Kit”, IDEXX Laboratories, Inc. Maine, USA).

Lentivirus infection was detected in five of 29 lions tested (17%). All other animals resulted negative for both retrovirus infections. Three out five lions seropositive were female and two were males. One out of three seropositive females had been kept with one positive male, the second female was kept in a group of animals lentivirus negative and the last female, as well as the remaining positive male, was maintained along with negative individuals. These negative individuals eventually died with a history of neoplastic disease and emaciation; however no lentivirus infection was suspected in these animals.

All positive animals showed episodes of anemia and/or leucopenia during their lifetime. The average time in zoo for positive lions was 15 yr, while negative animals had an average time of 9 yr and 50% of these had been at the São Paulo Zoo for less than 4 yr. Considering that there was no possibility of segregating the seropositive animals, all positive animals were submitted to euthanasia and material was preserved for future studies. Due to the endemic aspects of

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lentivirus infections in African lions, associated with the chronic characteristics of lentivirus infections in this group of animals, all felids will be tested periodically.

#### **ACKNOWLEDGMENTS**

This work was supported by a fellowship provided by Universidade Paulista and had the staff support of Fundação Parque Zoológico de São Paulo. Financial support: CAPES

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## EXOGENOUS INFLUENCES ON SERUM TESTOSTERONE CONCENTRATION IN CAPTIVE BLACK RHINOCEROS (*Diceros bicornis*)

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### Abstract

While captive breeding of rhinoceros species has met with some success, difficulties are encountered. Different rhinoceros species in the wild show varying sociosexual behaviors. Indian (*Rhinoceros unicornis*) and black rhinoceros (*Diceros bicornis*) tend to remain solitary except during the breeding season or when concentrating around resources. White rhinoceros (*Ceratotherium simum*) are more gregarious. Captive management strategies attempt to mimic these natural relationships as much as possible, but no study has evaluated the effect of captive sociosexual environment on hormonal concentrations. Testosterone, for example, is essential for spermatogenesis, development of primary and secondary sexual characteristics, and libido, all of which play key roles in fertility. This study investigates reproductive hormones in rhinoceros species in different sociosexual settings to determine the importance of social groupings on fertility. Our overall hypothesis is that reproductive hormone concentrations in male rhinoceros reflect reproductive performance and can be used to assess potential reproductive success. Our specific hypothesis for this study is that serum hormones related to reproduction in male rhinoceros will show variation with age, time of year, and sociosexual status.

Questionnaires and requests for serum samples were sent to 72 AZA institutions in the United States and responses were received from 63 institutions. As of January 2004, 442 samples had been received from collaborating institutions. To date, samples have been analyzed for testosterone using enzyme immunoassays (EIAs) already validated for these species. Preliminary data collected from 382 black rhinoceros serum samples show a significant difference between testosterone concentrations in juvenile and adult samples ( $P < 0.05$ ). These data do not show a correlation between time of year and testosterone concentration. Preliminary findings show that individual black rhinoceros males may experience a change in testosterone concentration if sociosexual status changes. One male housed with a single female rhinoceros, for example, showed a significant rise in testosterone concentration when a second female was introduced ( $P < 0.05$ ). In addition, individual males showed higher numeric concentrations when housed with females than when housed separated by a barrier or isolated completely. Further study is indicated to verify these relationships in black rhinoceros and evaluate their influence on white and Indian rhinoceros.

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## ISOLATION OF *Malassezia sympodialis* FROM OTIC SECRETIONS OF HEALTHY WILD FELIDS

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### Abstract

The objective of this study was to determine the presence of different species of the genus *Malassezia* in the healthy external auditory canal of wild felids maintained in captivity. Fifty-five adult animals (110 samples of otic secretion), 29 males (52.7%) and 26 females (47.3%), were studied: 26 lions (*Panthera leo*), 13 tigers (*Panthera tigris*), 6 leopards (*Panthera pardus*), 6 jaguars (*Panthera onca*), 2 cheetahs (*Acinonyx jubatus*), and 2 pumas (*Puma concolor*). Samples were obtained by introduction of a sterile swab into the auditory canal after cleaning the auricle with alcohol-ether solution. The swabs were seeded onto Petri dishes containing modified Dixon medium and dextrose Sabouraud agar with chloramphenicol and the plates were incubated at 35°C for 2 wk. The isolates were analyzed regarding macro- and micromorphology and identified through catalase tests and growth on Tween 20, 40, 60 and 80. *M. sympodialis* was isolated from 33 of the felids studied (60.0%) and from 53 samples of otic secretion (48.2%). Twenty-four strains (45.3%) were isolated from the right auditory canal and 29 (54.7%) from the left. The incidence of fungi was higher in lions, with yeast being isolated from 25 of 26 animals (96.2%). No other species of the genus *Malassezia* was isolated. This fact calls attention since *M. pachydermatis* is the species considered a member of the microbiota of the mammalian external auditory canal. The present results suggest that the main species of the genus *Malassezia* participating in the microbiota of the external auditory canal of large felines is *M. sympodialis*.

### ACKNOWLEDGMENTS

This project was funded by a UNIP Grant – Individual Research Project for Teachers.

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## DISEASE IN A STRUCTURED POPULATION: BOVINE TUBERCULOSIS IN AFRICAN BUFFALO

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### Abstract

Bovine tuberculosis (BTB, *Mycobacterium bovis*), an airborne bacterial pathogen, is re-emerging in wildlife and livestock worldwide. We study slow-moving epidemics of BTB in the buffalo (*Syncerus caffer*) populations of the Kruger National Park (KNP) and Hluhluwe-Umfolozi Park (HUP) in South Africa to develop a better understanding of disease spread in structured populations. The prevalence of BTB continues to increase in KNP, and the epidemic front is moving northwards from its introduction from cattle in the south. Buffalo are a reservoir host, maintaining the disease at high prevalence (~60%), while predators such as lions and leopards appear to be spill-over hosts. It is unclear how BTB, with its wide range of potential hosts, will affect these ecosystems. As an exotic disease, managers would like to control or eradicate this disease via culling, vaccination, or some combination of the two. Preliminary modeling and data suggest that neither vaccination nor culling is likely to eradicate the disease individually, but Hluhluwe-Umfolozi Park has begun to control prevalence by removing positive animals. We combine mathematic models, field data, buffalo and BTB genetics, and GIS to assess buffalo management options as well as the probable impacts.

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## INTEGRATING ASSOCIATION AND DISEASE DYNAMICS USING EMPIRICAL DATA

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### Abstract

Socially structured wildlife populations, such as the African buffalo (*Syncerus caffer*), are poorly characterized by either traditional disease models that assume random mixing or spatial disease models that assume limited dispersal between fixed groups. Dynamic network models in combination with data on who spends time with whom, however, more accurately reflect connections within and between groups and the spread of disease between associating individuals. We used 2 yr of radio-tracking data on 64 African buffalo to estimate monthly association matrices. These matrices were then used as a substrate to model disease dynamics in the buffalo system and investigate the importance of the topology of connections in the network as well as the variation in the frequency of contact between individuals. In agreement with previous studies on static networks, we found that topology was very important and that only a small proportion of connections between groups are necessary to create a ‘small-world’ network. Increasing the variation in frequency of contact between individuals had little impact upon disease dynamics in this system. Cluster analyses of the association matrices demonstrated that herds are not as well-defined as previously thought and are increasingly amorphous over time. Buffalo associations were more tightly clustered in 2002 than 2003, perhaps due to drier conditions in 2003 forcing herds out of previously habitable areas. As a result, we predict diseases to spread faster through the buffalo population during drought conditions due to both increased stress and increased population mixing.

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## SERUM ENZYME ACTIVITIES OF CAPTIVE STELLER'S EIDERS (*Polysticta stelleri*) AND SPECTACLED EIDERS (*Somateria fischeri*)

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### Abstract

Steller's and spectacled eider populations have declined drastically in recent decades and were listed as threatened under the U.S. Endangered Species Act in the 1990's.<sup>1-2</sup> Although serum chemistry values including enzyme assays have been used to evaluate the health and organ function of several species of birds,<sup>3</sup> no reference values have been established for Steller's and spectacled eiders. Therefore, we collected serum from captive eiders at the Alaska SeaLife Center in 2003 to establish reference ranges for clinically healthy captive birds on consistent diets. We measured alkaline phosphatase (ALKP, EC 3.1.3.1), gamma-glutamyltransferase (GGT, EC 2.3.2.2), aspartate aminotransferase (AST, EC 2.6.1.1), lactate dehydrogenase (LDH, EC 1.1.1.27), and creatine kinase (CK, EC 2.7.3.2) enzyme activities using a VetTest 8008 analyzer (IDEXX Laboratories, Inc.) and compared results among seasons and genders using the  $\alpha$ -level of 0.05. Gamma-glutamyltransferase activity was not detected in the serum of eiders. Seasonal differences in mean serum enzyme concentrations were detected in ALKP for Steller's eiders and in CK and AST for spectacled eiders. In January, male spectacled eiders had higher mean serum AST concentrations than females, but no other differences were detected between genders. Annual mean ( $\pm$  SD) concentrations were calculated when no seasonal or gender differences were detected. For Steller's eiders, the mean ( $\pm$  SD) serum concentration of AST, CK, and LDH were 11.5 IU/L ( $\pm$  28.6 IU/L), 203 IU/L ( $\pm$  68.3 IU/L), and 1031 IU/L ( $\pm$  246 IU/L), respectively. For spectacled eiders, the mean ( $\pm$  SD) serum concentrations of ALKP and LDH were 199 IU/L ( $\pm$  157.9 IU/L) and 1222 IU/L ( $\pm$  1138 IU/L), respectively. A significant correlation between CK and LDH was found in both species. Electrophoretic separation into isoenzymes has been used to characterize the source of enzyme activity, and our findings to date suggest that CK in eiders separates into two main isoenzyme fragments, and that a third isoenzyme fragment may be present (Beckman Paragon Electrophoresis system, Beckman Instruments, Inc.). In the future, reference intervals of enzyme and isoenzyme activities will be used to evaluate the health and organ function of wild Steller's and spectacled eiders as part of a cooperative research effort to determine the cause of their population declines.

### ACKNOWLEDGMENTS

The authors would like to thank the Alaska SeaLife Center Eider Research, Avian Husbandry, and Veterinary Services departments for their help with this project.

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## DIROFILARIASIS IN A FREE-RANGING SMALL LITTLE-SPOTTED-CAT (*Leopardus tigrinus*)

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### Abstract

The little-spotted-cat (*Leopardus tigrinus*) is the smallest Neotropical Brazilian species of felid, similar to the domestic cat in size and body structure. This species is listed in CITES Apêndice I and it is considered insufficiently known by IUCN. Information about occurrence of diseases and infections in such a species are sparse. Dirofilariasis has been reported worldwide, mainly in domestic dogs, but also in other carnivores. Among felids, occurrences have been reported in tiger (*Panthera tigris*), lion (*Panthera leo*), leopard (*Panthera pardus*), clouded leopard (*Neofelis nebulosa*) and snow leopard (*Uncia uncia*). The etiologic agent, the nematode *Dirofilaria immitis*, when adult, usually lives inside the heart and pulmonary artery of the host. Though domestic cats can lodge adult dirofilaria without signs of disease, severe complications and sudden death are frequent. *Hematophagous* mosquitoes are intermediate hosts. A wild adult female little spotted cat was found recumbent, with hemoptysis and anisocoria in a veterinary clinic in the southeastern coastal town of Ubatuba, São Paulo, Brazil, where there are protected areas of Atlantic Rainforest. Although receiving medical care, the animal died and necropsy was performed at LAPCOM, FMVZ-USP. Macroscopic examination revealed that the animal had an excellent body condition, was lactating, and had no sign of trauma, which was confirmed by post mortem radiography. Small and large intestines were heavily infested with worms, as well some were found in the heart. The right ventricle was enlarged, with eccentric hypertrophy at the histopathologic examination, pulmonary lesions suggested secondary hypertension due to parasitism, including endoarteritis and the presence of four intravascular microfilaria in small arteries. The cardiac nematodes were identified by morphologic methods as *Dirofilaria immitis*. Two males and one female were found at the right ventricle and a female at the left ventricle. Because of the uncommon localization at left ventricle, we suggest the occurrence of sudden death by cardiac parasitism. From our knowledge, this is the first reported case of *Dirofilaria immitis* infection in a Neotropical felid. It is not possible to determine if the little-spotted-cat is a natural host or if the agent was introduced in the region by domestic carnivores, considering that

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there are many confirmed cases of domestic canine dirofilariosis in Ubatuba. We suggest adoption of control measures for domestic dog populations that can act as reservoirs of several infectious agents for wild animals.

#### **ACKNOWLEDGMENTS**

Aquário de Ubatuba, CENAP-IBAMA; Financial support: CAPES.

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## SEROPREVALENCE OF HEPATITIS A IN NEOTROPICAL PRIMATES

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### Abstract

Hepatitis A virus (HAV) is a picornavirus that causes hepatitis A, a zoonotic disease. This virus has only human and nonhuman primates as its natural hosts. Just one serotype is known, but several strains have been recognized. The infection route of the HAV is fecal-oral. After the ingestion of the virus by infected food or contaminated objects, the virus replicates in the liver of the animal, reaches the intestines through the bile, and is eliminated with the feces. The disease in primates is mainly asymptomatic, but when present is unspecific and varies from mild signs to death. The diagnosis is made by serologic tests or identification from the viral antigen in sera or feces at the acute phase of the disease. The presence of anti-HAV IgM shows acute or recent infection. On the other hand, anti-HAV IgG is found from the convalescent phase of the disease through several years. The aim of this project was to research the seroprevalence of anti-HAV antibodies in New World primates and detect the viral antigen in feces from those animals that had acute infection. Sera from 421 animals of 32 different species were tested. From these animals, 13.5% (57/421) were wild animals, 29.7% (125/421) were from the Centro de Primatologia do Rio de Janeiro (CPRJ), 4.0% (17/421) from breeders, 3.8% (16/421) from Departamento de Parques e Áreas Verdes (DEPAVE) and 48.9% (206/421) were zoo animals. The sera were tested by immune-enzymatic tests for the presence of IgM and total anti-HAV antibodies. All the sera were negative for IgM, which means that no animal had acute infection when tested. All wild animals were negative for total anti-HAV, as were the animals from DEPAVE. Four percent (5/125) from the CPRJ animals and 7.6% (17/223) from the zoos'/breeders' animals were positive for total anti-HAV, showing that a number of captive animals have already been in contact with the virus. The prevalence of anti-HAV antibodies found in this study was lower than expected, as it is known that the number of positive animals in captivity is high. The possible reasons for such low prevalence are discussed. Our results lead us to think that hepatitis A is not a disease of high risk for either wild or zoo New World primates kept in our conditions. To our knowledge, this is also the first report of hepatitis A in animals belonging to the genus *Leontopithecus*.

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## **ACKNOWLEDGMENTS**

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP.

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## DETERMINING NORMAL THYROID HORMONE STATUS IN GALAPAGOS TORTOISES, THEN COMPARING NORMAL LEVELS TO THYROID LEVELS OF GALAPAGOS TORTOISES (*Geochelone elephantopus*) SUSPECTED OF HYPOTHYROIDISM

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### Abstract

### Introduction

The number of suspected hypothyroid cases occurring in giant tortoise species is increasing with the numbers being kept in captivity. Clinical signs reported in these animals have included anorexia, lethargy, myxedema of subcutaneous tissues, and fibrous goiter.<sup>1</sup> Goiter is most likely a misnomer given the location of the thyroid gland. In turtles and snakes the gland lies ventral to the trachea and just anterior to the heart.<sup>4</sup> The cause of these clinical signs has been associated with hypothyroidism secondary to inappropriate husbandry and diet.<sup>1,5</sup> However, there have been no clinical studies done to confirm this diagnosis.

### Methods

The Oklahoma City Zoo maintains an established group of five clinically normal adult galapagos tortoises, two male and three female. Each animal had blood collected from the brachial vein 4-6 times over a 6-mo period for a total of 22 blood samples. This group of animals was used to establish total T<sub>4</sub> reference ranges: 4.74–26.5 nmol/L, mean=10.39 nmol/L, standard deviation=18.72 nmol/L.

The Oklahoma City Zoo received three galapagos tortoises back from a loan in early 2002. These animals were approximately 10 yr old and on return to the Oklahoma City Zoo displayed the characteristic myxedema associated with tortoises suspected of being hypothyroid. These animals were bled three times over a 6-mo period for a total of 12 blood samples. The total T<sub>4</sub> levels of these three tortoises were: 2.43–6.02 nmol/L, mean=3.94 nmol/L, standard deviation=1.06 nmol/L.

Initial analysis of the data indicates a statistically significant difference between the T<sub>4</sub> levels of the assumed normal tortoises and the assumed hypothyroid tortoises ( $P < 0.05$ ). Total T<sub>4</sub> levels

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for all tortoises were determined by the Veterinary T<sub>4</sub> Elisa kit (Oxford Biomedical Research, 2165 Avon Industrial Drive, Rochester Hills, MI 48309 USA).

## Results and Discussion

Thyroid hormones are involved in the regulation of nutrient assimilation, metabolism, calorogenesis, growth and development, and reproduction.<sup>3</sup> There are various factors that can affect the normal function of the reptilian thyroid gland such as age, sex, diurnal changes, seasonal changes, day length, diet, environment, breeding season, exposure to sunlight, and stress.<sup>2,3,6</sup> Because of the diversity of things that can affect thyroid hormones in reptiles, point-sample determination of thyroid levels may not accurately diagnose thyroid status, however, having an established range of normals is an essential first step in the diagnosis of thyroid disease.<sup>2</sup>

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**PREDICTIVE VALUE OF EASILY MEASURED PARAMETERS FOR COMMON MURRE (*Uria aalge*) SURVIVAL TO RELEASE DURING THE LUCKENBACH OIL SPILL IN NORTHERN CALIFORNIA, NOVEMBER 2001-JULY 2002**

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**Abstract**

In 1957, the S.S. Jacob Luckenbach sank 17 miles outside San Francisco Bay. It has now been tied by chemical fingerprinting to “mystery spills” along the coast for more than a decade, where episodic and varying numbers of oiled birds have been found on beaches from Monterey to Bodega Bay. A recent event, which ran from November 2001 until July 2002, affected over 2100 seabirds, the majority of which were common murres (*Uria aalge*). The purpose of this study is to evaluate medical records from more than 900 live-stranded common murres affected by this spill to evaluate the utility of easily-measured parameters in predicting survival or death during rehabilitation, and thus increase the ability of rehabilitation staff to make humane treatment decisions for each affected animal during future spills. Parameters to be examined include: packed cell volume, total plasma protein, blood glucose, body temperature, body condition, degree of oiling, and hydration status. In addition, since common murres frequently become anemic during treatment, birds will be followed over time to determine a lower limit of PCV below which survival was unlikely. All parameters will be considered singly and in combination for the development of a predictive statistical model using SPSS software.

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## MISSISSIPPI AUDUBON PROJECT: HUMANITIES AND IN SITU CONSERVATION

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### **Abstract**

The humanities have come to play an important role in the future conservation strategy of the Strawberry Plains Audubon Center. Sisters Ruth Finley and Margaret Finley Shackelford who shared a common passion for birds and wildlife bequeathed this historic 2500-acre former cotton plantation to the Audubon Society in 1982. Audubon Mississippi took possession of the property in 1998 and established the Strawberry Plains Audubon Center the following year.

Thru well focused and clearly thought out programs the Audubon Center continues to grow as an education and conservation resource serving Holly Springs, north Mississippi and the southeast's diverse ethnic and socioeconomic communities. The Audubon Mississippi newsletter, published by the Mississippi State Office of the National Audubon Society, started just 3 yr ago, dramatically improves with each new issue. This newsletter reports for example on the growing September Hummingbird Migration Celebration and the Amateur Naturalist Camp at Strawberry Plains, the Great Backyard Bird Count, and honors the efforts and activities of Audubon Chapter volunteers throughout Mississippi.

The Strawberry Plains Audubon Center discovered early in its growth process the rich past of this former working farm. In 2002 a planning grant, matched by the Audubon Center, was awarded by the Mississippi Humanities Council to help connect the cultural heritage of the property and the mission of the Audubon Center. This grant received a special 30<sup>th</sup> Anniversary Award designation from the Mississippi Humanities Council celebrating 30 yr of public humanities programming in Mississippi. The grant provided for the visit and study of the Audubon property by three scholars from the disciplines of archaeology, vernacular or folk architecture, and oral history. Each scholar enlightened the Audubon Center staff illuminating its rich heritage and many program possibilities. Thru this grant the Audubon staff became acutely aware of its great potential to incorporate the humanities as a way of forming a bridge to the community, and in effect allow the community and conservation to converge.

The humanities disciplines, as defined by Congress, include languages and literature, history, archaeology, jurisprudence, philosophy, ethics, comparative religion, history and criticism of the arts, and social sciences employing historic and philosophic approaches.<sup>1</sup>

This novel approach has led to two additional MHC grants to begin projects in archaeology and oral history. These grants, matched by the Audubon Center, have resulted in bringing

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humanities scholars and graduate students from the University of Mississippi, the University of South Carolina and Hampton University together to meet with local citizens and community leaders at the Strawberry Plains Audubon Center.

The immediate goals of the Audubon Center include conservation and restoration of the natural ecosystem and building relationships between peoples and nature, focusing on birds other wildlife, and their habitats, for the benefit of humanity and the earth's biologic diversity. The Strawberry Plains Audubon Center is now ready to undertake a new effort; the restoration of up to three of its long abandoned former tenant or sharecropper's homes to serve as educational areas for the Center. Of nearly a dozen former tenant homes on the property there are several that can be saved and restored to their original condition for educational reuse. These homes can be utilized to tell the story of a time when people lived in close association with the land and how this relationship impacted plant and animal populations. One has only to stand inside a tenant home to feel the strength and courage of those who lived there and begin to understand the odyssey from slavery to freedom and restoration. Of critical significance will be to learn these relationships so that the Audubon Center can provide an accurate history of this historic property to guests at its visitor's center and for participants in educational programs.

Following restoration with the help of the local community, uses of the tenant homes may include:

- Playing recordings for visitors of the oral histories of the descendants of individuals who once lived there to enable reflection on our cultural heritage
- Conducting workshops for children and adults on native plants and their use in everyday life; or on restoration projects ongoing at the Center
- Provide settings for photography workshops and gallery shows
- Provide settings for teaching reading and creative writing through nature and history
- Providing gallery space for young local southern artists to paint and display wildlife art
- Provide space for blues and local roots music programs to connect individual imagination to shared experience
- Conduct seasonal writing, drawing, painting and sculpture workshops
- Display artifacts found from archaeological studies to provide insights about the past from early Native Americans to present day culture
- Demonstrate historic culinary and food preparation skills and techniques

The Strawberry Plains Audubon Center is located in Holly Springs, Mississippi, nearby Rust College, just 1 hr by automobile from Memphis, and 45 min from the campus of the University of Mississippi.

#### **LITERATURE CITED**

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## PRELIMINARY EVALUATION OF A PORTABLE CLINICAL ANALYZER TO DETERMINE BLOOD GAS AND ACID-BASE PARAMETERS IN MANATEES (*Trichechus manatus*)

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### Abstract

This study was conducted to validate the i-STAT machine (Heska Corp., Ft. Collins, CO, USA) for analyzing manatee blood gases and acid-base parameters. Blood was collected from captive (n = 2) and wild (n = 12) manatees to determine preliminary reference ranges for the i-STAT. Values from captive animals were compared to standard blood gas, clinical chemistry and complete blood cell count machines. Also blood values were compared between captive and wild manatees. The i-STAT machine reported sodium ( $140.0 \pm 2.92$ ) and chloride ( $97.80 \pm 2.17$ ) as lower and higher, respectively, than the reference machines. Additionally wild manatees had higher values for lactate ( $14.84 \pm 3.42$ ), glucose ( $90.50 \pm 22.39$ ) and a lower pH ( $7.08 \pm 0.14$ ) than captive manatees.

### Introduction

Capture and veterinary care of manatees frequently occurs in the field in relatively remote areas. The quick evaluation of blood gas parameters and acid-base status in manatees may aid in treatment of these animals in the field and during rehabilitation at oceanaria. Also, detection of significant respiratory or metabolic changes associated with capture may be used to assess and potentially modify capture designs to minimize physiologic stress. The recent development of portable clinical analyzers, such as the i-STAT (Heska Corp., Ft. Collins, CO, USA), have allowed point of care analysis in settings where this was previously impossible. The i-STAT has been validated for use in humans, domestic animal species, and northern elephant seals (*Mirounga angustirostris*) in field and acute care settings.<sup>1-4</sup> The i-STAT is a hand-held, self-powered, portable unit that utilizes a small amount of heparinized, whole blood instead of using serum or plasma. Since clot formation and separation are not required, results are available in less than 5 min from venipuncture. However, the i-STAT's methodology must be validated and compared to reference methodologies in each species to be examined prior to use. The i-STAT uses a different methodology, potentiometry and measurement of ionic charge, to determine analyte values than do standardized laboratory analyzers. The differences in sample processing

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and methodology between the i-STAT and laboratory machines can result in clinically significant differences in reported analyte concentration and reference intervals. This study was conducted to validate the i-STAT machine for use in manatees.

## **Materials and Methods**

Monthly blood samples were collected voluntarily, without restraint, from two trained, captive manatees held at Mote Marine Laboratory from the lateral brachial vascular plexus into lithium heparinized blood tubes. Blood samples were placed on ice and transported to Sarasota Memorial Hospital. At the hospital, samples were analyzed at the same time using the i-STAT and a standard blood gas machine. Samples were also processed by a standard chemistry and complete blood cell count machine within 1-3 hr of blood collection. Additionally blood samples were collected from twelve wild manatees captured during health assessment and satellite tagging research projects conducted by Florida Fish and Wildlife Conservation Commission and the Sirenia Project during 2003 and 2004. Animals were caught in the water by circle net and processed on a boat or moved to land. Blood samples were taken at the beginning of the capture period (Pre) and at least 20 min or more after the initial sample was taken prior to release (Post). Blood samples were analyzed in the field using the i-STAT as soon as possible after sampling. The i-STAT cartridges used were the CG4+, EC8+ and Crea cartridges. The following parameters were tested per cartridge: CG4+ = lactate, total carbon dioxide content (TCO<sub>2</sub>), hydrogen ion concentration (pH), partial pressure of carbon dioxide (PCO<sub>2</sub>), partial pressure of oxygen (PO<sub>2</sub>), bicarbonate ion concentration (HCO<sub>3</sub>), base excess (BE), hemoglobin saturation with oxygen (SO<sub>2</sub>); EC8+ = glucose (Glu), blood urea nitrogen (BUN), sodium (Na), potassium (K), chloride (Cl), anion gap (AnGap), hematocrit (Hct), hemoglobin (Hb), pH, PCO<sub>2</sub>, HCO<sub>3</sub>, BE; Crea = creatinine (Crea). Means and standard deviations were calculated for all values measured. Means were compared between the i-STAT and standard blood gas, clinical chemistry and complete blood cell count machines at Sarasota Memorial Hospital for the captive manatees using one-way analysis of variance (ANOVA) with Bonferroni-adjusted post hoc comparisons. For wild manatees means from Pre and Post samples for each i-STAT cartridge were compared using a paired t-test. Additionally i-STAT variables were compared between captive animals and wild animals using ANOVA with Bonferroni-adjusted post hoc comparisons. If normality or equal variance was violated, variables were analyzed using Kruskal-Wallis tests. All statistical calculations were performed with the SPSS 11.0 software (SPSS Inc., Chicago, Illinois, USA). For all analyses, values of  $P \leq 0.05$  were considered significant.

## **Results**

Preliminary i-STAT reference ranges for certain blood parameters in captive manatees and wild manatees were determined (Table 1). For captive manatees, the mean time between the blood being sampled and being processed in the i-STAT machine was  $52.5 \pm 11.5$  min. For the wild manatees, mean processing time out of the water was  $66.08 \pm 11.7$  min. Mean time between Pre

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and Post samples being taken was  $34.42 \pm 12.5$  min. Mean time between the blood being sampled and being processed in the i-STAT machine was  $20.00 \pm 12.0$  min. For captive manatees the only significant difference in parameters measured by the i-STAT machine and the standard blood machines were found in Na, Cl, Hb and Crea (Table 2). For the wild manatees the only significant differences between Pre and Post samples were pH, Glucose, and Na (Table 3). Lastly, between the captive and wild manatees there were significant differences in Lactate, Ph, Glu, BUN, Na, and Cl. (Table 4)

## Discussion

The values generated by the i-Stat for  $\text{TCO}_2$ ,  $\text{HCO}_3$ , BE, and  $\text{SO}_2$  were calculated from human normograms based on temperature, pH, and hemoglobin affinity. These variables would be markedly different between humans and marine mammals. For this reason, these values were not analyzed in this study. Manatees were bled from a mixed arteriovenous plexus where shunting of blood may occur. Therefore, there may be a dramatic difference in  $\text{pO}_2$  and  $\text{pCO}_2$  simply because of the difference in arterial, venous or mixed blood values. We hope in the future to gather enough samples to separate arterial samples from venous samples based on  $\text{pO}_2$  to generate reference intervals for both arterial and venous samples. Currently however discrepancies in  $\text{pO}_2$  and  $\text{pCO}_2$  cannot be evaluated at this time due to low sample numbers.

There currently are not enough samples collected to generate an accurate reference interval and the one presented is only preliminary. The low animal numbers also preclude final determination of statistical relevance. However, some values are evidently different in the samples and are noteworthy. A significant decrease in sodium concentration and an increase in chloride concentration are present when measured by the i-STAT as opposed to the clinical chemistry machine. This is consistent with previous reports in pinnipeds and dogs using alternate standardized instruments.<sup>1,2</sup> This indicates that there is likely a significant difference in methods between the two machines and this difference should be considered when testing other marine mammals.

For wild animals there was a significant increase in glucose concentration when comparing initial values with release values in the same animal. In other species, this increase may be caused by endogenous glucocorticoid (cortisol/corticosterone) release which has not been well documented in manatees during capture operations. Additional significant differences for pH, Na, and Crea were noted between Pre and Post samples but these differences may not be biologically significant for the parameters tested. An increase in sample size will help determine if there are really significant differences in these parameters in the future.

Lastly, between the captive and wild animals there were several significant differences in certain blood values. In wild caught animals lactate and glucose were both increased. The increase in lactate is expected from an animal that is undergoing anaerobic glycolysis secondary to struggling during capture. The increased glucose may also be associated with capture stress. The decreased pH as compared to captive animals was striking in some individuals. This

apparent acidosis may be secondary to the sample being more venous in nature or it could be secondary to a possible dive reflex exhibited by captured manatees. Continued investigation into the mechanism behind this acidosis is ongoing. In conclusion the i-STAT appears to be a useful tool for clinically assessing manatees. However the i-STAT has some values that are significantly different from standard reference machines and i-STAT values should not be directly compared to standard blood gas or clinical chemistry machines but compared to i-STAT reference ranges established for each new species tested.

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**Table 1.** Preliminary i-STAT values for specific blood parameters in captive and wild manatees.

Captive (n = 6)	Mean	Stdev	Wild (n = 12)	Mean	Stdev
Lactate	0.73	0.15	Lactate	14.84	3.42
pH	7.37	0.02	pH	7.08	0.14
PCO <sub>2</sub>	86.73	4.76	PCO <sub>2</sub>	91.35	22.93
PO <sub>2</sub>	40.00	17.16	PO <sub>2</sub>	60.67	32.23
Glu	57.20	5.59	Glu	90.50	22.39
BUN	9.40	2.30	BUN	5.30	2.36
Na	140.0	2.92	Na	145.80	3.16
K	4.40	0.37	K	4.67	0.48
Cl	97.80	2.17	Cl	103.00	4.47
Hct	40.80	1.92	Hct	41.60	3.86
Hb	14.00	0.71	Hb	14.20	1.32
Captive (n = 4)			Wild (n = 5)		
Crea	2.25	0.13	Crea	2.24	0.54

**Table 2.** Preliminary comparison of i-Stat values with standardized reference methodologies in repeated blood samples from trained captive manatees.

i-STAT CG4+ (n = 6)			Blood Gas (n = 6)	Mean	Stdev	P-Values
	Mean	Stdev				
pH	7.37	0.02	pH	7.36	0.02	1.00
PCO <sub>2</sub>	86.73	4.76	PCO <sub>2</sub>	84.83	4.70	1.00
PO <sub>2</sub>	40.00	17.16	PO <sub>2</sub>	43.32	15.01	0.729
i-STAT EC8+ (n = 6)			Chemistry/CBC (n = 6)			
Glu	57.20	5.59	Glu	61.00	6.20	0.284
BUN	9.40	2.30	BUN	10.50	1.64	0.379
Na	140.0	2.92	Na	145.83	2.48	0.008
K	4.40	0.37	K	4.57	0.33	0.450
Cl	97.80	2.17	Cl	93.33	1.97	0.006
Hct	40.80	1.92	Hct (CBC)	39.22	2.74	0.998
Hb	14.00	0.71	Hb (CBC)	12.30	0.77	0.012
i-STATCrea (n = 4)			Chemistry (n = 4)			
Crea	2.25	0.13	Crea	1.67	0.23	0.002

**Table 3.** Preliminary comparison of i-Stat values collected at the beginning of capture (Pre) and just prior to release (Post) for wild manatees.

Pre 4+ (n = 12)	Mean	Stdev	Post 4+ (n = 12)	Mean	Stdev	P-value
Lactate	14.84	3.59	Lactate	14.11	4.73	0.595
pH	7.08	0.14	pH	7.12	0.16	0.031
PCO <sub>2</sub>	91.35	22.93	PCO <sub>2</sub>	76.7	18.17	0.068
PO <sub>2</sub>	60.67	32.23	PO <sub>2</sub>	93.08	36.11	0.078
Pre 8+ (n = 12)			Post 8+ (n = 12)			
Glu	90.50	22.39	Glu	115.83	23.87	0.0001
BUN	5.30	2.36	BUN	5.30	2.36	1.0
Na	145.80	3.16	Na	143.30	3.83	0.023
K	4.67	0.48	K	4.82	0.77	0.552
Cl	103.00	4.47	Cl	102.7	3.27	0.671
Hct	41.60	3.86	Hct	42.4	5.19	0.210
Hb	14.20	1.32	Hb	14.50	1.84	0.279
Pre Crea (n = 5)			Post Crea (n = 5)			
Crea	2.24	0.54	Crea	1.84	0.45	0.016

**Table 4.** Mean differences between blood values measured using the i-STAT in trained captive vs. wild captured manatees.

Captive 4+(n = 6)	Wild 4+ (n = 12)		Mean	Stdev	P-Value	
	Mean	Stdev				
Lactate	0.73	0.15	Lactate	14.84	3.42	0.001
pH	7.37	0.02	pH	7.08	0.14	0.001
PCO <sub>2</sub>	86.73	4.76	PCO <sub>2</sub>	91.35	22.93	0.638
PO <sub>2</sub>	40.00	17.16	PO <sub>2</sub>	60.67	32.23	0.165
Wild 8+ (n = 12)						
Captive 8+(n = 6)						
Glu	57.20	5.59	Glu	90.50	22.39	0.006
BUN	9.40	2.30	BUN	5.30	2.36	0.007
Na	140.0	2.92	Na	145.80	3.16	0.004
K	4.40	0.37	K	4.67	0.48	0.292
Cl	97.80	2.17	Cl	103.00	4.47	0.030
Hct	40.80	1.92	Hct	41.60	3.86	0.673
Hb	14.00	0.71	Hb	14.20	1.32	0.759
Wild Crea (n = 5)						
CaptiveCrea (n = 4)						
Crea	2.25	0.13	Crea	2.24	0.54	0.973

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## HELMINTH ASSEMBLAGES OF ROSS' AND WHITE-FRONTED GEESE WINTERING IN SOUTH TEXAS

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### Abstract

Helminth community structure and pattern were assessed in 16 Ross' (*Chen rossii*) and 46 white-fronted geese (*Anser albifrons*) collected during winter 1999-2000 in Kleberg County, Texas. Infracommunities in Ross' geese ranged from 2-6 species and 3-71 individuals, and averaged  $3.6 \pm 0.3$  (SE) species and  $42.6 \pm 7.6$  individuals. Ten species were found in the Ross' goose component community, in which *Amidostomum anseris*, *Epomidiostomum crami*, *Heterakis dispar*, and *Trichostrongylus tenuis* were the most prevalent and dominated numerically. *Amidostomum anseris*, *E. crami*, *H. dispar*, *T. tenuis*, *Tetrameres* sp., and *Capillaria* sp. found in the Ross' goose represent new host records. Infracommunities found in white-fronted geese ranged from 1-7 species and 4-117 individuals, and averaged  $4.2 \pm 0.2$  species and  $28.9 \pm 4.0$  individuals. Seventeen species were found in the white-fronted goose component community. *Dendritobilharzia pulverulenta*, *Paramonostomum* sp., and *Capillaria* sp. represent new host records in the white-fronted goose. *Epomidiostomum crami*, *Amidostomum spatulatum*, and *T. tenuis* were the most prevalent and dominated numerically. Rank abundance of *A. spatulatum* and *T. tenuis* varied by host age; *E. crami* and *T. tenuis* varied by host sex. Component communities between juvenile and adult white-fronted geese were most similar, followed by male and female white-fronted geese, and juvenile Ross' and white-fronted geese. Relatively low species richness, preponderance and numeric dominance by direct life cycle nematodes, and absence of helminths in a number of available microhabitats suggested that the mainly herbivorous diet of Ross' and white-fronted geese dramatically influenced helminth community structure and pattern on the wintering grounds.

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## HEALTH ASSESSMENT OF THE IN SITU POPULATION OF GUAM RAIL (*Rallus owstoni*)

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### Abstract

An investigation into the health of the *in situ* population of Guam rail was initiated to determine the prevalence of disease issues that could impact the *in situ* captive and release populations as well as zoological populations. This type of investigation had not been conducted on the *in situ* population since the repatriation program started in the 1990s. This investigation included a review of pathology records, analysis of the *in situ* captive diet, and clinical examination of two release populations (n = 100) as well as the captive breeding population (n = 50). In addition, clinical sampling of local domestic chicken (*Gallus gallus*) populations (n = 50) was performed to determine prevalence of diseases of concern to the rail population. Free-ranging chickens are common on the islands of Guam and Rota where rails are being released.

Mortality records for the *in situ* captive breeding population from 1994- 2003 were reviewed to determine prevalence of disease in the historic population. The *in situ* captive population diet was collected frozen for nutritional analysis. Diagnostic testing for the rails included complete blood count (CBC), plasma chemistry analysis, plasma protein electrophoresis, *Mycobacterium avium* complex (MAC) serology, arboviral serology (Eastern equine encephalitis, St. Louis encephalitis, Japanese encephalitis, and West Nile virus), enteric pathogen culture, fecal MAC antigen detection via polymerase chain reaction (PCR), and fecal acid- fast cultures. Diagnostic testing for the domestic chickens included serum arboviral serology (Eastern equine encephalitis, St. Louis encephalitis, Japanese encephalitis, and West Nile virus), enteric pathogen culture, and fecal acid- fast cultures. Findings from the health assessment were reviewed to identify: significant pathologic findings; significant disease noted on physical examination, CBC, plasma chemistries, and plasma protein electrophoreses; prevalence of arboviral antibody; prevalence of fecal acid- fast bacteria; and, prevalence of enteric pathogens such as *Salmonella* and *Campylobacter*.

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## **PATHOLOGIC REVIEW OF THE CHIMPANZEE (*Pan troglodytes*): 1990-2003**

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### **Abstract**

During 1990-2003, 94 chimpanzees (*Pan troglodytes*) died in zoos that are currently accredited by the American Zoo and Aquarium Association (AZA). Not all institutions were AZA members during the time period evaluated. However, all deaths from each institution were included to provide a more robust database.

In 2003, complete gross and histopathology reports were requested from the holding institutions by the Chimpanzee Species Survival Plan (SSP)<sup>®</sup> Veterinary Advisor. Limited medical history was requested for individuals where illness was directly associated with death. All 94 records were retrieved for a 100% survey return rate; however, the depth of reporting varied across the database (Table 1).

### **Methods**

Each report was reviewed for signalment (specimen gender and age at death), medical history at death, primary cause of death, and secondary pathologic findings. Primary causes of death and secondary pathologic findings were categorized by body system (cardiopulmonary, endocrine, gastrointestinal, integument, lymphatic, musculoskeletal, nervous, reproductive, urinary) and representative pathologic process (degenerative, anomalous, metabolic, neoplastic, infectious/inflammatory, toxic, traumatic).

Of the 94 animals, 22 (7.9, 23%) were neonates ( $\leq 1$  day of age), of which 10 (6.2, 45%) were stillborn, 17 (8.9, 18%) were infants (1 day to 1 yr of age), 20 (10.1, 21%) were juveniles (1-10 yr of age), 8 (5.3, 9%) were young adults (10-20 yr of age), 16 (4.1, 17%) were mature adults (20-35 yr of age), and 11 (3.8, 12%) were elderly adults ( $> 35$  yr of age). The primary cause of death is presented by age categories and representative pathologic process. Specific primary etiologies are provided separately (Table 2). Secondary or incidental findings were too numerous to be effectively tabulated. However, certain pathologic trends were observed.

### **Results and Discussion**

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Cardiovascular disease was identified as an important cause of disease in chimpanzees, particularly for adults (27 of 35 animals, 77%) (Table 3). This is also a disease concern reported in captive gorillas (*Gorilla gorilla*).<sup>3</sup> Intra-abdominal abscessation, recently reported as a serious disease process in gorilla, was not a notable finding in chimpanzee mortalities.<sup>2</sup> Parasitism was identified frequently ante-mortem, with *Balantidium coli* identified as an endemic parasite of minimal pathogenicity, except at times of stress, from a survey of 35% of AZA-accredited institutions (n = 40) currently housing chimpanzees.<sup>1</sup> *B. coli* was similarly the most often observed parasite post-mortem. Parasitism itself was secondarily reported in only 16% of all chimpanzee mortalities (Table 4). Identified at nearly the same frequency of affected individuals, neoplasia was identified in 15% of the chimpanzees (Table 5). This pathology was exclusively identified in animals greater than 10 yr with a mean of 32.7 yr of age. Exhibit-related mortalities included drowning (n = 7), post-escape recovery efforts (n = 3), and entrapment in an exhibit prop (n = 1), or 12% of the total deaths, with an average age of 15.2 yr of age (range = 15 mo to 37 yr). As the predominant cause in this category was drowning, the current Chimpanzee SSP<sup>®</sup> recommendation against water moats as primary containment was supported.

The summary of this information will contribute to the development of differential diagnoses for presented clinical cases. Adjustments to medical management can be made from the determination of the more common causes of death. Assessment of necropsies from the captive population will provide a comparative database for those performed in wild populations. To extend the utility of this evaluation, continued updates will be generated for the Chimpanzee SSP<sup>®</sup> annual report using these parameters for consistency.

#### ACKNOWLEDGMENTS

The authors appreciate the cooperation of the following institutions' veterinary staffs in providing the records for this review: North Carolina Zoological Park (Asheboro, NC), Gladys Porter Zoo (Brownsville, TX), Busch Gardens (Tampa, FL), Lincoln Park Zoo (Chicago, IL), Cleveland Metroparks Zoo (Cleveland, OH), Cheyenne Mountain Park Zoo (Colorado Springs, CO), Dallas Zoo (Dallas, TX), Detroit Zoo (Detroit, MI), Sequoia Park (Eureka, CA), Fort Worth Zoological Park (Fort Worth, TX), Chaffee Zoo (Fresno, CA), Hogle Zoo (Salt Lake City, UT), Jackson Zoological Park (Jackson, MS), Kansas City Zoological Park (Kansas City, MO), Knoxville Zoo (Knoxville, TN), Little Rock Zoo (Little Rock, AR), Los Angeles (Los Angeles, CA), Henry Vilas Zoo (Madison, WI), Sunset Zoo (Manhattan, KS), Montgomery Zoo (Montgomery, AL), Oakland Zoo (Oakland, CA), Metroparks Zoo (Portland, OR), Sacramento Zoo (Sacramento, CA), Riverside Zoo (Scottsbluff, NE), Sedgwick County Zoo (Wichita, KS), Saint Louis Zoo (St. Louis, MO), Toledo Zoological Gardens (Toledo, OH), Tulsa Zoo and Living Museum (Tulsa, OK), and Lion Country Safari (Loxahatchee, FL).

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**Table 1.** Pathology reporting for chimpanzee mortalities (1990-2003).

Full report	40
Gross only	12
Histopathology only	2
Medical records only	35
Death report only	5
Total	94

**Table 2.** Primary cause of death by age class for chimpanzee mortalities (1990-2003).

Cause of death	≤1 day	<1 day > 1yr	1-10 yr	10-35 yr	35 yr<
Conspecific trauma	4	3	3	0	0
Trauma	2	1	1	1	0
Premature birth	3	0	0	0	0
Malpositioning	1	0	0	0	0
Failure to thrive	0	1	0	0	0
Spina bifida	0	1	0	0	0
Cerebral amoebiasis	0	0	1	0	0
Non-infectious CNS disease	0	1	1	0	0
Diabetes insipidus	0	0	1	0	0
Clostridial enteritis	0	0	1	0	0
Internal abdominal abscess	0	0	0	2	0
Glossal actinomycosis	0	0	0	1	0
Systemic amyloidosis	0	0	0	1	0
Systemic coccidioides	0	0	0	0	1
Disseminated granulomatosis	0	0	0	0	1
Chronic renal disease	0	0	1	3	0
Acute renal failure	0	0	0	0	1
Non-infectious cardiac disease	0	0	0	5	2
Aneurysm	0	0	0	0	2
Respiratory failure	0	0	0	1	0
Encephalomyocarditis	0	0	1	0	0
Aspiration pneumonia	0	0	1	1	0
Infectious pneumonia	0	4	0	0	1
Metabolic bone disease	0	0	1	0	0
Hepatocellular carcinoma	0	0	0	2	1
Autolysis	1	2	0	0	0
Exhibit-related <sup>a</sup>	0	0	4	6	1
Unknown	11	4	4	1	1
Totals	22	17	20	24	11

**Table 3.** Types of cardiovascular disease by age for chimpanzee mortalities (1990-2003).

Cardiovascular disease	<1 day > 1yr	1-10 yr	10-35 yr	35 yr <
Congestive heart failure	0	0	1 <sup>a</sup>	1 <sup>a</sup>
Endocardiosis	0	0	3	3
Myocardial fibrosis	0	1	9 <sup>a</sup>	4 <sup>a</sup>
Atherosclerosis	0	0	1	1
Arteriosclerosis	0	0	1	1
Viral myocarditis	1	1 <sup>a</sup>	1	0
Aortic aneurysm	0	0	0	1 <sup>a</sup>

<sup>a</sup>Primary lesions in cardiovascular disease was found in congestive heart failure (10-35 yr, n = 1, 35yr<, n = 1), aneurysm (35yr<, n = 1), myocardial fibrosis (10-35yr, n = 4, 35 yr<, n = 1), and viral myocarditis (1-10 yr, n = 1).

**Table 4.** Internal parasites identified at pathologic review of chimpanzees (1990-2003).

Parasite	Location	Incidences
<i>Balantidium coli</i>	Small/large intestine	6
<i>Enterobius</i> sp.	Large intestine	4
<i>Trichuris</i> sp.	Intestine	1
<i>Cryptosporidia</i> sp.	Large intestine	2
Nematode,unidentified	Small/large intestine	3
Ciliate,unidentified	Intestine	1
Amoeba,unidentified <sup>a</sup>	Brain	1

<sup>a</sup>This parasite was primary cause of animal's death, rather than an incidental finding.

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**Table 5.** Types of neoplasia identified for chimpanzee mortalities (1990-2003).

Neoplasia	Organ	Metastasis <sup>a</sup>	Age	1° vs 2° <sup>b</sup>
Carcinoma	Liver	N	10 yr	1°
Carcinoma	Liver	N	31 yr	2°
Carcinoma	Unidentified	Y	39 yr	1°
Carcinoma	Unidentified	Y	43 yr	2°
Leiomyoma	Uterus	N	35yr	2°
Leiomyoma	Uterus	N	47 yr	2°
Leiomyoma	Uterus	N	43 yr	2°
Adenoma	Liver	N	13 yr	2°
Adenoma	Intestinal tract	N	37 yr	2°
Fibroma	Uterus	N	28 yr	2°
Fibroma	Uterus	N	36 yr	2°
Lipoma	Fat	N	23 yr	2°
Lipoma	Fat	N	36 yr	2°
Adenoma	Cervix	N	37 yr	2°

<sup>a</sup>N (metastasis not observed), Y (metastasis observed).

<sup>b</sup>1° (primary cause of death), 2° (secondary/incidental finding).

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## ECOLOGICAL SIGNIFICANCE OF GIZZARD NEMATODE SEX RATIOS IN WATERFOWL

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### Abstract

Current hypotheses concerning nematode reproduction strategies focus on the component population level (individuals in all hosts collectively) and predict that sex ratios should be female biased at low prevalence and mean intensity and approach 1:1 as prevalence and mean intensity increase. We observed sex ratios of nematodes at both the component and the infrapopulation level (within each host individual), using 46 white-fronted geese (WFG) and 16 Ross' geese (RG) collected in 1999-2000 and 60 blue-winged teal (BWT) collected in 2002. Nematodes were removed from gizzard linings and identified, and the sex of each worm was recorded.

Three species were found in the WFG: *Epomidiostomum crami* (prevalence 96.7%, mean intensity 14.0, sex ratio 1 female: 1 male), *Amidostomum spatulatum* (75.0%, 6.5, 1.2:1), and *A. anseris* (31.7%, 2.5, 1.3:1); two species were found in Ross' Geese: *E. crami* (87.5%, 13.6, 1.2:1), and *A. anseris* (68.8%, 4.1, 1.3:1). Blue-winged teal also contained three species: *A. acutum* (96.7%, 7.5, 1.1:1), *Streptocara crassicauda* (56.7%, 2.9, 2.9:1), and *E. uncinatum* (21.7%, 2.5, 1.9:1). At the infrapopulation level, sex ratios from WFG were *E. crami* (18% equal, 43% male biased, 32% female biased, and 7% single sex infections), *A. spatulatum* (6%, 43%, 43%, 24%), and *A. anseris* (30%, 0%, 0%, 70%); sex ratios from RG were *E. crami* (8%, 38%, 38%, 16%) and *A. anseris* (10%, 20%, 20%, 50%). Within BWT sex ratios were *A. acutum* (11%, 37%, 41%, 11%), *S. crassicauda* (15%, 0%, 18%, 67%), and *E. uncinatum* (8%, 0%, 23%, 69%). The trend of sex ratios within the component population to approach 1:1 as prevalence and mean intensity increased concur with predictions of models finding less female biased sex ratios with increased prevalence and intensity of infection. At the infrapopulation level, the high incidence of single sex infections at low prevalence suggests that propagation of those component populations is achieved by fewer hosts than shown by component level analyses and stresses the importance of infrapopulation dynamics in community based evaluations.

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**EFFECTS OF TRANSLOCATION ON THE BLOOD CHEMISTRY, HEMATOLOGY, AND ENDOCRINOLOGY OF CRITICALLY ENDANGERED TURKS AND CAICOS IGUANAS, (*Cyclura carinata*)**

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**Abstract**

In January 2002, 158 adult Turks and Caicos iguanas were translocated from islands where they are threatened to islands without existing iguana populations. To study the stress of translocation on the iguanas, we measured the body mass and baseline blood chemistry, hematology, and corticosterone of iguanas at the time of translocation and then re-sampled translocated (experimental) and source (control) populations at one, five, and 12 mo post-translocation. Most of the animals that were moved decreased in mass 1 mo after translocation but subsequently rebounded and exhibited increased growth rates at five and 12 mo post-translocation. None of the eighteen blood chemistry parameters monitored exhibited a significant change as a result of translocation at 1, 5, or 12 mo. For standard CBCs, the number of azurophils seen increased significantly after translocation and remained elevated throughout the study period, whereas all other measures did not change significantly. Corticosterone levels of experimental animals were highest 1 mo after translocation and subsequently decreased but remained significantly higher than controls throughout the study period. Despite this, successful reproduction occurred on all translocation islands during the study period. Our studies indicate that translocation can cause measurable and protracted stress on Turks and Caicos iguanas but also suggest that this stress does not significantly impede growth or reproduction.

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## IDENTIFICATION OF A GAMMA HERPESVIRAL INFECTION IN NORTHERN ELEPHANT SEALS (*Mirounga angustirostris*)

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### Abstract

Northern elephant seals (*Mirounga angustirostris*) can be found on the offshore islands and along the coast of central California during their breeding and moulting seasons.<sup>1</sup> These phocids were hunted almost to extinction by the early 20<sup>th</sup> century, and the population has since rebounded and now includes approximately 150,000 individuals. The population may be increasing at a rate of 6% annually.<sup>2</sup>

The Marine Mammal Center, a rehabilitation facility in central California, admits approximately 100 elephant seals pups each year for treatment. Ten weaned pups died between 1998 and 2002 with ulcerative lesions on the tongue or palantine mucosa and inflamed tonsils. The causes of death identified included verminous pneumonia and arteritis due to *Otostrongylus circumlitos* infection, as well as endotoxemia secondary to acute enterocolitis. Histologic examination of the oral ulcerative lesions revealed eosinophilic to amphophilic intranuclear inclusions bodies suggestive of a herpesviral infection. Electron microscopic examination supported the presence of a herpesviral infection characterized by nonenveloped intranuclear 90-110 nm diameter icosahedral nucleocapsids that occasionally contained central dense core, and enveloped extracellular virions were detected. Polymerase chain reaction, using previously published degenerate primers on samples collected from three of these animals, detected the presence of herpesviral DNA in these tissues.<sup>3</sup> An elephant seal herpes specific primer pair was then developed to further analyze samples collected from elephant seals. This molecular analysis showed that the new herpesviral isolate was most similar to three viruses: an unidentified herpesvirus from a black rhinoceros, *Chlorocebus rhadinovirus* 1 from African green monkeys, and *Alcephaline herpesvirus* 1 (malignant catarrhal fever virus from wildebeeste). This places the elephant seal herpesvirus in the gamma herpesvirus subgroup.

Identical herpesviral DNA was also detected in blood and mucosal swabs collected from five healthy pups that were released. These data suggest that this gamma herpesvirus can be found in

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the secretions and tissues from healthy elephant seals as well as in ulcerative lesions. Therefore, further work needs to be done to understand the epidemiology and pathogenicity of this infection in northern elephant seals.

#### **ACKNOWLEDGMENTS**

We would like to thank the staff, volunteers and interns from The Marine Mammal Center for help collecting samples, especially Denise Greig, Marty Haulena and Christy McKnight. We are also grateful to the residents and pathologists at UC Davis and AFIP for assisting in the histopathologic examination of tissues, especially Katie Colegrove. Thanks to Brian Aldridge and Ken Jackson for help with the phylogenetic analysis. Samples were collected under the authority of Marine Mammal Protection Act permit number 932-1489-00

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## MOLECULAR IDENTIFICATION OF A NOVEL GAMMA HERPESVIRUS IN HAWAIIAN MONK SEALS (*Monachus schauinsland*)

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### Abstract

The Hawaiian monk seal (*Monachus schauinslandi*) was listed as an endangered species in 1976. The population declined 60% from 1958 to 1992 and remained relatively stable from 1993 to 2000, but numbers began to decline again in 2001.<sup>2</sup> The total population has been estimated at about 1300 seals. Known reasons for monk seal mortality and the lack of population growth over the last 2 decades include entanglement in marine debris, male aggression, shark predation, human disturbance, and malnutrition. Epidemiologic surveys since 1998 have not demonstrated disease to be a significant impediment to population recovery, although studies have implicated traumatic injuries and infectious diseases, such as parasitism and bacterial infection, as contributors to individual animal deaths.<sup>1</sup> While limited data are available relative to the role of viral diseases in compromising monk seal health, the possible association of a morbillivirus infection with a mass mortality event in Mediterranean monk seals (*Monachus monachus*) in 1997 demonstrates the potential impact that a viral infection might have on an endangered population of animals.<sup>3,4</sup>

Serologic evidence for the existence of a herpesvirus infection in Hawaiian monk seals has recently been detected utilizing an ELISA assay developed to measure antibodies to phocine herpesvirus-1 (PhHV-1) in harbor seals (*Phoca vitulina*). Although the monk seal sera were positive for anti-herpesviral antibodies, these antibodies were unable to neutralize the PhHV-1 isolate in serum neutralization tests.

The goal of this study was to identify and characterize this putative monk seal herpesvirus in nasal swab samples collected from free-ranging and captive Hawaiian monk seals. Previously published degenerate primers were used to initially identify the novel sequence.<sup>5</sup> All samples

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were then analyzed with a monk seal herpes specific primer pair. This analysis identified a previously unknown gamma herpesviral molecular isolate in 20% (19/95) of the animals. The isolate was similar to gorilla rhadinovirus and porcine lymphotropic virus. This newly identified monk seal virus has not yet been associated with disease.

#### **ACKNOWLEDGMENTS**

This work was supported by grants from the Marine Mammal Health and Stranding Program, Office of Protected Resources, National Marine Fisheries Service as well as The Marine Mammal Center and the UC Davis Wildlife Health Center. The authors wish to thank the many dedicated researchers from the Marine Mammal Research Program, Honolulu Laboratory, National Marine Fisheries Service for their technical support and help with collection of field samples and the U.S. Fish and Wildlife Service and the State of Hawaii Department of Land Natural Resources for their logistical support. Thanks also to Sea World of Texas for providing samples from the captive seals. We would also like to thank Jerry Saliki for providing Phocine herpesvirus-1 and 2 isolates from Atlantic harbor seals and Don King for providing the Otarine herpesvirus-1 isolate from a California sea lion for comparative purposes. Thanks to Ken Jackson for help with the phylogenetic analysis. Samples were collected under the authority of Marine Mammal Protection Act permit number 848-1135.

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## WEST NILE VIRUS IN MIGRATORY DUCKS

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### Abstract

West Nile virus (WNV) first entered the United States in 1999. In 2001, WNV found its way south to Louisiana. As part of a study to understand the epidemiology of WNV in Louisiana, heads from ten species of hunter killed ducks in the 2002 and 2003 hunting season from Lacassine National Wildlife Refuge in southeastern Louisiana were submitted to the Louisiana State University School of Veterinary Medicine. Migratory ducks were selected for the study to understand the epidemiology of WNV in Louisiana because migratory birds are believed to have been responsible for introducing WNV in parts of the Middle East and are speculated to have a role in WNV transmission in the United States.<sup>1,2</sup> From the 2002 collection, 190 ducks were submitted for testing while 261 duck heads were submitted for testing in 2003. Duck heads were identified by sex and species prior to having brain tissue removed and sectioned into two pieces for testing. One section of brain was submitted for testing for WNV via nested RT-PCR while the other section was frozen at -70°C for virus isolation.

RNA was extracted from brain tissue submitted for WNV testing by nested RT-PCR using the Trizol extraction method. After the RNA was extracted, nested RT-PCR was performed on 187 of the 190 samples submitted in 2002 using the Qiagen 1-step RT-PCR. Eleven of the 187 samples tested were WNV positive by nested RT-PCR. The eleven samples were then submitted for virus isolation. No virus was isolated on culture or subculture for the 11 WNV nested RT-PCR positive samples. Duck brains from the 2003 hunting season are currently being processed. It is hoped that these results will provide information on the role migrating ducks may play in the spread and transmission of WNV.

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## SERUM CORTISOL IN CAPTIVE AFRICAN LIONS (*Panthera leo*) DURING CHEMICAL RESTRAINING AND ELECTROEJACULATION

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### Abstract

The serum level of cortisol is been associated with different degrees of stress in a number of wild animals species in the last decades. The physical and chemical restraining are well known as stressful procedures although they are necessary in many situations like electroejaculation for reproductive research purposes. The aim of this study was to determine the variations in the serum cortisol levels in African lions, comparing both sexes during the chemical restraining, and just for the males, before and after electroejaculation. We studied 33 adult African lions, been 14 females and 19 males, belonging to the Fundação Parque Zoológico de São Paulo. The animals were restrained by the use of the association of xylazine (Rompun®, Bayer do Brasil, São Paulo, Brazil; 2 mg/kg i.m.) and ketamine (Vetaset®, Fort Dodge, São Paulo, Brazil; 10 mg/kg i.m.), delivered by dart gun. Three blood samples were serially collected every 20 min as soon as the animal was accesible. To asses the serum cortisol before and after electroejaculation, we performed the procedure in 15 adult male lions. After chemical restraining, according the same protocol already described, two blood samples were collected, before and after the electroejaculation. The serum obtained in the two experiments were frozen and stored at - 20°C. The dosages were performed by the use of radioimmunoassay (RIA) with comercial kits for cortisol (Cortisol DPC MEDLAB®, Los Angeles, California,USA). The inter and intra assay coeficients of variation were lower than 10%. The results were statistically analised by the Student *t* test (95%). The mean concentrations and standard deviations for serum cortisol during restraining were  $12.02 \pm 6.05$  µg/dl and  $9.95 \pm 4.73$  µg/dl, for males and females, respectively ( $P > 0.05$ ). Regarding the electroejaculated group the results obtained before and after the procedure were  $12.13$  µg/dl  $\pm$  7.15 and  $10.80$  µg/dl  $\pm$  6.25 showing no significant variation ( $P > 0.05$ ). In conclusion, based on the serum cortisol levels, there were no difference between sexes regarding the intensity of the stress response during the chemical restraining procedure. Similarly, there were no significant changings in the degree of stress before and after electroejaculation as well.

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## ASSESSMENT OF AFLATOXIN TOXICITY IN GRANIVOROUS AVIAN SPECIES

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### Abstract

Aflatoxin is a widely occurring and dangerous mycotoxin, which can potentially affect wildlife that consumes contaminated grain.<sup>2,3,5</sup> Unfortunately, grain that has been condemned for human and domestic animal consumption typically gets marketed as supplemental feed for wildlife.<sup>1</sup> Avian species are exposed to aflatoxins from contaminated grain supplied at deer feeders and at backyard feeders.<sup>1-2</sup> Because of the potential deleterious effects of aflatoxin (i.e., carcinogen, mutagen, teratogen),<sup>4</sup> a limit of 50 parts per billion (ppb) of aflatoxin arbitrarily has been set for wildlife feed (?) in Texas. Our objective was to determine the level of aflatoxin that negatively affects normal physiologic responses and induces acute morbidity and mortality in northern bobwhite (*Colinus virginianus*) and northern cardinals (*Cardinalis cardinalis*). Wild-caught, adult bobwhites (n = 100) and cardinals (n = 100) from southern Texas were maintained at the Texas A&M University-Kingsville aviary and were randomly assigned to a treatment group (2.5% fat diet = Trial 1 and 5.0% fat diet = Trial 2). Bobwhites were given 0, 100, 500, 1,000, and 2,000 ppb aflatoxin (Trials 1 and 2); cardinals given 0, 100, 500, 1,000, and 2,000 ppb aflatoxin (Trial 1) or 0, 25, 50, and 75 ppb aflatoxin (Trial 2). Weekly bird weight and daily feed consumption were determined throughout each 28-day experiment. Blood plasma chemistries were determined at the onset and end of each 28-day experiment for bobwhites and only at the end of the experiments involving cardinals. Aflatoxin, derived from *Parasiticus flavus*, was orally administered once per week for 4 wk. Control birds (0 ppb of aflatoxin) received an equivalent amount of aflatoxin solvent (Dimethyl sulfoxide). A white blood cell proliferation test was conducted postmortem using spleen tissue to determine the effect that aflatoxin had on the function of the immune system.

Mortality due to aflatoxin was <20% in bobwhites and <20% in cardinals that received 100 ppb aflatoxin but >47% in cardinals that received >100 ppb aflatoxin. Aflatoxin did affect plasma parameters associated with liver, kidney, and immune system function. Beta globulins and creatinine decreased while gamma glutamyltransferase and uric acid increased in birds given aflatoxin. However, a dose-dependent effect with aflatoxin concentration was not evident in blood plasma parameters. Bird mortality during the study may have confounded this effect. White blood cell proliferation was greatly suppressed at aflatoxin concentrations as low as 50 ppb. Short-term, acute doses of aflatoxin are deleterious to the health of bobwhites and cardinals, and it potentially can cause death in immune-challenged birds.

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## ACKNOWLEDGMENTS

Support was provided by the Ben and Rachel Vaughan Foundation, Tim Hixon, and the Caesar Kleberg Wildlife Research Institute.

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## CONGENITAL VESTIBULAR DISEASE IN TWO RELATED LITTERS OF SUMATRAN TIGERS (*Panthera tigris sumatrae*)

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### Abstract

The Sumatran Tiger (*Panthera tigris sumatrae*) is critically endangered due to habitat loss and poaching. The captive population of Sumatran tigers is subject to a carefully managed breeding strategy as part of a comprehensive conservation program. We report on two related litters of Sumatran tiger cubs with a congenital vestibular disorder characterized by ataxia, head tilt and rolling. Diagnostic elimination tests suggest damage due to in utero viral infection or hereditary factors. Given the close relationship between the sires of each affected litter and the high level of inbreeding in the Australasian population of Sumatran tigers, a genetic cause is likely. In order to define this disorder further, retrospective pedigree mapping and ongoing monitoring of all new cubs is needed.

### Case Reports

A litter of three Sumatran tiger cubs (two males, one female) was born at Perth Zoo, Western Australia, as the result of the first breeding event of sire and dam. Early video footage of the cubs was of variable quality, however keepers frequently commented on the “clumsiness” of the cubs. The cubs were first examined closely at the time of their primary vaccinations at 6 wk of age. Cub 1, (the female) had noticeable ataxia, head tilt to the left and ventral strabismus of the left eye. Abnormal nystagmus was not evident, menace response was poor and visual response was considered reduced. Cub 2 appeared normal. Cub 3 had a mild head tilt to the right, but no other signs. Conscious radiographs of the heads of cubs 1 and 2 did not reveal any abnormalities.

A veterinary neurologist subsequently examined all three cubs. Cub 1 remained ataxic but had no discernable head tilt or strabismus. Cub 2 showed no abnormal signs. Cub 3 now showed marked ataxia and a dramatic right- sided head tilt. All cubs’ postural reactions and visual responses were considered normal. CBC and serum chemistry were run on all cubs. All three cubs had low PCV’s (0.26, 0.21 and 0.22 L/L, reference levels 0.371 L/L, SD 0.059),<sup>4</sup> low RBC (4.4, 4.7 10<sup>12</sup>/L, reference levels 6.4 L/L, SD 1.13)<sup>4</sup> and low Hb (83, 71, 74 g/L, reference levels 124g/L, SD 22)<sup>4</sup>. Serum biochemistry was unremarkable and *Toxoplasma* titers on cubs 1 and 2 were negative. *Toxoplasma* titers were not done on cub 3.

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Over the next few months the degree of ataxia and head tilt evident in cubs 1 and 3 varied from day to day. Ataxia and head tilting were more obvious when the cubs were lifted up and then put down. Cub 1 showed no evidence of vestibular disturbance by the age of 8 mo as assessed by observation. Ataxia and head tilt of cub 3 had resolved by 5.5 mo of age. Cub 2 remained unaffected. Investigation into the parents' medical history revealed that the sire of this litter had suffered from similar clinical signs, which resolved by 5 mo of age. In his case, a diagnosis of vestibular disease due to inflammation was reached based on a high percentage of macrophages in cerebrospinal fluid (CSF), though blood contamination was recorded.

A second litter of cubs (two males and one female) was born 3 yr later at Taronga Zoo, Sydney, Australia, to different parents, though this was also their first pairing. The grand-dam on the father's side was the litter-mate of Perth Zoo's sire, though she showed no abnormal signs. Twenty-four-hour closed circuit television monitoring was available from the den and nestbox areas. Within one day of an uneventful birth, all three cubs were showing unusual behavior. Each cub held its head twisted and flexed to one side. Deliberate progressive forward movement was uncoordinated, often resulting in repeated rolling to the side. The cubs' righting response was poor and they would often end up on their backs with their forelegs extended and paddling, and their heads twisted over their back. The cubs were otherwise strong, of normal size, and once attached to the teat they suckled well. Maternal care was excellent and the cubs' growth rates were normal. Despite the adult diet ration having adequate thiamine, extra daily supplementation of the dam's meat ration with 312 mg thiamine (Vitajek Vitamin B<sub>1</sub>, Jurox Pty. Ltd., Rutherford, NSW, Australia) was instituted for a period of 3 wk. The cubs were examined briefly at 3 wk of age. All still showed uncontrolled rolling and head tilting. Two cubs had their eyes open, and had a mild bilateral ventral strabismus.

At 6 wk of age, each cub was given a detailed examination by a veterinary neurologist. All cubs showed symmetric ataxia in all four limbs and would fall to either side and roll continuously. They had variable degrees of head tilt, and showed wide side-to-side head movements as they moved forward. If held and lowered to the ground, they circled tightly. Pupillary responses were normal, menace responses poor and there was no abnormal strabismus or nystagmus. No other abnormalities were seen on cranial nerve examination, postural reactions were difficult to assess and spinal reflexes were not tested. Any vision and hearing deficits could not be determined, though subjective clinical assessment suggested the cubs could see and hear. CBC showed all cubs to have low PCV (0.24, 0.21, 0.28 L/L) compared to reference values.<sup>4</sup> Serum chemistry was within normal limits. FeLV, FIV and paired *Toxoplasma* IgG titers were negative for two cubs. The third was not tested.

At 4 mo of age, the cub with the most marked vestibular signs was anesthetized for a detailed physical examination including ophthalmoscopic and otoscopic examinations. Findings were unremarkable. Magnetic Resonance Imaging (MRI) of the head did not reveal any abnormalities in the brain or middle and internal ear structures. CSF was normal and no organisms grew on bacterial or fungal culture. *Toxoplasma* titers on CSF were negative. Feline Coronavirus antibody titer was negative.

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By 5 mo of age, all the cubs remain in good health but still have intermittent and variable head tilts, and occasionally fall to one side or circle when changing direction or when distressed.

## **Discussion**

Congenital vestibular disorders have been described in several breeds of domestic dogs and cats.<sup>1-3,7</sup> An undiagnosed neurologic disease thought to be toxic or metabolic has been seen in successive litters of Sumatran tigers at London Zoo,<sup>8</sup> but no reports were found involving congenital neurologic conditions in tigers.

Vestibular disease can be peripheral, affecting the semi-circular canals or the vestibular nerve; or central, affecting the vestibular nuclei in the brainstem, the cerebellar peduncles or the flocculonodular lobe of the cerebellum. Clinical signs indicating central vestibular disturbance (paresis, cranial nerve abnormalities, altered mentation and postural reactions) were absent in the cubs although postural reactions were difficult to assess. Variable head tilt (not consistently to one side), symmetric ataxia and side-to-side sweeping head movements are characteristic of bilateral vestibular disease. It was determined from the neurologic examinations that affected cubs of both litters suffered from a congenital bilateral vestibular disorder with the precise location of any lesion undetermined.

Hearing deficits have been reported in domestic cats and dogs with congenital vestibular disease.<sup>2,7</sup> Hearing deficits may be present in these cubs but have not been accurately assessed. A hearing deficit may be associated with a central abnormality, or abnormality of the cochlea, or cochlear nerve, which are closely associated with the semi-circular canals and the vestibular nerves. It is difficult to accurately assess hearing by clinical examination. Any response to noise may be influenced by fear, cues from litter-mates, or inadvertent visual or olfactory cues. In order to assess hearing in the tiger cubs, a brainstem auditory evoked potential (BAEP) test is planned.

Vestibular signs in some affected animals appear to regress by about 5 mo of age.<sup>1,7</sup> Apparent clinical resolution may be attributable to central compensatory mechanisms rather than restored function of the vestibular apparatus. Redevelopment of signs in the few months following recovery is not uncommon. In domestic cats and dogs, where present, deafness is permanent.<sup>1,2,7</sup>

Trauma, vascular incident, neoplasia, immune mediated inflammation, and otitis interna due to spread of bacteria from the middle ear (via the Eustachian tube or external ear canal) were ruled out as causes based on history, ancillary tests and the age and geographic separation of the two litters. Malformations, including middle and inner ear structural abnormalities and cerebellar hypoplasia, were not evident on MRI performed on one Taronga cub, and skull x-rays were normal in two of the Perth cubs.

Sub-acute thiamine deficiency can produce degeneration in the cerebellar and vestibular nuclei. In both affected litters, dietary thiamine intake of the dam was presumed to be adequate. The

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presence of signs from birth and the absence of response to additional thiamine supplementation of the dam at Taronga did not suggest thiamine deficiency as a likely cause.

A congenital viral or protozoan infection co-incident to both litters is possible. No infectious agents were isolated via cytologic analysis or bacterial and fungal culture of CSF on one cub. No attempt was made to culture mycoplasma. Negative *Toxoplasma* titers on five cubs did not support a diagnosis of toxoplasmosis. Both dams were in good health throughout their pregnancies. Both had current vaccination status for feline panleucopenia, feline rhinotracheitis and feline calicivirus, and neither received any toxic drugs or live virus vaccines. Two cubs tested negative for FIV and FeLV. There was no evidence of concurrent or multi-systemic disease. All cubs of both litters were mildly anaemic. The cause of this was unknown, but could represent iron deficiency common in milk dependent animals. The anaemia had resolved within 3 wk in two out of three cubs at Taronga Zoo.

All affected cubs are still alive, and no histopathology data is available. Pathologic examination of a litter of Doberman dogs with congenital vestibular disease revealed labyrinthitis, although no infectious agent was identified using ancillary tests.<sup>3</sup>

A genetic cause is highly likely given the close relationship between the sires of the two affected litters. The Australasian captive population of Sumatran tigers is derived from 9 founders. Unequal contributions by the founders have added to a high rate of inbreeding. The distribution of known affected animals could fit with a single gene, autosomal recessive mode of inheritance. In order to determine the nature of a genetic cause for this disorder, a retrospective pedigree indicating affected animals should be compiled. This may prove difficult since mildly affected cubs may have been overlooked due to the practice of non-disturbance of cubbing female tigers to prevent mis-mothering; variation in severity of signs and early compensation. There are numerous anecdotal references to tiger cubs with head turning and stargazing, which could be analogous with the syndrome reported here.<sup>6</sup>

The Australasian and European populations of Sumatran tigers are managed cooperatively. A potential genetic disease with clinically apparent signs, where permanent deafness may be present, represents a significant threat to a successful captive breeding program. As a precaution, consideration should be given to preventing affected tigers from breeding until the exact nature of the disease is known. Close monitoring of all future Sumatran tiger cubs along the following guidelines is suggested:

- All cubs should be monitored from birth by closed circuit video.
- All cubs should receive a full neurologic exam co-incident with the first vaccination.
- Tissues/blood samples should be collected from all cubs and stored for future genetic work.
- All affected cubs should have hearing assessed by BAEP testing along with examination by a veterinary ophthalmologist to detect visual defects.

#### ACKNOWLEDGMENTS

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The authors wish to acknowledge the assistance of veterinary neurologists Georgina Child BVSc DACVIM (Neurology), Fiona Haining BVMS MVM, and David Pepper-Edwards ASMP Tiger Coordinator (Australasia).

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**FIELD TECHNIQUE: A METHOD FOR OBTAINING TRUNK WASH MYCOBACTERIAL CUTURES IN ANESTHETIZED FREE-RANGING AFRICAN ELEPHANTS (*Loxodonta africana*)**

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**Abstract**

The *Guidelines for the Control of Tuberculosis in Elephants 2003 (Guidelines)* of the National tuberculosis Working Group for Zoo and Wildlife Species were written to protect the health and safety of captive elephants together with their handlers and the viewing public.<sup>1</sup> The *Guidelines* specifically address the display and transport of captive elephants but do not address the unique situation of free-living elephants being imported and subsequently displayed to the public. Although the *Guidelines* describe a technique for collecting and handling a trunk wash in a trained, standing, non-anesthetized elephant, it does not describe a similar technique for anesthetized elephants in lateral recumbency. In an attempt to detect active mycobacterial infection in a group of 3 male and 8 female free-ranging African elephants scheduled for import into the United States, a technique was developed for collecting trunk washes in recumbent, anesthetized elephants for mycobacterial culture.

A South African game-capture crew, experienced in translocating elephants, anesthetized elephants in groups via remote drug delivery and from a helicopter. The ground crew accomplished multiple simultaneous procedures including anesthesia maintenance and monitoring, physical and reproductive examinations, collection of general diagnostic and investigative samples, and trunk washes for mycobacterial cultures. This was accomplished while the capture crew was preparing animals for loading into specially designed trailers for transport to a holding boma. Little time was available for any one of procedure with multiple animals being attended to at one time.

Once an elephant was stable in lateral recumbency, a 3-m foal stomach tube, prepackaged and sterilized, was inserted into the dependent side of the trunk tip. It was then gently fed up the trunk approximately 2.5 m. A 50-ml sample suction trap was attached to the end of the foal tube. The suction trap was then attached to a battery powered, portable aspirator pump designed for emergency medical care. The aspiration pump was activated to collect secretions from the most proximal portion of the trunk. If little or no secretions were collected by this means, the system was disconnected between the sample trap and the foal tube. Then, 100 ml of sterile saline was placed into raised end of the foal tube allowing it to drain toward the tip through gravity. The suction trap and aspiration pump were reattached to collect a sample in the sample trap. Then,

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the sample trap was replaced with a new trap, and the foal tube was inserted into the oral pharynx for collection of a separate oropharyngeal sample. This same procedure was repeated with each elephant.

#### **ACKNOWLEDGMENTS**

So African veterinarians, Mike Bester, Larry Killmar, Janet Payeur, ARC/OVI, Thomas Hildebrant, Eric Zeehandelaar, Kevin Reily, Denise SoFranko.

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## SUCCESSFUL TREATMENT OF URSICOPTIC MANGE IN A BLACK BEAR (*Ursus americanus*) USING IVERMECTIN

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### Abstract

Mange is a dermatopathy of captive and wild black bears (*Ursus americanus*) caused by *Demodex* spp., *Sarcoptes* spp., and *Ursicoptes* spp.<sup>3,4</sup> *Ursicoptes americanus* is an audycoptid mite that lives in hair follicles.<sup>2,5</sup> The morphology of *U. americanus* has been well described,<sup>5</sup> however, a limited number of clinical descriptions of Ursicoptic mange exist in the literature. Reports of clinical presentation of Ursicoptic mange have been variable and may include mild to severe facial alopecia<sup>2</sup> associated with chronic lesions of the muzzle and forehead,<sup>5</sup> crusting,<sup>3</sup> and pruritis.<sup>3,5</sup> Distribution of alopecia has also been variable and may be limited to the head<sup>2</sup> or include the neck, thorax and limbs.<sup>5</sup>

A juvenile female black bear presented to The Wildlife Center of Virginia in November 2003. On physical examination the bear weighed 6.25 kg, was emaciated and dehydrated. A clean wound approximately 4 cm long with thickened skin edges was present in the left axilla. Moderate fecal staining was present around the hindquarters. Large areas of bilateral alopecia with a diffuse thinning of the hair were also noted. The alopecia was more significant on all limbs, particularly in the flank region of the hind limbs, the ventral thorax and abdomen, and ventral to the ears. There was minimal alopecia on the face and along the spine. Small crusty scabs with associated erythema were present throughout the skin. There was no evidence of hyperkeratosis. Initial therapy consisted of basic wound management, rehydration and antibiotic therapy with ceftiofur (Excenel<sup>®</sup>, Pharmacia & Upjohn, Kalamazoo, MI 49001 USA; 2 mg/kg) administered intramuscularly.

Complete diagnostic investigation was staged over the next 3 days and included hematology, serum chemistry profile, direct and floatation fecal examinations, survey radiographs, skin scrapings and skin biopsies. Hematology showed a severe anemia and severe panleukopenia. Serum chemistry panel showed a mildly decreased creatinine and calcium. Fecal examination revealed moderate to heavy numbers of coccidia, strongyloides, pinworms and ascarids. *Baylisascaris transfuga* infection was confirmed based on egg measurement and morphology from fresh fecal samples submitted to the Southeastern Cooperative Wildlife Disease Study (SCWDS). Radiographs were within normal limits. Skin scraping revealed several audycoptid

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mites identified as *Ursicoptes americanus*.<sup>5</sup> Multiple skin biopsies were submitted to SCWDS for histopathologic examination. Microscopic examination identified golden brown pigment, interpreted to be mite feces, within hair follicles and along the skin surface. Occasionally, partial profiles of mites were noted on the epithelial surface and within some hair follicles. These mites were generally round with cuticular spines. Mite legs were not observed. Large numbers of yeast and fewer mixed bacteria were also noted on the skin surface and in some follicles; however, no associated inflammation was reported.

Treatment of Ursicoptic mange was initiated with ivermectin (Ivomec<sup>®</sup>, Merial, Iselin, NJ USA; 0.3 mg/kg s.c.). Repeat ivermectin treatments were administered at 2 wk (0.3 mg/kg p.o.), 3 wk (0.3 mg/kg s.c.) and 6 wk (0.3 mg/kg s.c.) after initial therapy. Three weeks after initial therapy, dermatologic examination of the bear under anesthesia showed no improvement and a secondary pyoderma was observed. Skin scrapings showed one dead *Ursicoptes americanus* mite. The bear was treated with trimethoprim sulfamethoxazole (Sulfamethoxazole and Trimethoprim Tablets, USP<sup>®</sup>, Mutual Pharmaceutical Co., INC, Philadelphia, PA 19124 USA; 32 mg/kg p.o. b.i.d.) for 7 days. Six weeks after initial ivermectin therapy, the bear cub was again examined under anesthesia and dermatologic evaluation showed generalized fur growth with only occasional crusted areas. Skin scraping showed one dead *Ursicoptes americanus* mite. Visual examination of the bear over the next 4 mo showed a continued improvement in the pelage with no signs of pruritis. Steady weight gain and a generalized improvement in body condition were also noted.

Few reports describe the successful treatment of Ursicoptic mange in black bears.<sup>1,2</sup> Currently, the recommended therapy consists of multiple treatments with Amitraz.<sup>2,3</sup> The use of ivermectin has been reported to be unsuccessful in treating ursicoptic mange.<sup>1</sup> However, this report describes the successful treatment of ursicoptic mange in a black bear using repeated high doses of ivermectin, which can now be considered as an alternative treatment.

While *Ursicoptes americanus* has been identified in free-ranging black bears in the nearby state of Pennsylvania (M. Ternent, pers. communication), this is the first report of *Ursicoptes americanus* associated with clinical mange in a free-ranging black bear in Virginia (D. Martin, pers. communication). Further surveillance is planned in order to determine the prevalence of this mite infestation in black bears in Virginia.

#### **ACKNOWLEDGMENTS**

We thank the Virginia Department of Game and Inland Fisheries for their cooperation and the staff of The Wildlife Center of Virginia for their excellent animal care.

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## THE FIRST EUROPEAN ELEPHANT MANAGEMENT SCHOOL IN HAGENBECK'S TIERPARK, HAMBURG, GERMANY

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### **Abstract**

Since 2003 the First European Elephant Management School takes place at Tierpark Hagenbeck in Hamburg, Germany and will be held on an annual base. This 9-day course results out of the idea of Elephant Business and Hagenbeck's Tierpark. Its goal is to teach husbandry, medical care and training techniques on Free Contact & Protected Contact elephants and also to be able to exchange experiences among professional elephant people from all over the world.

The curriculum covers a broad variety of important subjects and is divided up into theoretic lectures and a large scale of practical experiences with the animals themselves. The main emphasis of this program is to teach through learning by doing.

Hagenbeck's Tierpark has been keeping and taking care of elephants for more than one century. Hagenbeck keeps 10 Asian cows under full contact and one mature bull under Protected Contact conditions. This special environment provides the participants with the opportunity to compare and interact with these forms of husbandry. The differences in safety, husbandry and handling are important matters that need to be outlined. Besides that the major topics of the daily routine (care, safety, training) are discussed as well as foot care, medical problems, reproductive tasks, training, conservation and transport. Therefore, a special selection of international experts assembles in Hamburg to provide the participants with a broad spectrum of information.

The course is meant for professional elephant keepers, elephant curators, zoo veterinarians and zoo directors. The curriculum consists of the following.

### **General Tasks**

- Problems and danger of "hands on" and "hands off" elephant management.
- Elephant management in Europe and in the United States (current situation).
- Future strategies of the EAZA/AZA.

### **Elephant Management**

- Elephant program design.
- Working routines (morning wash routine, exercise routines).

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- Facility design (exhibit design, natural breeding facilities, elephant bull handling, etc.).
  - Facility and equipment maintenance.
  - Appearance (uniforms, general appearance, dialogue).
  - Vision and goals of a proper elephant program.
  - Handling (restraint, commands, hook usage, rope slicing and knot tying, chaining, target training, shows and demonstrations).
  - Behavioural enrichment.

### **Medical Topics**

- Medical prevention techniques and body hygiene (foot care, skin care, tusks and teeth, ears and eyes, tail care, trunk care, bed and pressure sore treatment, sleeping strategies, medical training, veterinary inspections (e.g., skin mapping).
- Medical procedures (foot problems, immobilization, tooth extraction).
- Diseases (herpes, tuberculosis, pox).

### **Breeding and Reproduction**

- Herd dynamics.
- Fertility control.
- Cycle monitoring.
- Ultrasound.
- Semen collection.
- Artificial insemination.
- Birth (preparations, protocols, caesarean, births within elephant group, handling and training of baby elephants).

### **Conservation**

The next course will take place in November 2004 at Tierpark Hagenbeck in Hamburg.  
For further information please visit: [www.elephant-management.com](http://www.elephant-management.com).

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## PARTIAL CRANIAL CRUCIATE LIGAMENT RUPTURE IN A JAGUAR (*Panthera onca*) REPAIRED USING THE TIBIAL PLATEAU LEVELING OSTEOTOMY PROCEDURE

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### Abstract

A 2.5-yr-old male jaguar (*Panthera onca*) presented acutely lame on the right hind leg during the third week of a 4-wk quarantine at Denver Zoological Foundation. Records review from the previous institution revealed this jaguar had presented with a similar lameness when 1-yr-old. Following examination the diagnosis at that time was a partial rupture of the cranial cruciate ligament (CrCL). The jaguar was treated conservatively with caprofen (Rimadyl®, Pfizer Animal Health, Exton, Pennsylvania 19341, USA; 1 mg/kg, p.o., s.i.d. 14 days) and followed after completing that treatment course with Cosequin® (Nutramax® Laboratories Inc., Edgewood, Maryland 21040, USA; 750 mg, p.o., s.i.d., 15 days). The lameness continued on and off for the next 6 mo at which time the jaguar had normal ambulation for the next 12 mo.

Quarantine examination and radiography at the Denver Zoo revealed effusion of the right stifle, atrophy of the hamstrings, and a palpable click on flexion and extension of the stifle but was negative on the cranial drawer and tibial compression tests for a ruptured CrCL. Seven days later partial rupture of the CrCL and a longitudinal tear of the medial meniscus (MM) was confirmed during computed tomography and arthroscopy. The few remaining fibers of the CrCL were severed and partial menisectomy of the caudal MM was performed.

Ten days later the instability of the right stifle was corrected using the tibial plateau leveling osteotomy procedure (TPLO).<sup>1</sup> TPLO surgery was developed by Drs. Barclay Slocum and Theresa D. Slocum (U.S. patent number 4,677,973, Slocum Enterprises, Eugene, Oregon 97404, USA) due to dissatisfaction with the current intra-articular and extracapsular techniques used to repair the CrCL.<sup>1</sup> Preoperative radiographs were taken of the right stifle. A lateral view of the stifle was obtained to determine the amount of tibial plateau rotational correction that would be required to approximate a tibial plateau that is perpendicular to the center of motion for the stifle and the hock. In this jaguar the correction angle was determined to be 25°. An anterior-posterior radiograph of the right stifle was also taken to determine if there was any evidence for genu varum or valgum, which would negatively impact this surgical procedure.

A medial incision was made from mid femur to the proximal third of the tibia. A medial meniscal releasing procedure was performed prior to the TPLO. The tibial plateau was then

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bluntly dissected and a Slocum jig (Slocum Enterprises, Eugene, Oregon 97404, USA) fixed to the tibia with two pins to act as guide for the Slocum osteotomy saw (Slocum Enterprises) in order to make a perfect cylindrical cut in the proximal tibia. The tibial plateau was then rotated 10.4 mm which resulted in actual correction to 12°. In the surgeons opinion more correction would risk the stability of the fixation. The tibial plateau was secured to the tibia using a Slocum tibial leveling osteotomy plate (Slocum Enterprises).

It is critical during the first 4 wk postoperatively to severely restrict the patient's activity while the osteotomy site begins to heal. We dosed the jaguar with the tricyclic antidepressant amitriptyline HCl (Generic brand, Mutual Pharmaceutical Co. Inc., Philadelphia, Pennsylvania 19124, USA; 100 mg, 1.7 mg/kg, p.o., b.i.d.). At this level of sedation the jaguar would eat, drink, and defecate but spent most of the day resting peacefully.

Four and 8 wk postoperatively the jaguar was immobilized to reevaluate the right stifle by palpation and radiography. The anterior-posterior view revealed the initial formation of bony callous at the osteotomy site at 4 wk. In order to encourage additional callous formation and minimize the occurrence of "joint freeze" we discontinued the use of amitriptyline HCl and started regularly (three to four times daily) exercising the jaguar using operant conditioning. At 8 wk we noted more callous formation radiographically and the jaguar was virtually limp free. We are hopeful with more time the jaguar may make a complete recovery.

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## DISEASE-RISK ASSESSMENT FOR FREE-RANGING GECKOES (*Gecko monarchus*, *Gehyra mutilata*) IN A CAPTIVE REPTILE STOCK

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### Abstract

In zoos, free-ranging animals may go beyond barriers segregating captive breeding stock thereby increasing the risk of transmission of infectious agents. In order to assess the role of free-roaming geckoes (*Gecko monarchus*, *Gehyra mutilata*), in the epidemiology of infectious diseases in the Reptile House of Jersey Zoo, a bacteriologic, parasitologic, and virologic screening of 32 free-roaming geckoes was conducted. The animals were caught from exhibit and non-exhibit rooms where they had free access to by open doors and the ventilation system. Blood samples by cardiocentesis were taken under anaesthesia (isoflurane 5%) and pooled of two to four animals to obtain enough serum to be tested for ophidian paramyxovirus (OPMV-1, OPMV-7) by haemagglutination inhibition. After euthanasia by pithing, a complete post mortem examination was conducted. Bacterial cultures of intestines, liver, and any organs showing abnormalities were done. Parasitologic examinations of intestinal content were conducted by microscopy of direct smears, sporulation of coccidia in potassium dichromate 2.5%, and Ziehl-Neelsen staining. Apart from physiologically occurring enterobacteria and protozoa, cryptosporidia could be identified. In addition, 70% of pooled blood samples tested positive for ophidian paramyxovirus (OPMV-1, OPMV-7). More detailed investigations would be necessary to prove the definite vector role of the geckoes in specific diseases. They can, however, increase prevalence of opportunistic agents and exposure of the captive breeding stock to diseases and should be taken into account when dealing with infectious disease problems in captive reptile collections.

### ACKNOWLEDGMENTS

The author would like to thank A. Allchurch, BVSc, MRCVS, and A. Thomasson for the generous support to realize this paper as a product of a work placement student project at the Durrell Wildlife Conservation Trust.

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**VARIABILITY OF *Mycoplasma gallisepticum* ISOLATES FROM HOUSE FINCHES DETECTED BY RANDOM AMPLIFICATION OF POLYMORPHIC DNA (RAPD) AND AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP)**

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**Abstract**

*Mycoplasma gallisepticum* (MG) conjunctivitis emerged in 1994 as a disease of free-ranging house finches (*Carpodacus mexicanus*) in the eastern United States and has since spread to house finches throughout their entire eastern range. The resulting epidemic of MG conjunctivitis produced an unprecedented decline of eastern house finch populations, and the endemic disease remains associated with repeating seasonal peaks of disease and limitation of host populations. Random amplification of polymorphic DNA (RAPD) had indicated a single RAPD profile among house finch isolates, suggesting a single point source of origin and one 'strain' common to this outbreak. However, genomic variability of MG house finch isolates has recently been identified by PCR-RFLP and nucleotide sequencing of the *pvpA* gene. These findings suggested that house finch MG isolates may be more polymorphic than previously recognized and/or evidence of molecular evolution.

We have seen some evidence of genomic variability among MG isolates by RAPD fingerprinting. However, RAPD 'fingerprints' are prone to variability, and may be difficult to reproduce and standardize, making interpretation challenging and somewhat subjective. To explore the possibility of genomic variability among house finch isolates of *M. gallisepticum*, we selected samples from our archive of isolates to analyze by RAPD and amplified-fragment length polymorphism. Amplified-fragment length polymorphism (AFLP) analysis is a selective restriction fragment amplification technique based on the ligation of adapters (linkers and indexers) to genomic restriction fragments followed by a PCR-based amplification with adapter-specific primers. This analysis may approach the ideal genotyping method, which produces results that are consistent from laboratory to laboratory and allows unambiguous comparative analyses and the establishment of reliable databases. The AFLP technique has been successfully used to explore the genomic variability of several *Mycoplasma* spp. Analyses of MG house finch isolates by RAPD and AFLP allows us to more definitively explore the potential genomic variability of these isolates and their molecular epidemiology especially with respect to temporal and geographic relationships. These analyses also generate comparative data between RAPD and AFLP methodologies, providing an opportunity to evaluate the utility of AFLP for MG typing.

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## HUMERAL FRACTURE IN A NEWBORN ASIAN ELEPHANT CALF (*Elephas maximus*)

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### Abstract

### Introduction

The Asian elephant (*Elephas maximus*) is threatened with extinction mainly because of conflict with human activities.<sup>2,13</sup> Half of the world's remaining Asian elephants share the present range of 20% of the world's human population.<sup>13</sup> Mahouts are decreasing in number and aging.<sup>3</sup> Zoos may become the sole repository of the species. Much effort is invested in the breeding of Asian elephants. Peri-natal injuries by the mother are not uncommon in captive animals.<sup>11</sup> This report describes the successful management of an oblique midshaft humeral fracture in a neonate Asian elephant calf at the Night safari, Singapore.

### Case History

On the 18<sup>th</sup> April 1998 a newly born female calf weighing 108 kg was found unable to stand. The left shoulder was swollen and crepitus could be felt. An oblique midshaft fracture of the humerus with approximately 10 cm overlapping of distal and proximal fragments was diagnosed on X-rays. Myoglobinuria due to the injured muscles of the foreleg persisted for 48 hr.

An intravenous catheter was inserted in an ear vein. Fluid therapy was initiated alternating Ringers® and Dextrose 5%® (Thai Otsuka Pharm. Co., Thailand) at 50 ml/kg/day for a total of 9 L in 48 hr. Her rectal temperature was 39.8°C (normal 36 to 37).<sup>6</sup> To accelerate heat loss the animal was maintained wet and ice packs were positioned between the limbs. The rectal temperature dropped to 38°C in 24 hr and remained in that region for the next 5 mo.

Serum from the mother (65 ml) was injected subcutaneously. oxytocin 100 IU were administered i.v. to the mother to assist milking. Warm colostrum (800 ml) was administered to the calf via a stomach tube.

Amoxicillin 1650 mg (Betamox LA, Norbrook laboratories) was injected intra-muscular every 48 hr for four treatments preventively. Flunixin 1 mg/kg i.m. (Flunixin®, Troy Laboratories) was administered for analgesia.

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A custom-made modified Thomas splint was manufactured. The calf was supported in a sling for approximately 10 hr per day. The remaining time the animal was recumbent laterally on a padded area.

After 1 mo of trial and error with various formulas, a diet inspired from Mikota, et al.<sup>8</sup> was selected. It consists of the following.

- UHT cow's milk in boiled water 1:1, progressively concentrated to pure cow's milk over 2 wk
- Vit C 0.5 g/L of milk
- Pediatric re-hydration solution (Repalyte®, Drug house of Australia) 4 g/L of milk
- Calcium carbonate powder 1g/L of milk

The formula was offered every 3 hr and uptake was recorded.

After 3 mo in the sling the leg felt strong. It could not be ascertained on radiographs whether the fracture had healed satisfactorily. An equine veterinarian from the Singapore Turf Club and an orthopaedic human specialist were consulted. Plans for a surgical exploration of the fracture site were made.

The left leg was scrubbed twice daily for 3 days before the surgery. The calf was transported to the Singapore Turf Club equine Hospital on the 31<sup>st</sup> July 1998. Xylazine 0.08 mg/kg i.m. (Ilium Xylazine®, Troy Laboratories)<sup>1,8,12</sup> was followed 20 min later by induction with ketamine i.v. to desired effect (1.15 mg/kg). A 16-mm cuffed endotracheal tube was passed and anaesthesia maintained with halothane 1 to 1.5%. Phenylbutazone (150 mg; Tomanol®, Vet Schering-Plough) was given i.v. preoperatively. Ringers solution was administered at a rate of 5 ml/kg/hr.

A 30-cm incision was made laterally, from the shoulder joint to the elbow. The fracture site was exposed by dissection of the arm muscles. The overlapping fragments were firmly joined by their extremities. A 1-cm gap between the fragments was filled with thick fibrous tissue. The fibrous bridge was curetted and the periosteum elevated. The fragments were adjoined using three compression screws. After recovery the calf was placed back in the sling.

Physiotherapy was started 2 wk after surgery in the form of swimming and walking in a large pool. The duration of exercise was increased gradually from 20 min to several hours per day. After 8 wk of physiotherapy she was allowed to ambulate freely. The right fore leg arched outwards to compensate for the shorter length of the left foreleg. By the age of 13 mo the two legs were of the same length.

## **Discussion**

Several successful fracture cases in elephants are reported.<sup>8-10</sup> In elephant the scapula and humerus are straight.<sup>5,12</sup> Thomas splint are particularly well suited for the treatment of vertical

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bones.<sup>4,8,14</sup> A Thomas splint was custom-made to fit the anatomy of the elephant. Two metal rings were connected by three metal rods. The proximal ring was angled such that it rested on the sternum and enclosed the shoulder laterally. A Robert-Jones bandage protected the limb. Muraleedharan Nair, et al. (2002).<sup>9</sup> later reported the successful treatment of two cases involving fractures of the tibia and radius and ulna using a similar contraption.

Internal fixation of the fracture immediately after birth was complicated by the incompletely mineralised state of the neo-natal bone and by the considerable swelling of the surrounding tissues. The priority was to confer passive immunity and to keep the calf alive. Many milk formulas have been tried with varying degrees of success.<sup>7,8,11,17</sup> The mother's milk changes with the stage of lactation<sup>15</sup> Mercy (2002)<sup>7</sup> reported that cow's milk cause severe diarrhea. The diet used at the Night Safari and described above produced satisfactory results. It has been used since to raise three other calves.

Good quality radiographs of the humerus proved difficult to obtain due to the thickness and density of the tissues and the difficulty of consistent positioning of the animal. The consulting specialists feared a non-union. The animal was in good health and a surgical exploration of the fracture site was decided. With the benefit of hindsight the surgical intervention appears unnecessary, but so was running the risk of a re-fracture. The procedure lasted 50 min. Anaesthesia was unremarkable and after reversal with yohimbine at 0.125 mg/kg i.v. the calf regained consciousness.

Physiotherapy was important, as the animal had never walked until the age of 18 wk. Physical re-education was carried out in water to reduce the weight borne while allowing a natural and full range of movements. Swimming is a mild and complete exercise that comes natural to elephants.<sup>1</sup>

## **Conclusion**

The overall cost of the treatment from birth was calculated to be in excess of US\$ 40,000, including manpower. The successful outcome of this case was the result of the collective commitment within the organisation. In zoological medicine inter-departmental collaboration and communication make the difference between failure and success.<sup>14</sup>

## **ACKNOWLEDGMENTS**

We are grateful to Dr Rod Richards and Dr Ong Leong Boon for their assistance with the surgery and the elephant keepers for their nursing feat.

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## TREATMENT OF UNILATERAL PLEURAL EFFUSION IN A BLACK AND WHITE RUFFED LEMUR (*Varecia varecia*) WITH RUTIN

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### Abstract

### Introduction

Chylothorax is a rare and complex disorder. Causes include neoplasia, thoracic trauma, fungal infection, dirofilariasis, cardiac disease, cranial vena caval thrombosis, and congenital anomaly of the thoracic duct. Most cases are idiopathic which are difficult to resolve medically or surgically.<sup>1,2</sup> Currently, thoracic duct ligation is the preferred technique, but invasive surgery and guarded prognosis for resolution makes this procedure less than desirable.<sup>3</sup> Rutin, a bioflavonoid has been utilized as an alternative to traditional treatments in domestic species with reports of substantial improvement.<sup>4,5</sup>

### Case Report

During routine preshipment examination of a 19-yr-old female, black and white ruffed lemur (*Varecia varecia*), inhalant anesthetic irregularities, decreased SpO<sub>2</sub>, and thoracic radiograph abnormalities were encountered.

Thoracocentesis, thoracic ultrasonography and echocardiography were performed to confirm unilateral (right-sided) pleural effusion. The fluid was ultimately determined to be chylous, but as no cause was identified, idiopathic chylothorax was diagnosed.

Rutin, (General Nutrition Corp., Pittsburgh, PA 15222, 500 mg, p.o., s.i.d) was initiated and patient compliance was excellent. Physical exam, thoracic radiographs, and thoracocentesis, when appropriate, were performed twice monthly to monitor progress and resolution.

### Results and Discussion

At 6 mo post-diagnosis, a negligible amount of pleural effusion remains with no associated clinical signs. Rutin administration is planned for at least 12 mo and recheck interval has been extended to 3-6 mo. The incidence of idiopathic chylothorax is unknown in non-domestic species.

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Rutin is a naturally occurring compound found in buckwheat, black tea, and apple peels. Literature indicates that rutin may have antioxidant, anti-inflammatory, anticarcinogenic, antithrombotic, cytoprotective and vasoprotective activities.<sup>6</sup>

In the treatment of chylothorax, the exact mechanism of action for rutin is poorly understood. It is thought to reduce vessel leakage, increase protein removal by the lymphatic vasculature, stimulate phagocytosis by macrophages, increase the number of macrophages, inhibit lipid proteolysis, and increase lymph proteolysis and removal from tissue.<sup>7-9</sup>

Rutin is generally well tolerated. Reported adverse reactions include gastrointestinal signs, such as diarrhea and nausea. Rare reports of headache and mild tingling of the extremities are found in the human literature. From this case, rutin appears as a safe and effective alternative to more invasive treatments for chylothorax in nonhuman primates.

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**PAPILLOMAVIRUS-ASSOCIATED BASOSQUAMOUS CARCINOMA IN AN EGYPTIAN FRUIT BAT (*Rousettus aegyptiacus*)**

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**Abstract**

An approximately 5-yr-old, female Egyptian fruit bat (*Rousettus aegyptiacus*) was acquired by The Organization of Bat Conservation, in Bloomfield Hills, MI, from a privately owned colony of 20-25 Egyptian fruit bats in February, 2003. Upon arrival, the bat had a small raised pigmented mass located at the lateral canthus of the left eye. Over the next 6 mo, the mass progressively increased in size; however, due to pregnancy, surgical excision was delayed to allow for parturition and weaning of the pup. The facial mass was surgically removed in January, 2004. At the time of surgery, the mass was 12 mm in diameter, and extended 6 mm into the underlying subcutaneous tissue. Further examination identified multiple (approximately 6) variably sized, raised, smooth to cauliflower-like skin masses randomly distributed throughout the left wing membranes. An additional three masses were removed in February, 2004. All excised tissues were submitted to the Diagnostic Center for Population and Animal Health at Michigan State University for microscopic examination.

All four masses appeared microscopically similar and were characterized by elongate to polygonal neoplastic cells with prominent intracellular bridging that were arranged in lobules and thick pegs extending from the overlying hyperplastic epithelium. Neoplastic cells had variable amounts of eosinophilic cytoplasm and large vesiculate nuclei with prominent, sometimes multiple nucleoli and surrounded central areas of keratinization with numerous intermixed dyskeratotic cells. There were 1-3 mitoses per high power field (HPF). All masses were diagnosed as basosquamous carcinomas. Immunohistochemistry for papillomavirus on the four examined masses detected positive intranuclear staining in all tumors. DNA extracts from formalin fixed-paraffin embedded tumor tissue were tested by PCR, using degenerate primers designed to amplify a 450 bp segment of the L1 region of the human papilloma virus genome. A 450-bp product was obtained and directly sequenced. A BLAST analysis of the sequence data showed that there was 42.9% sequence identity with the L1 region of human papillomavirus.

This is the first report of a papillomavirus-associated carcinoma in a bat. Papillomaviruses have been associated with a number of hyperplastic and neoplastic lesions in a wide variety of vertebrate species, including humans.<sup>2</sup> Whereas papillomavirus in bovines most commonly

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results in benign lesions, such as fibropapillomas and papillomas, papillomavirus-induced lesions in humans may progress to squamous cell carcinomas.<sup>2</sup> A similar progression to carcinoma has been documented for other species-specific papillomaviruses, such as canine oral papillomavirus, cottontail rabbit cutaneous papillomavirus (*Shope papillomavirus*), and rodent (*Mastomys natalensis*) papillomavirus. Interestingly, bovine papillomaviruses (BPV-1, BPV-2) have been associated with neoplastic lesions in other species, such as cutaneous sarcoids in horses.<sup>1</sup> In the case presented here, papillomavirus antigen was detected in all four examined carcinomas, strongly suggesting a role of this virus in tumorigenesis. It is uncertain whether the papillomavirus identified in this bat represents a novel species-specific bat papillomavirus, or cross-infection of a known papillomavirus from another species. Further genomic sequencing is in progress.

#### **ACKNOWLEDGMENTS**

We would like to thank The Organization of Bat Conservation, Cranbrook Institute of Science, 39221 Woodward Ave, Box 801, Bloomfield Hills, MI 48303, for the care and commitment to sick and injured bats, and for their work to promote bat conservation in Michigan.

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**RETROSPECTIVE STUDY OF CAUSES DEATH IN GIANT ANTEATER (*Myrmecophaga tridacyla*) AT FUNDAÇÃO PARQUE ZOOLOGICO DE SÃO PAULO (FPZSP) - FROM 1964 TO 2003**

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**Abstract**

The giant anteater (*Myrmecophaga tridacyla*) belongs to family Myrmecophagidae, order Xenarthra. They are animals of terrestrial and solitary habits found in fields, savannahs and rain forests from Guatemala to Argentina. In nature, their diet is based on termites and ants, but in captivity they receive different kinds of food. Biologic data allied to the incidence with different morbid cases in free-living animals, are important tools for disease prevention and control, allowing the better understanding of their impact.

This study presents a retrospective analysis of causes of death of giant anteaters registered at FPZSP, from 1964 to 2003. Necropsy reports of 74 animals were analyzed at the veterinary division of FPZSP. The main diagnosis of death causes were 22,97% (17/74) for caquexia and/or malnutrition, 13,51% (10/74) cardio-pulmonary failure, 22,97% (17/74) hipovolemic shock, 6,76% (5/74) trauma, 10,71% (8/74) pneumonia, 1,35% (1/74) hepatitis, 5,41% (4/74) endoparasitosis, 6,76% (5/74) septicemia, 1,35% (1/74) renal failure, and 8,11% (6/74) couldn't be determined because of advanced autolysis. Due to the lack of published data about Xenarthra's pathology, studies like this can be helpful in elucidating problems involving these animals, leading to better therapeutic approaches and guidelines to improve the animal's welfare.

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## ***Leucocytozoon* sp. INFECTION IN WILD ROCK PTARMIGANS (*Lagopus mutus*) IN JAPAN**

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### **Abstract**

*Leucocytozoon* sp. was detected in wild rock ptarmigans (*Lagopus mutus*) which are designated as an endangered species in Japan and inhabit alpine areas.<sup>3</sup> Nine ptarmigans were captured with the permission of the Ministry of the Environment of the Japanese Government on Mt. Tateyama (36° 35' N, 137° 36' W) at about 2,400 m elevation in April and on Mt. Jiigatake (36° 35' N, 137° 45' W) at about 2,650 m elevation in June 2002. Eight of nine adult birds (88.9%) tested positive for *Leucocytozoon* sp.. The percentage of infected cells in 400 leukocytes was 0.32-12.1%, and Ashford scale<sup>1</sup> was 1-3. No mixed infections with other hematozoa were observed. The birds infected with the hematozoa appeared to be healthy, and anemia was not diagnosed upon hematologic examination. For comparison, two captive adult rock ptarmigans hatched in a breeding facility of the Ohmachi Alpine Museum at the foot of the mountains were examined, and no hematozoa were detected. The oval-shaped gametocytes with long fusiform projections extending from both ends were observed by light microscopy. The form was similar to *L. lovati* detected from the red grouse (*L. scoticus*) in Britain,<sup>2</sup> but the species could not be determined because there was variability in gametocyte morphology. Using cytochrome *b* gene sequences of mitochondrial DNA of *Leucocytozoon* sp. by nested PCR,<sup>4</sup> phylogenetic relationships and genetic divergence among the hematozoa from ptarmigans and selected Japanese wild birds were investigated. Analysis of these samples based on the partial *cyt b* sequences of 750bp in length suggested that *Leucocytozoon* sp. found in Japanese rock ptarmigan is genetically different from the other *Leucocytozoon* spp. in wild birds in Japan. Considering the historic distribution of the ptarmigan, this clear division suggests independent co-evolution among the *Leucocytozoon* sp. and the bird species. *Leucocytozoon* sp. infection in the rock ptarmigan in Japan has not been previously reported.

### **ACKNOWLEDGMENTS**

The authors are indebted to Mr. Masanori Kitahara of The Institute for Alpine Environment and Biota, the members of Ptarmigan Research Group of Toyama Prefecture and the staff of Ohmachi Alpine Museum for sampling at Mt. Tateyama, Mt. Jiigatake and the Museum. Partial funding for this project was obtained through a research grant from the College of Bioresource Sciences, Nihon University.

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## EARLY PREGNANCY DETECTION IN RHINOCEROS SPECIES USING SERUM GLYCOPROTEINS

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### Abstract

There are currently five rhinoceros species still surviving. The Javan (*Rhinoceros sondaicus*), Sumatran (*Dicerorhinus sumatrensis*), Indian (*Rhinoceros unicornis*), black (*Diceros bicornis*), and white (*Ceratotherium simum*) are each critically endangered and all but the Javan has captive populations in North American zoological institutions. Habitat destruction and poaching for the rhino's horn have led to the decreased numbers in the wild; therefore, captive populations need to be self-sustaining with successful reproduction programs. All of the rhino species are difficult to breed in captivity with early embryonic death, stillbirths, and uterine leiomyomas as part of the problems. In fact, fewer than 10% of sexually mature animals have successfully reproduced and hence are not represented in the current captive population. Therefore, an early detection pregnancy test would allow animal care staff to determine first if a rhino has conceived. The test can then be followed up with additional tests to see if she maintains the pregnancy through the first month as well as confirming the pregnancy at 5 mo with the progesterone spike characteristic of rhino pregnancies.

ECF or Early Conception Factor is a protein that is released when an egg is fertilized by a sperm cell that will help with pregnancy detection and diagnosis of conception problems. A lateral flow assay test has been developed for use in horses to detect the ECF protein by Concepto Diagnostics Corporation™. The test can detect the ECF glycoprotein factor in horse serum between 3 and 30 days after breeding. The ECF kits test mare serum using specific monoclonal-polyclonal antibodies with colloidal gold as the indicator. We hypothesize that because rhinoceros are part of the equid family the Concepto Horse ECF™ Test will help detect early pregnancy in captive Indian, black, white, and Sumatran rhinoceros species.

Using a cycling, rhinoceros female with recent breeding activity for the test, we collected fresh blood from her using the saphenous vein of the front leg. The blood was then centrifuged for 10 min at 3000 rpms to separate the serum. The serum was then pipetted out and one drop was placed on a paper towel and the second drop placed in the circular test window on the ECF test kit. The serum was then followed a few seconds later with two drops of assay buffer. The kit was then laid flat, undisturbed at ambient room 25°C for 2 hr. After 2 hr, the kit was read under fluorescent light to detect all lines. A red line was present in the "C" or control region of the indicator window meaning the test had been set up correctly. If the rhino is pregnant a second

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red line, which can be faint will form in the “T” or test region, which was seen on the suspected pregnant rhino female. This procedure was then tested on a cycling, non-breeding, white rhinoceros female as a control for the test and she was negative for ECF on the test kit. To test the efficacy of the test on frozen serum the samples collected from each rhino, (both the pregnant female and the control female), were frozen for five days, thawed to room temperature, and vortexed vigorously for 5 min. The protein can stick to the sides of the serum sample tubes causing a false negative result in frozen samples so sufficient vortexing is necessary. The results produced using frozen serum were consistent with those from the fresh samples.

Using test kits, on further breedings and other rhinoceros species, determination will be made as to the accuracy of the test, the approximate testing window to detect ECF protein in the different rhino sp., and the viability of the test on fresh versus frozen serum. We will then confirm any positive or pregnant females by collecting serum progesterone levels, which should peak at 5 mo in pregnant rhinoceros. The ECF test is a revolutionary new way for animal care managers to confirm early pregnancy as well as early embryonic death and could lead to further conclusions that elucidate the cause of captive breeding problems in this difficult to breed equid group. Likewise, once the test is validated for rhino sp. the test can be tried on other endangered equid species.

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## IMMOBILIZATION OF BABIRUSSA (*Babyroussa babyroussa*) USING A BUTORPHANOL-TILETAMINE-ZOLAZEPAM COMBINATION

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### Abstract

Numerous protocols have been described for immobilization of captive exotic suid species at zoological institutions.<sup>1,3-5</sup> A commercially available tiletamine-zolazepam combination (Telazol<sup>®</sup>; Fort Dodge, Inc., Fort Dodge, Iowa 50501 USA) has been used extensively, and is adequate for immobilization of most exotic suids.<sup>1</sup> Its usage offers the advantages of being relatively inexpensive and readily available. However, prolonged and rough recoveries are seen with tiletamine-zolazepam,<sup>1,5</sup> and adverse effects are most commonly noted at doses exceeding 3 mg/kg.<sup>1</sup> In some cases, rough recoveries have resulted in death.<sup>5</sup> The use of adjunct anesthetic agents facilitates the usage of lower doses of Telazol<sup>®</sup>, and therefore minimizes secondary, drug-dependent adverse effects.<sup>1</sup> Most documented anesthetic combinations used in suids contain an alpha<sub>2</sub>-adrenergic agonist, and some require a large volume for successful induction.<sup>4</sup> Alpha<sub>2</sub>-adrenergic agonist combinations may cause bradycardia, which is likely a physiologic response to changes in peripheral vascular resistance induced by this class of drugs. Physiologic parameters seen in response to various anesthetic combinations have been well documented in domestic swine.<sup>3,6</sup> Species-specific variations in sensitivity to drug effects have been reported in some exotic swine,<sup>7</sup> although not extensively characterized for all species.

The purpose of this prospective clinical trial was to develop a reliable anesthetic protocol to be used in babirusa (*Babyroussa babyroussa*) as an alternative to the use of alpha<sub>2</sub>-adrenergic agonists. A combination of tiletamine-zolazepam (Telazol<sup>®</sup>, combined dose of  $1.26 \pm 0.3$  mg/kg) and butorphanol (Torbugesic<sup>®</sup> 10 mg/ml, Fort Dodge Laboratories, Fort Dodge, Iowa 50501 USA;  $0.37 \pm 0.04$  mg/kg) administered intramuscularly by blowdart was used in a total of 16 immobilizations performed on animals undergoing elective medical procedures. This combination of butorphanol-tiletamine-zolazepam (BuTZ) resulted in a smooth induction and a stable plane of anesthesia to facilitate minor elective procedures. Mean time to anesthetic induction was  $8.1 \pm 5.6$  min, and was inversely correlated to drug dose, with higher doses resulting in shorter induction times. A light plane of anesthesia or heavy sedation was achieved in all cases, and was sufficient for non-invasive procedures (transport, hoof trims, relocation, venipuncture, examination, ultrasonography, radiography). Heart rate values ranged from 80 to 120 bpm, which was higher than values reported in a xylazine-telazol combination previously reported in this species.<sup>2</sup> Neither respiratory depression nor hypoxemia was observed when BuTZ was used. Pulse oximetry saturation values ranged from 92 to 100% without oxygen supplementation. Indirect mean blood pressure readings ranged from 100-150 mm Hg, and were consistent throughout all immobilization events.

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Partial antagonism with intramuscular naltrexone (Trexonil, Wildlife Pharmaceuticals, Inc.; 0.34 ± 0.2 mg/kg) was used to decrease recovery times, with most individuals ambulating voluntarily within 60 min of induction. The BuTZ protocol was adequate for elective, non-invasive medical procedures, and offers the advantages of a low volume for induction, relatively low cost, and stable cardiorespiratory function. Anesthetic recoveries can be prolonged with this protocol, but are shorter than combinations that rely on higher doses of tiletamine-zolazepam. This protocol offers a reliable, safe alternative to the use of alpha-2 based protocols in babirusa, and should be useful in the clinical management of cases where this class of drugs would be contraindicated.

#### ACKNOWLEDGMENTS

The author acknowledges the efforts of the Saint Louis Zoo's keepers and technicians who assisted during immobilization events. Special thanks go to Martha Fischer, Diane Wilson, Randy Junge and R. Eric Miller for providing logistic support and feedback during this project.

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## MOLECULAR CHARACTERIZATION OF REPTILE GASTROENTESTINAL AMOEBAE; THE USE OF MOLECULAR TECHNIQUES IN COLLECTION MANAGEMENT

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### Abstract

Using molecular techniques, we have been able to characterize different species of amoeba in reptile feces and tissues to assist in epidemiologic investigations. Fatal amoebiasis was diagnosed post-mortem in five individual reptiles representing four different species of lizards (blue-tailed monitor, *Varanus doreanus*; caiman lizard, *Dracaena guianensis*; banded ground skink, *Eugongylus albobfasciolatus*; blue tongue skink, *Tiliqua scincoides intermedia*). The histologic lesions and morphology of the amoebae in these lizards were consistent with *Entamoeba invadens* infection. The amoeba from the affected lizards was confirmed by PCR on DNA extracted from either frozen or formalin-fixed tissues to be *E. invadens* with 100% nucleotide identity. Coincidentally, during routine medical screening, amoebae were identified in feces of clinically normal Fiji Island banded iguanas (*Brachylophus fasciatus*), thus raising suspicion of them as a source of infection for the fatal amoebiasis cases. PCR for *Entamoeba* sp. was performed on DNA extracted from feces of five of these iguanas. Molecular characterization of PCR results identified a previously undescribed species of *Entamoeba* in all five iguanas. The *Entamoeba* found in the Fiji iguanas was most similar genetically to *Entamoeba coli* with 74% homology vs 59% homology to *E. invadens*. This analysis allowed us to exclude the Fiji iguanas as the source of the fatal *Entamoeba invadens* infections in the other lizards. Application of molecular techniques can be a vital ancillary tool to traditional morphologic evaluations in disease investigations and management decisions.

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## SEROLOGIC SURVEY OF THE GREATER PRAIRIE CHICKEN IN WISCONSIN AND MINNESOTA

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### **Abstract**

This report provides the preliminary results of a 5-yr serologic survey of Greater Prairie Chickens (*Tympanuchus cupido pinnatus*) in Wisconsin and Minnesota. Adult, juvenile and chicks were captured as part of a long-term field research project, Prairie Chickens & Grasslands: 2000 and Beyond (PCG2B), supported by Society of Tympanuchus Cupido Pinnatus, Ltd. (STCP). A 2-5 ml blood sample was taken via venipuncture from each prairie chicken. Blood samples were drawn into heparinized syringes or placed in lithium heparin tubes. Starting in August 2000, samples were divided into serum tubes and heparinized tubes.

Four hundred plasma samples collected from January 1998-August 2001 were tested for antibodies to *Pasturella multocida*, *Salmonella typhimurium*, and *S. pullorum*, and no antibody titers were identified. Plasma samples were screened for antibodies to *Mycoplasma gallisepticum* and *M. synoviae* using a plate test, and positive samples were re-tested using a more sensitive test, hemagglutination inhibition (HI) using turkey red blood cells. Of the 415 samples from 1998-2001, the plate test yielded over 183 positive samples but when re-evaluated using the HI assay, only two samples had very low titers for *Mycoplasma gallisepticum*.

Six hundred twenty-five plasma samples collected from August 2001-January 2003 were tested for New Castles Disease and Avian Influenza. All samples were negative for these viruses. One hundred thirty-eight serum samples collected in 2002 from Wisconsin and Minnesota have been tested for antibodies to West Nile virus (WNV). Three samples from Minnesota have low titers, and all other samples are negative for WNV.

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## COMPARATIVE EVALUATION OF THE RENAL EFFECTS OF A SEVEN-DAY THERAPY WITH FLUNIXIN MEGLUMINE, KETOPROFEN AND MELOXICAM IN BUDGERIGAR (*Melopsittacus*)

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### Abstract

### Introduction

Nonsteroidal anti-inflammatory drugs (NSAID) are widely used for the treatment of inflammatory diseases and pain relief in birds clinics practice. The NSAIDs promote analgesic effects, but also present adverse effects. However, little is known about their effect in birds. Low doses of flunixin meglumine caused glomerular injuries after a 7-day treatment in northern bobwhite (*Colinus virginianus*).<sup>3</sup> Renal injuries, renal and visceral gout and death were described in siberian crane followed treatment with flunixin meglumine.<sup>1</sup> But renal injury was not observed in quails (*Coturnix coturnix japonica*) treated with flunixin meglumine at 3.4 mg/kg and in quails treated with meloxicam at 1.0 mg/kg for 3 days or 5 days.<sup>4</sup> Currently meloxicam and ketoprofen are often used in avian clinics practice, since it is believed to produce less clinically important adverse effects than flunixin meglumine.

### Material and Methods

Thirty-two healthy budgerigars (young male and females) were used in this study. Food and water were available *ad libitum*. Birds were randomly assigned to 1 of 4 treatments: group 1- distilled water alone; group 2-flunixin meglumine (Banamine®) at 5.5 mg/kg; group 3- ketoprofen (Ketofen®) at 2.5 mg/kg and group 4-meloxicam (Bioflac®) at 0.1 mg/kg. All drugs were dissolved in distilled water at such concentrations that the dose was delivered in a volume of 0.04 ml for all experimental groups. All birds were injected at chest muscle alternately each 24 hr for 7 days. Following treatment period, blood samples were collected to perform plasma uric acid dosage, and each bird was immediately euthanatized. Kidneys were assessed by macroscopic examination, following histologic processing, staining with hematoxilin-eosin and microscopic examination.

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## Results and Discussion

Plasma uric acid values did not present significant differences among groups. Others authors reported no significant differences on plasma uric acid values in northern bobwhite quails, although renal injuries were present.<sup>3</sup> Therefore, a normal uric acid value does not mean healthy kidneys.<sup>2</sup> Increasing on plasma uric acid level in birds only occurs during extensive tubular disease or due to severe dehydration.<sup>6</sup> No bird on control group presented either glomerular or tubular injuries. Birds treated with ketoprofen presented glomerulate vacuoles (12.5%) and tubular dilatation (12.5%).

Macroscopically examination revealed one bird (1/8) presenting renal paleness after treatment with meloxicam. Furthermore, 12.5% of birds presented glomerular vacuoles, 12.5% glomerular congestion and 12.5% glomerular congestion after treatment with meloxicam. Even NSAID has been selective inhibitor of COX-2 as meloxicam, it produced adverse effects over kidneys, whereas the advantage of using specific COX-2 inhibiting NSAIDs is related to only gastrointestinal tract.<sup>5</sup>

Birds treated with flunixin meglumine presented mesangial hypocellularity (25%) and tubular necrosis (75%). Some authors found similar results in northern bobwhite quails treated with flunixin meglumine such as glomerular injuries, amorphous mineralized deposition, granular and basophilic on renal glomeruli in all birds treated,<sup>3</sup> whereas more aggravated injuries than our results were found in this study. A possible explanation should consider the sensibility of this bird specie as well as absence of hydric fasting in this study. Since other author did not observe renal injury in healthy quails treated with flunixin meglumine at 3.12 to 3.43 mg/kg support this idea.<sup>4</sup> Tubular necrosis was significant in birds treated with flunixin meglumine, which demonstrates that this NSAID has more potential to cause tubular damage in budgerigars when compared to ketoprofen and meloxicam, therefore it should not be used. Further studies are necessary to evaluate the effects of treatments using ketoprofen and meloxicam at long lasting administration periods.

## Conclusion

Ketoprofen, meloxicam and flunixin meglumine present great potential to cause renal injuries in clinically healthy budgerigars submitted to 7-days treatment, whereas the flunixin meglumine presents higher potential to cause tubular and glomerular injuries, followed by meloxicam and ketoprofen.

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## COMPUTED TOMOGRAPHY (CT) AS A COMPLEMENTARY EXAM FOR THE EVALUATION OF AN ORANGUTAN (*Pongo pygmaeus*) WITH SOFT PARESIS OF THE PELVIC LIMBS: DESCRIPTION OF A CASE

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### Abstract

Computed tomography (CT) is a sectional image obtained free of superposition by overlaying structures. Images may be obtained in transverse, dorsal, sagittal and oblique planes. The use of CT in the evaluation of the vertebral spine has been indicated after myelogram. The tomography exam can highlight the sight of the lesion and spinal cord compression. This work was conducted to describe the use of computed tomography in the clinical examination of an adult male orangutan (*Pongo pygmaeus*), belonging to Fundação Parque Zoológico de São Paulo, which had flacid paresis of the pelvic limbs after being observed during ten days in a restraining cage for dyspnoea treatment. The animal was sent to Diagnostic of Imaging Service of Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo for survey radiography, myelogram and CT. Ketamine hydrochloride (Dopalen®, vetbrands, 10mg/Kg) and midazolam (Dormire®, Cristália, 0.5 mg/kg) intramuscularly were used to induce anesthesia and maintenance was done with isoflurane in 100% oxygen. Survey films were taken of the spine, then myelogram and CT were performed. The survey radiographs revealed spondylosis at the thoracolumbar and lumbosacral regions. The myelogram detected contrast at the cervical region, and progression could not be determined. In thoracic and lumbar regions of the CT (5 mm thick slices) the interruption of the contrast column progression was seen between T9-T10. Soft tissue attenuation mass was observed in the right portion of the medullar canal, between T8-T9, extending cranially for approximately 1.5 cm, causing significant extradural compression. Therefore, CT was fundamental in diagnosing the extradural compression that was accountable for the pelvic limb flacid paresis of this orangutan. Neoplasia was suspected as the cause of the illness. Surgery was conducted successfully and histopathology is being done to define the type of tumor affecting this orangutan.

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## PROLIFERATIVE PNEUMONIA DUE TO INTRACELLULAR PROTOZOA IN RADIATED TORTOISES (*Geochelone radiata*)

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### Abstract

Infectious causes of pneumonia in tortoises include viruses, bacteria, fungi, and parasites. An uncommonly recognized cause of pneumonia in tortoises is an intranuclear protozoa reported in several species of captive tortoises in the United States.<sup>3</sup> This has been described in two captive-bred juvenile radiated tortoises (*Geochelone radiata*), two adult radiated tortoises, one wild caught adult impressed tortoise (*Manouria impressa*), one captive-bred juvenile leopard tortoise (*Geochelone pardalis*), and three Travancore tortoises (*Indotestudo forstenii*).<sup>1-3</sup> All tortoises had intranuclear coccidial parasites in a variety of epithelial tissues. Inflammation of the lung was noted in five tortoises and one had a proliferative pneumonia.<sup>1-3</sup>

Over a period of 4 mo, four young adult radiated tortoises became weak and lethargic with mouth breathing before they died. The animals were kept together in the same exhibit and had been moved to this enclosure 5 mo earlier. The first two tortoises of the outbreak had a proliferative pneumonia with inflammatory exudate and intranuclear inclusions within respiratory epithelial cells. These inclusions could be found in a variety of other epithelial cells (bile ducts, gastric mucosa, and thyroid follicular epithelium). By electron microscopy it was determined that the protozoa were within the nucleus and some appeared to be invaginating or pushing into the nucleus. Their morphology is compatible with an apicomplexa protozoan. These protozoa reacted weakly with *Sarcocystis neurona* antisera and were negative by immunohistochemistry for *Toxoplasma gondii*, *Neospora*, and *Sarcocystis falcatula*.

Various therapies and supportive care were used with little effect. The last two tortoises also developed pneumonias; however, the previously described epithelial inclusions were not recognized. Both of these tortoises were given potentiated sulfonamides (Tribrissen® Schering-Plough Animal Health Corporation, 1095 Morris Ave., Union, New Jersey, 07083, USA) as part of their therapies. Four other radiated tortoises and one Madagascar angulated tortoise (*Geochelone yniphora*) penned with these four, were relocated and have survived.

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## PREVALENCE OF PATHOGENIC ENTERIC BACTERIA IN WILD BIRDS ASSOCIATING WITH AGRICULTURE IN HUMBOLDT COUNTY, CALIFORNIA

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### Abstract

Assessing the prevalence of pathogenic bacteria wild birds carry is important to understanding their role in disease transmission, especially in environments that bring these birds close to domestic livestock. Cloacal swabs from 243 wild birds from five dairy farms in Humboldt County, California (USA), were cultured for enteric bacteria between July 2002 and February 2004. Fecal swabs from 80 dairy cattle were also cultured for enteric bacteria to determine if the cattle and birds shared similar bacterial species, indicating possible transmission. Birds sampled included 34 European starlings (*Sturnus vulgaris*), 56 brown-headed cowbirds (*Molothrus ater*), 65 house sparrows (*Passer domesticus*), 29 white-crowned sparrows (*Zonotrichia leucophrys*), 16 Brewer's blackbirds (*Euphagus cyanocephalus*), and 43 red-winged blackbirds (*Agelaius phoeniceus*). Among all birds, the most prevalent enteric bacteria were *Escherichia coli* (40%), *Enterobacter* spp. (19%), *Citrobacter* spp. (11%), *Proteus* spp. (8%), *Pseudomonas* spp. (6%), *Klebsiella* spp. (5%) and *Yersinia* spp. (5%). *Escherichia coli* (92%) was by far the most common bacterium cultured from cattle feces; followed by *Citrobacter* spp. (20%), *Klebsiella* spp. (11%) and *Proteus* spp. (11%). Preliminary analysis indicates that bacterial species composition varied more among cattle on different farms than among bird species on different farms, although birds carried a wider range of enteric bacteria than cattle. Furthermore, few bacteria were shared between birds and cattle. However, both birds and cattle carry potentially pathogenic bacteria at low prevalences.

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## PRELIMINARY STUDY OF THE HEMATOLOGIC PROFILE OF BRAZILIAN BIRDS OF PREY IN SÃO PAULO CITY

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### Abstract

Birds of prey are predatory birds, characterized by strong beaks and talons, belonging to Falconiform and Strigiform orders.<sup>9</sup> Latin America has a large diversity and number of these animals; however, hematologic data for the wildlife species in this area are few, in contrast to the data available for wildlife species from other areas. The aim of this research was to develop a hematologic profile for birds of prey in São Paulo city (Brazil) in an attempt to establish reference ranges for diagnostics.<sup>3-7</sup> Thus, blood samples from 68 animals of four different species presenting to DEPAVE-3 were collected between July and December, 2003. Birds received a physical exam under manual restraint and information on origin, age, sex (if possible), nutritional condition and disease status were noted on a clinic form. Blood samples were collected from the jugular or wing vein and were stored both in tubes containing EDTA and tubes without anticoagulant. These samples were processed on the day of collection in the Laboratory of Wildlife Comparative Pathology, College of Veterinary Medicine, São Paulo University. Blood stored without anticoagulant was used to prepare blood smears. Smears were stained (Rosenfeld)<sup>1</sup> and reviewed to identify and quantify erythrocytes and leukocytes and estimate thrombocyte numbers. Total erythrocyte count (TRBC) and total leukocyte count (TWBC) were determined using a hemocytometer (Natt and Herrick's method).<sup>2,8</sup> Hemoglobin concentration was measured by the cyanmethemoglobin method using the Lab Test<sup>®</sup> kit. Packed cell volume (PCV) and total plasma protein (TPP) were determined via microhematocrit centrifugation and the use of a refractometer.<sup>2,8</sup> In order to facilitate analysis of data, the individuals from each species were classified as healthy and unhealthy (most often trauma). Table 1 shows the hematologic values (arithmetic mean and standard deviation) for the four species: striped owl (*Rhinoptynx clamator*; n = 26), tropical screech owl (*Otus choliba*; n = 17), common caracara (*Polyborus plancus*; n = 14), and roadside hawk (*Rupornis magnirostris*; n = 11).

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**Table 1.** Hematologic values (arithmetic mean  $\pm$  standard deviation) for four species of birds of prey found in São Paulo city, Brazil.

Parameter	Striped Owl ( <i>Rhinoptynx clamator</i> ) n = 26		Tropical Screech Owl ( <i>Otus choliba</i> ) n = 17		Common Caracara ( <i>Polyborus plancus</i> ) n = 14		Roadside Hawk ( <i>Rupornis magnirostris</i> ) n = 11	
	Healthy n = 10	Unhealthy n = 16	Healthy n = 10	Unhealthy n = 7	Healthy n = 8	Unhealthy n = 6	Healthy n = 7	Unhealthy n = 4
TRBC ( $\times 10^{12}/L$ )	1.95 $\pm$ 0.58	2.13 $\pm$ 0.76	2.45 $\pm$ 0.74	1.99 $\pm$ 1.46	2.53 $\pm$ 0.43	2.58 $\pm$ 0.51	2.96 $\pm$ 1.58	2.94 $\pm$ 1.02
Hb (g/dl)	8.18 $\pm$ 2.09	9.08 $\pm$ 1.47	8.69 $\pm$ 1.96	7.9 $\pm$ 1.71	9.54 $\pm$ 3.49	12.03 $\pm$ 2.11	10.32 $\pm$ 2.22	9.92 $\pm$ 1.91
PCV (%)	33.1 $\pm$ 4.62	35.5 $\pm$ 3.07	31.7 $\pm$ 4.34	34.57 $\pm$ 8.50	36.62 $\pm$ 7.78	39.83 $\pm$ 5.45	36.57 $\pm$ 6.62	42.75 $\pm$ 4.92
MCV (fl)	209.23 $\pm$ 170	188 $\pm$ 75.71	136.1 $\pm$ 29.14	231.07 $\pm$ 160.32	146.63 $\pm$ 7.78	159.57 $\pm$ 37.20	148.58 $\pm$ 57.08	155.95 $\pm$ 41.55
MCH (pg)	51.71 $\pm$ 43.99	48.44 $\pm$ 20.49	38.18 $\pm$ 14.95	58.1 $\pm$ 37.53	37.95 $\pm$ 13.24	48.97 $\pm$ 15.89	33.55 $\pm$ 14.71	36.55 $\pm$ 13.79
MCHC (%)	24.58 $\pm$ 5.23	25.66 $\pm$ 3.95	27.44 $\pm$ 5.74	23.41 $\pm$ 4.52	25.86 $\pm$ 6.5	30.21 $\pm$ 3.44	29.06 $\pm$ 9.3	23.31 $\pm$ 4.44
TWBC ( $\times 10^9/L$ )	16.85 $\pm$ 9.55	17.50 $\pm$ 12.27	8.4 $\pm$ 3.16	12.42 $\pm$ 9.25	14.18 $\pm$ 13.27	13.16 $\pm$ 3.23	10.78 $\pm$ 3.74	6.62 $\pm$ 3.44
Heterophils (%)	63.22 $\pm$ 19.81	70.62 $\pm$ 13.97	38.7 $\pm$ 18.69	54.71 $\pm$ 15.38	77.75 $\pm$ 11.11	91.16 $\pm$ 4.75	53.85 $\pm$ 19.84	55 $\pm$ 26.85
Lymphocytes (%)	16.88 $\pm$ 10.94	17.62 $\pm$ 9.79	21.7 $\pm$ 4.39	13.28 $\pm$ 11.04	19.12 $\pm$ 9.43	6.5 $\pm$ 4.18	14.7 $\pm$ 12.27	12 $\pm$ 8.28
Eosinophils (%)	14.88 $\pm$ 19.27	6.68 $\pm$ 5.38	33.6 $\pm$ 16.6	25.14 $\pm$ 18.66	0.37 $\pm$ 0.74	0.83 $\pm$ 1.16	20.28 $\pm$ 17.10	24.75 $\pm$ 15.30
Monocytes (%)	5 $\pm$ 3.12	4.93 $\pm$ 5.28	5.5 $\pm$ 6.02	6.71 $\pm$ 6.1	2.75 $\pm$ 2.54	1.16 $\pm$ 1.47	10.57 $\pm$ 7.13	7.5 $\pm$ 5.06
Basophils (%)	0	0.12 $\pm$ 0.5	0.5 $\pm$ 0.97	0.14 $\pm$ 0.37	0	0.33 $\pm$ 0.81	0.57 $\pm$ 1.13	0.75 $\pm$ 0.95
Thrombocytes ( $\times 10^9/L$ )	24.94 $\pm$ 11.07	29.13 $\pm$ 12.84	31.21 $\pm$ 13.01	24.16 $\pm$ 18.92	29.18 $\pm$ 10.36	31.97 $\pm$ 10.01	37.97 $\pm$ 34.69	30.17 $\pm$ 17.65
TPP (g/dl)	4.54 $\pm$ 0.71	4.3 $\pm$ 0.96	5.57 $\pm$ 0.56	5.27 $\pm$ 0.64	4.48 $\pm$ 0.73	4.15 $\pm$ 0.72	5.35 $\pm$ 0.65	5.95 $\pm$ 0.7

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**PHAEOHYPHOMYCOSIS IN A FREE-LIVING EASTERN BOX TURTLE (*Terrapene carolina carolina*)**

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**Abstract**

In December 2003, an adult male Eastern box turtle (*Terrapene carolina carolina*) was presented to The Wildlife Center of Virginia (WCV) after approximately 2 mo in a rehabilitation facility for marked swelling of the right hindlimb. In September 2003, a mass from the area of the right proximal tibia was removed and histopathologically diagnosed as chromomycosis. On presentation, the turtle was 450 g, well hydrated, and in good body condition. Swelling with localized edema was visible around and distal to the right stifle, and a circumferential mass was palpable around the right proximal tibia. The lateral two digits were missing from the right hind foot. Radiographs revealed marked soft tissue swelling of the entire right hindlimb, particularly the caudal and plantar aspects. No additional abnormalities were noted. The turtle was euthanatized due to the severity of the soft tissue involvement.

On gross necropsy, the liver was diffusely pale, and a black branching line was visible on the surface of the right lobe. Multifocal darkened areas of the lungs were also noted. There was an adhesion between the skin and underlying tissue on the caudolateral aspect of the right hindlimb at the level of mid-tibia, and a remnant of suture material remained cranially. Subcutaneously, an approximately 3 cm × 1 cm encapsulated mass ran the entire caudal aspect of the lower right hindlimb. The capsule was filled with a dark brown-black, friable necrotic material. On histopathology, there were pale and clear hepatocytes in the liver. There was no microscopic evidence in any of the submitted tissues (lung, heart, esophagus, stomach, spleen, pancreas, liver, kidney, testicle, intestine, cloaca, and right hindlimb integument) of a systemic fungal infection. Histopathology of the subcutaneous soft tissue of the right hindlimb showed a mass-like granulomatous inflammatory process including a mix of lymphoid cells, eosinophils and histiocytes. Included were numerous multinucleated histiocytic giant cells, often arranged in ringed groups around necrotic debris. Numerous fungal elements were seen within the necrotic centers and associated with multinucleated cells. The fungi were phaeoid (brown) hyphae and yeast-like cells. With Gomori methenamine silver stains, they appeared as chains of ovoid yeast-like bodies (conidia) as well as short rectangular hyphae with occasional right-angle budding. In addition, *Torulopsis (Candida) glabrata* and *Exophiala (Phialophora) jeanselmei* were isolated from fungal culture of the empty lesion capsule and a section of necrotic tissue. The final diagnosis was phaeohyphomycosis of soft tissues.

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*Torulopsis glabrata* is a yeast that is normal flora of the mouth, gut, or urinary tract (in humans) but, in weak or immunocompromised patients, can cause opportunistic infection.<sup>6</sup> We suspect that *T. glabrata* was a contaminant, as none of the yeast were identified in the histopathology of any of the submitted tissues. *Exophiala jeanselmei* is a saprophytic dematiaceous (pigmented) fungus that is most commonly found in decaying wood and soil that is enriched with organic waste, as well as polluted water and sewerage.<sup>3,5,8,9</sup> It is also considered opportunistic, but phaeohyphomycosis caused by *Exophiala* species has been reported in both immunosuppressed and immunocompetent patients.<sup>10</sup>

Chromomycosis is a general term for a group of clinicopathologic syndromes including superficial chromomycosis, chromoblastomycosis, and chromohyphomycosis (used interchangeably with phaeohyphomycosis).<sup>1,8,9</sup> The distinction lies in tissue location (cutaneous vs. extracutaneous)<sup>7,8</sup> and fungal form (hyphal vs. muriform cells).<sup>1,3,8,9</sup> It is most often associated with traumatic inoculation<sup>2,5,8,10</sup> and/or immunocompromised hosts.<sup>1,7</sup> The condition has been described in mammals, reptiles, amphibians, crustaceans, fish, and birds.<sup>1,2</sup> Infection can be diagnosed cytologically or histologically, but the causative agent must be isolated by culture.<sup>1,7</sup> Many different courses of chemotherapy are described, but many agree that efficacy is questionable.<sup>10</sup> Excision or debulking is recommended before chemotherapy begins and is potentially curative.<sup>3,5,7,8,10</sup> Some consider itraconazole to be the drug of choice<sup>3,5,7,10</sup> while others report it to be ineffective.<sup>8</sup> Ancillary flucytosine,<sup>3,7,10</sup> terbinafine,<sup>5</sup> or ketoconazole<sup>4,5</sup> are also sometimes used. Fluconazole and Amphotericin B appear to be subject to resistance<sup>1</sup> or simply ineffective.<sup>7</sup>

When dissected, there was a small opening in the caudodistal portion of the subcutaneous capsule and although the integument showed no obvious evidence of trauma, the absent digits of the right hindlimb suggested such; however, we were unable to determine definitively whether this infection was the result of direct inoculation, immunosuppression, or both.

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## USE OF UNPASTEURIZED HONEY FOR TREATMENT OF A DEEPLY INFECTED WOUND IN AN AFRICAN ELEPHANT (*Loxodonta africana*)

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### Abstract

#### Case Report

A 26-yr-old female African elephant (*Loxodonta africana*) received a deep laceration to the neck from the tusk of another elephant. The wound originated approximately 10 cm caudal to the middle of the right pinna, extended ventromedially, and penetrated multiple muscle layers. The wound was approximately 10-12 cm wide and 25-30 cm deep.

Initial treatment involved wound lavage with sterile saline twice daily, sulfadimethoxine/ormetoprim (Primor<sup>®</sup>, Pfizer Animal Health, Exton, Pennsylvania 19341, USA; 8.5 mg/kg p.o., b.i.d.), and ibuprofen (Pharmacia and Upjohn, Kalamazoo, Michigan 49001, USA; 2 mg/kg p.o., b.i.d. as needed). There was purulent discharge from the wound on day 5, therefore topical wound dressing was initiated. After wound lavage, the wound cavity was packed with laparotomy sponges coated with a 1:1 mixture of 1% silver sulfadiazine cream (BASF Corporation, Mount Olive, New Jersey 07828, USA) and an anti-inflammatory ointment (hemorrhoidal ointment, CVS Pharmacy Inc., Woonsocket, Rhode Island 02895, USA). Despite aggressive topical and systemic therapy, the wound became progressively more purulent, necrotic, and malodorous. On day 11, the wound dressing was changed from silversulfadiazine cream / hemorrhoidal ointment to laparotomy sponges coated with unpasteurized honey (Eisele's Raw Honey, Westfield, Indiana 46074, USA). On day 16, oral antibiotics were changed from sulfadimethoxine / ormetoprim to enrofloxacin (Baytril<sup>®</sup>, Bayer Corporation, Shawnee Mission, Kansas 66201, USA; 1.5 mg/kg p.o., s.i.d.) based on culture and sensitivity results. After 5 wk of therapy (day 51), enrofloxacin was discontinued due to poor patient compliance. Wound care from day 52 until completion of healing (12 additional weeks) consisted of twice daily wound lavage and dressing with unpasteurized honey. By day 101, wound care was decreased to once daily. On day 138 wound care was discontinued, and on day 143 the wound was considered healed.

Within 4 days of beginning topical treatment with honey, subjective scores of purulent exudate, necrotic tissue, and malodor began to improve. By day 29, the wound was no longer malodorous. Minimal necrotic tissue remained in the wound on day 37, and purulent discharge had resolved by day 90.

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A single-dose oral enrofloxacin pharmacokinetic study was performed to evaluate serum and milk levels of the drug. Following oral administration of enrofloxacin at 1.5 mg/kg, serum levels were subtherapeutic at all time points over 24 hr.

## **Discussion**

Unpasteurized, or raw, honey has been used as a medicine for centuries. Many ancient cultures used honey to treat skin wounds, gastric ulcers, diarrhea, eye disorders, and cough.<sup>7</sup> There are many reports in the human medical literature of wound dressing with unpasteurized honey, but there are very few reports of its use in clinical veterinary medicine.<sup>4,5</sup>

The success of unpasteurized honey as a wound dressing is due to its antibacterial, anti-inflammatory, immune-stimulating, tissue-debriding, and tissue-nourishing properties. High osmolality, phytochemicals, production of hydrogen peroxide, and stimulation of leukocyte activity contribute to the overall antibacterial activity of honey. Raw honey reduces inflammation by eliminating bacterial production of pro-inflammatory antigens and cytotoxins,<sup>8</sup> reducing local edema by osmosis,<sup>6</sup> and contributing antioxidants that scavenge free radicals.<sup>2</sup> Immune system stimulation includes activation of neutrophils, stimulation of lymphocyte proliferation,<sup>1</sup> and release of immune-mediator compounds by monocytes.<sup>11</sup> Dressing wounds with raw honey often eliminates the need for surgical debridement.<sup>10</sup> Honey improves tissue regeneration by stimulating the development of new capillary beds, thereby increasing nutrient delivery and oxygen supply to tissues.<sup>3,6</sup> Raw honey also provides the moist environment necessary for proliferation of epithelial cells and fibroblasts.<sup>8</sup>

Honey is easy to use as a wound dressing. It can be spread directly onto wounds, soaked into gauze, or used to fill cavities. It generally causes no pain upon application.<sup>7</sup> Plasma or lymph is drawn out of tissues by osmosis, creating a layer of dilute honey in contact with the wound surface; there is minimal adhesion of bandage materials to cause pain or tissue damage when dressings are changed.<sup>6</sup> Honey dressings can be changed daily, but can be changed more frequently if the wound is infected or contaminated; bandages can be changed less frequently if the wound is clean and dry.<sup>6</sup> Any residual honey is easily removed with warm water. Solidified honey can be returned to the liquid form by warming to 37°C. Honey should not be heated excessively because the enzyme that produces hydrogen peroxide is easily inactivated by heat.<sup>6</sup> Although honey may contain clostridial spores, there are no published reports of wound botulism.

In this case, no adverse effects resulted from using unpasteurized honey as a wound dressing. Necrosis and malodor were greatly decreased within 16 days and purulent discharge was drastically reduced within 23 days of beginning treatment with honey. Subjectively, the wound healed faster and with less scar tissue than expected for this elephant as well as in comparison to wounds in other elephants. Raw honey likely provided the primary antibacterial activity during

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wound healing since enrofloxacin serum levels were subtherapeutic. Unpasteurized honey should be considered for topical treatment of deep, infected wounds in elephants.

#### **ACKNOWLEDGMENTS**

The authors thank Ellen Clark, RVT, Jennifer Niederlander, RVT, David Hagan, Barre Fields, Don Nevitt, Jill Sampson, Leslie Mackie, Adam Cheek, Niki Jordan, and Shea Earley for assistance with wound management, and Susan Mikota, DVM for consultation regarding the case.

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## A SURVEY OF BACTERIAL MICROFLORA OF SOUTHERN SEA OTTERS (*Enhydra lutris nereis*): PRELIMINARY FINDINGS

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### Abstract

For the southern sea otter (*Enhydra lutris nereis*), a federally-listed threatened species that has a limited geographic range, the determination of putative “normal” and potentially pathogenic bacterial microflora is crucial to understanding the health of the population and assessing risk of disease. From 1998 to 2003, standardized sampling protocols were employed to identify microorganisms present on or in tissues and feces of live-captured and necropsied southern sea otters. Samples for evaluation of bacterial microflora from live and dead otters were submitted to the UC Davis School of Veterinary Medicine, where standardized methods and media were employed for evaluation of microflora present in each sample. All areas of the southern sea otter range are represented in the study, including San Nicholas Island. Data was analyzed for relationships between isolates and sea otter location, age, sex and live or dead status at time of sampling. All recognized human and terrestrial animal fecal pathogens selected for study were identified in sea otter feces with the exception of *E. coli* (0157:H7). Lists of putative “normal” and potentially pathogenic bacterial microflora of southern sea otters have now been established.

### ACKNOWLEDGMENTS

The studies included in this survey were supported by a variety of sources, including the Town of Pacific Grove, California Department of Fish and Game, Monterey Bay National Marine Sanctuary, National Oceanic and Atmospheric Administration/National Marine Fisheries and United States Geological Survey. In particular, we recognize the outstanding fieldwork of the following individuals: Jack Ames, Michelle Staedler, Jennifer Coffey, Deborah Brownstein, Michael Murray, David Jessup, Christine Kreuder, Michael Harris, Heather Harris, and Alisha Kage.

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## THE ZOONOTIC DISEASE PREVENTION PROGRAM IN THE MASOALA RAINFOREST ECOSYSTEM EXHIBIT AT ZURICH ZOO

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### Abstract

In June 2003, Zurich Zoo in Switzerland opened a new rainforest exhibit based on and linked to the Masoala National Park in Madagascar. The “MASOALA RAINFOREST” ecosystem at Zurich Zoo exhibits on an area of 11,000 m<sup>2</sup> animals and plants from the Madagascan rainforest. The free roaming animal collection comprises representatives of 30 different species from insects (e.g., *Gromphadorhina portentosa*), amphibians (e.g., *Dyscophus antongilii*), reptiles (e.g., *Geochelone gigantea*, *Phelsuma madagascariensis*) birds (eg *Scopus umbretta*) and mammals, including different species of primates (e.g., *Pteropus rodricensis*, *Varecia variegata rubra*). Temperatures in the ecosystem vary from 18°C during night time up to 40°C during the day with an ambient relative humidity above 65%. The unique animal collection, tropical climate, possible public animal contact, size of the exhibit and semifree condition make veterinary care challenging especially in regards of zoonotic disease prevention.

The zoonotic disease prevention program in the Masoala rainforest ecosystem at Zurich Zoo regulate that all arriving animals, even animals from within the country, have to pass through a quarantine procedure according to state veterinarian regulations or an extended protocol with additional clinical and laboratory investigations. The quarantine procedure is carried out at the veterinary clinic and quarantine facility at Zurich Zoo, which is accredited by USDA and is physically separated from the zoo. In addition, animals within the exhibit are screened for potential diseases on a regular basis, especially primate faeces is examined for pathologic bacteria and parasites biannually. In cooperation with the state physician an annual health check for all employees was established and carried out by a consulting physician, specialized for zoonotic diseases. The consulting physician is also available on demand for all staff members year around and is informed about the situation within the zoo. A potential health risk was especially examined for the exhibit’s water distribution system. Standing water in puddles and water pipes for fog and artificial rain can reach temperatures between 20 to 40°C due to high environmental temperatures in the exhibition and carries an increased risk for *Legionella* ssp. colonization. Therefore water pipes are emptied after every use and water quality is tested within the rain/fog system, as well in the river and lake system for *Legionella* ssp. quarterly. Based on the current experiences the applied zoonotic disease prevention program appears effective in identifying potential pathogens during quarantine and within the ecosystem.

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**TONGUE TIP CONSTRICTION DUE TO WOOD FIBERS IN A GIANT ANTEATER  
(*Myrmecophaga tridactyla*)**

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**Abstract**

An approximately 10-yr-old male giant anteater (*Myrmecophaga tridactyla*) exhibited partial, intermittent anorexia, soft stool and bleeding from the mouth over a 4-wk period. The giant anteater was anesthetized with xylazine (3.4 mg/kg; Rompun,<sup>®</sup> Bayer AG, Leverkusen, Germany) and ketamine (13 mg/kg; Narketan<sup>®</sup> 10, Vétoquinol AG, Belp Bern, Switzerland) by intramuscular injection for diagnostic investigations. A constriction due to wood fibers was detected by endoscopy and removed in the distal part of the tongue. All other clinical findings were normal and hematology and blood biochemistry results were within reference range. Treatment was effective and the animal returned to normal health and feeding behavior within five days.

Wood fibers were present in peat, which was included as a dietary supplement to improve stool consistency. The giant anteater diet consisted of fatless meat, low fat curd cheese, seasonal fruits, tomatoes, oatmeal, dog pellets, boiled eggs, bruised shrimp, chitin replacement and peat. Further investigations revealed that a switch to another commercial source of peat had resulted in a peat type which included elongated fibers. The risk for further problems was eliminated by careful sieving of the peat before inclusion in the diet. To prevent future tongue injuries in giant anteaters it is recommended to avoid any elongated fibers in the diet by careful chopping or sieving of all dietary elements.

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## THE SEABIRD ECOLOGICAL ASSESSMENT NETWORK: A PROJECT OF TUFTS UNIVERSITY CENTER FOR CONSERVATION MEDICINE

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### Abstract

The overall goals of SEANET are to:

- Form a network of researchers and citizen scientists
- Perform beached bird surveys
  - volunteers, school science class participation
  - compile a database of seabird information
  - population estimates
  - seasonal distribution patterns
  - disease outbreaks
  - mortality events
  - ecological and anthropogenic threats
  - patterns of environmental contaminants in birds
  - pollutant source locations (heavy metal, petroleum)
  - regional oil shipping and distribution patterns
  - oil spill locations
- Formulate GIS maps of above patterns (searchable on internet, with NBII, US EPA)
- Maintain an on-going web-based strandings reporting system (with NBII)
- Hold annual workshops (Northeast Regional Fish and Wildlife Conference)
  - dissemination of information to network participants
  - development of regional plans for seabird and habitat protection (with NAWCP)

### Conservation Medicine, SEANET, and Human Health

Conservation medicine is a relatively new discipline, focusing on the health of humans, animals, and ecosystems. Human development, population growth, and modern technologies inevitably lead to altered landscapes, increased demands on limited resources, and decreased air and water quality. The human influence on the environment has health implications for wildlife, domestic animals, and ourselves in the form of emerging disease, species extinctions, and the loss of ecosystem services upon which we rely.

The Seabird Ecological Assessment Network (SEANET), a project of the Tufts Center for Conservation Medicine, the Lloyd Center for Environmental Studies, the Mass Audubon Wellfleet Bay Wildlife Sanctuary, Wildlife Trust and several other collaborators, aims to make the link between marine ecological health and human health by monitoring seabird mortality in

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the northeastern U.S. and Atlantic Canada. Numerous threats contribute to mortality, including disease, fisheries operations, organic pollutants, heavy metals, offshore development (potentially wind farms), and oil pollution. These risks to seabirds also threaten the coastal and marine environments used by humans for respite and ecological services, such as food production, waste elimination, and flood protection. Pinpointing areas of concern enables SEANET and our collaborators to focus on specific causes of mortality or ecological degradation, and propose policy and conservation measures to counteract the threats.

#### Examples

- Seabird distribution has been known to be an **indicator of fisheries resources** in time and space, allowing resource managers to predict future catches in some cases.
- Seabirds also are long-lived, near the top of the food chain, and **serve as good indicators or sentinels to alert us to threats that might not be obvious**. For instance, the extent and timing of the damage from **oil or contaminant spills** is often difficult to pinpoint, but seabirds can be used as indicators of the risks to human health posed by eating seafood from or spending time in the affected marine and coastal environment. Because dead birds can be examined for signs of internal oiling and feathers and other tissues can be analyzed for contaminant levels long after a spill or discharge has taken place, they are useful indicators of subtle ecological damage. Technology also exists to pinpoint sources of oil: samples can be easily obtained from feathers, and polluters can be prosecuted.
- Beached bird mortality can be used to **pinpoint spread of diseases**, particularly those that could pose a risk to other species and humans. Although little is known about the impacts of harmful algal blooms (marine biotoxins, from events like “red tides”) on seabirds, there have been large-scale marine bird mortality incidents that were traced to harmful algal blooms. Birds that eat species of fish or shellfish that contain biotoxins can be indicators of risk to humans, and might also be used to track “hotspots” of blooms which potentially could be mitigated by reducing human generated run-off.
- Wind farms pose a new form of development in the marine and coastal environment that have the potential to disrupt bird migrations, and even to cause substantial mortality. **Beached bird surveys provide baseline data on the numbers, species, and geographic locations of marine (and other) bird mortalities and deposition on beaches before wind farm development**. If wind farms or even test towers are then built in these areas, levels of bird mortality that are recorded could be compared to the pre-wind farm baseline. Particularly for offshore wind energy development, very little is known about bird mortality impacts, so such data could prove very useful and applicable to the rapidly emerging issue of offshore development.
- Seabirds are sometimes victims of marine fisheries operations, caught as bycatch along with the desired resource, but it is difficult to estimate the magnitude of this problem. By collecting seabird specimens that have been caught in gillnets and by other means, we hope to develop a descriptive pathology for such birds, and gather baseline data on levels of disease, contaminants, and biotoxins in a wide range of species. Seabirds that seem to have been caught in gillnets or other fishing gear sometimes wash up on beaches in fairly

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large numbers, but there is no definitive pathology associated with specific fisheries as causes of death. With the **development of a standard pathology, cause of death could be more accurately determined, and the impacts of bycatch could be better assessed.** With knowledge of timing, seasonality, location, and frequency of bycatch by particular fishing methods on different species, **impacts of fisheries operations on seabirds could be mitigated by shifting season, location and/or timing of fishing.**

Overall we hope to pinpoint geographically and temporally some of the most detrimental threats to marine bird populations, target specific conservation measures to alleviate those threats, and educate the public about conservation of the larger marine ecosystem.

### Summary of Progress

1. Established monthly **beached bird surveys** conducted by an Atlantic coastal network of trained volunteers and students collecting data on seabird mortality. Approximately 50 volunteers are walking 50 miles of beach in Massachusetts and Rhode Island; in addition, several agency and non-profit personnel are reporting mortality events. This winter, we hope to expand beached bird surveys to NH and ME and south to Delaware Bay.
2. Strengthened a **bycatch recovery** effort, in collaboration with the US National Marine Fisheries Service, to develop a descriptive pathology for such birds, and for baseline data on levels of disease, contaminants, and biotoxins in a wide range of species.
3. Planning for production of an **Atlantic guide to beached birds**, an important resource for everyone involved in data-gathering in the field, in collaboration with Bird Studies Canada and the Coastal Observation and Seabird Survey Team (COASST). Production should start this fall after successful fund-raising efforts by BSC and Tufts.
4. Initial stages of construction of **web-based, searchable databases and interactive GIS maps** for the assessment of risk factors and mortality patterns of seabird populations, in collaboration with the National Biological Information Infrastructure (of the USGS) and US EPA. This system also will house a web-based reporting system, allowing volunteers to enter data directly. As an initial focal region, we will start with Massachusetts. Completion of MA breeding seabird GIS maps, mortality data collected, and other associated environmental parameters is on target for this fall.

### Current Network Participants:

- Tufts Center for Conservation Medicine
- Lloyd Center for Environmental Studies
- Maine Coastal Program
- ReMaine Wild
- Avian Haven
- National Audubon Society Seabird Restoration Project
- Maine Audubon Society
- Manomet Center for Conservation Sciences
- Massachusetts Audubon Society

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- Massachusetts Division of Fisheries and Wildlife
  - HSUS Cape Wildlife Center
  - Wild Care
  - US Fish and Wildlife Service
  - US Environmental Protection Agency
  - National Marine Fisheries Service
  - National Biological Information Infrastructure (NBII), US Geological Survey
  - US National Park Service
  - MA Dept. of Coastal Zone Management
  - The Nature Conservancy
  - Bird Studies Canada
  - Wildlife Trust (NY)
  - NY State Department of Environmental Conservation
  - Volunteers for Wildlife (NY)
  - Wildlife Rescue Center of the Hamptons
  - Riverhead Foundation for Marine Research and Preservation
  - NY State Parks Department
  - NY State Office of Parks, Recreation, and Historic Preservation
  - NY City Parks Department
  - NY City Audubon Society
  - New Jersey Audubon Society
  - New Jersey Division of Fish and Wildlife
  - TriState Bird Rescue and Research

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## AN EXAMPLE OF CONSERVATION THROUGH COLLABORATION: LIMBE WILDLIFE CENTRE, CAMEROON

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### **Abstract**

For a conservation project to succeed, engagement with local communities and understanding of the local culture is vital. Long term goals must be set, and a level of trust and respect between project and community must be reached before real progress can be made.

The Nigerian based NGO Pandrillus, formed by Peter Jenkins and Liza Gadsby to help in their work with drill and chimpanzee rehabilitation, began working at Limbe Wildlife Centre (LWC) in 1994. The Government of Cameroon is a partner with Pandrillus through the Ministry of Forests and Environment. Most of the animals that come to the LWC are orphans, donated by their previous owners, or confiscated by wildlife officials. Primates are orphaned by hunters who shoot the mothers for bush-meat, or by loss of habitat through forest destruction. Cameroon is a relatively prosperous and stable society compared to its neighbours. As such, its mission statement reflects a goal that, in recent years, appears to be being achieved.

*LWC Mission statement: To secure the ultimate survival of threatened and endangered species, the Limbe Wildlife Centre focuses on conservation education to raise awareness and change attitudes towards Cameroon's unique wildlife.*

This mission has been successfully realized by concentrating on the three following areas.

### **Rescue**

Predominately primate species (including drills, chimpanzees, mandrills, gorillas and various guenons and mangabey species). All species are accepted however, and return to the wild of other bird and mammal species after rehabilitation is conducted similar to any wildlife rehabilitation centre worldwide. As members of the public are now volunteering animals, a more thorough history of where animals originate is possible. The centre currently houses 140 primates, with 20 new arrivals annually. Over the last 10 yr, hundreds of birds and non primate mammals have been released to suitable habitat, usually close to their origin. Good relations with the Cameroon Government are vital. Most of the workers at LWC are Government employees, including a wildlife official authorized to confiscate wildlife being held illegally.

Educational material is provided for animal donors on bushmeat and conservation in Cameroon, in local Pidgin, English and French. This is vital, as payment is never made for animals, and

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donors are never compensated for any materials, such as food, they may have provided the animal, to try and negate the perception of animals as an income source. Maintaining good relations allows periodic back up checks on donors, to make sure they are not obtaining more animals. International Funding sources – including individuals, zoos, other NGO's, charities and businesses. Full financial details are available on the web (Limbe produces an annual report) at [www.limbewildlife.org](http://www.limbewildlife.org).

### **Rehabilitation**

LWC has 22 Permanent Cameroonian staff, all with at least 4 yr primate husbandry experience. Several, including the senior education officer, have also trained at the Durrell Conservation Trust. Pandrillus volunteers, (a project manager and assistant, plus onsite veterinarian), act as advisers. Limbe has its own Cameroonian 'Conservator', with his own Government budget (a first for a Cameroonian wildlife organization). A major role for the on site veterinarian is staff training in basic veterinary techniques, as well as more specific training for a local Cameroonian vet. Veterinary protocols and care, produced by Pandrillus' veterinary advisor John Lewis, (International Zoo Vet Group), provides the basis for health care and disease surveillance of the animals and staff

### **Conservation Education**

Two years ago, LWC's education officers were able to instigate an outreach programme - travelling to villages throughout Southern Cameroon: 35,000 Cameroonians in 2002 and close to 50,000 in 2003 were reached. Bush Palaver, an anti-bushmeat play, written by the Cameroonian education officers in local pidgin has proven an effective icebreaker to broach the subject of bushmeat.

Natures Club, an onsite education programme for local school children has been running for a number of years. Bilingual (English and French) education material is provided, and many of the LWC staff assist with lectures and activities. Final year school children, who have been through the 'Natures Club' system, volunteer to show visitors around the Centre. This personalized system allows them to not only discuss individual animal rescues with visitors, but use them as ambassadors for the more general topics of bushmeat and habitat protection

While the output of a conservation project is relatively easy to measure, the impact of that project on peoples attitudes and actions locally is much more difficult to quantify. Conservation organizations worldwide are grappling with this problem, but information on impact is vital, if organizations like the LWC are to proportion severely limited funds and resources most effectively.

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## FOILING BIOTERRORIST ATTACKS IN ZOOS; RISK ASSESSMENT AND WILD LIFE FORENSIC APPROACHES

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### **Abstract**

Physical, biologic, and chemical agents or combinations of the three can be used in a bioterrorist attack on zoos. Risk assessment and wildlife forensics can serve as initial robust lines of defense in quantifying the risk, characterizing the hazard, and enabling risk management. Computer assisted medical decision making and scientific reasoning are essential. In an era of Info-Medicine, the foiling of a bioterrorist attack requires an interdisciplinary task force. One key member of the task force should be an expert in biowarfare. The combative motivations and skills of Bioterrorists should never be underestimated. A response to an attack on any zoo must be at the state and national levels due to the nature of bio-pathogens used as weapons and medico-legal factors. Maintaining optimal animal health and a highly reliable surveillance system are paramount components for countermeasures.

### **Introduction**

The high technology advances in communication and travel in the current Information Age have magnified the ease of asymmetric warfare. It is an unconventional warfare which is trans-global and includes macro-ecological niches and micro-ecological niches. Zoological parks, gardens or zoos as micro-ecological niches are susceptible to bioterrorist attacks.

Though in-house expertise should be utilized in foiling a bioterrorist attack on a zoo, the counterattack or preventive action has to be a well coordinated national and international effort. Though a zoo cannot afford a multi-disciplinary team of biomedical scientists and clinicians, the in-house veterinary staff can serve as coordinators for a team of specialists. The team members can consist of a toxicologist and risk assessor, a wildlife forensic pathologist, a zoo-veterinarian and a veterinary clinical bioinformatician. One significant member of the counter bio-warfare team will be an expert in counter-terrorism or asymmetric warfare with skills in chemical and biologic warfare.<sup>1</sup> The combative objectives of a bioterrorist attack should never be underestimated.<sup>3</sup> Animals at a zoo can be used as reservoirs for acute bio-pathogens or chronic bio-pathogens. For example, a number of mammals, including elephants and rhinoceroses can be inoculated with weaponized strains of the anthrax organism. Reptiles and birds can be inoculated with weaponized strains of *Salmonella typhimurium*.

The zoo veterinarian, the toxicologist and risk assessor, the wild life forensic pathologist, the veterinary clinical bioinformatician and bio-warfare expert can, as an effective coordinated team,

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develop a robust plan to foil a terrorist attack on a zoo. The 1986 thallotoxicosis at the Guyana Zoo example illustrates methods for improving approaches for nullifying bioterrorist attacks on zoos.

### **Modes of Attacks**

The agents used by bioterrorists to attack humans and animals at a zoo can be categorized as physical, chemical and biologic agents. Combinations of these agents can be employed to induce an epidemic in a zoo community and surrounding environment. For instance, the aerosol inoculation of captive and free ranging birds and reptiles with the highly pathogenic strains of *Salmonella* or *E. coli* 0157:H7 organism can be conducted covertly, and visitors to the zoo can be exposed to the organism from the body fluids and excrements of infected birds and reptiles. Feral rats and mice can be inoculated with the weaponized strains of the *Leptospira* organism and surreptitiously released in a zoo compound. Unless there is a successful rodent control program at the zoo or zoological park, susceptible humans and animals can be infected, and an epidemic can occur.

The bioterrorist can also use chemical agents to induce an epidemic with high mortality and low morbidity in a zoo population.<sup>2</sup> Thallium sulfate is colorless, odorless and tasteless. Depending on the exposure dose, the toxic effects can be acute, sub-acute, chronic or sub-chronic. The effective dose varies by species. During the 1986 thallotoxicosis at the Guyana Zoo, 75% of the animal population sampled were thallium positive. A forensic investigation by the author indicated that thallium was introduced into the foods of the animals when the foods were transported from the zoo kitchen to the animal enclosures. There were also animals with acute and chronic signs of thallium poisoning. One toucan and peacock exhibited paraesthesia, hind leg paralysis and diarrhea. A margay with ante-mortem signs of hemorrhagic diarrhea, epilation and shock was on necropsy examination found to have diffuse gastric and duodenal ulcers, hydrothorax, and ascites.<sup>13</sup> Thallium sulfate a homicidal agent has been used during the Saddam Hussein regime against dissidents and in the Warsaw pack countries during the Cold War.<sup>7</sup> More effective than ricin, thallium toxicosis has only been detected in victims via meticulous diagnostic work-up. The bioterrorists have a variety of methods of delivering the chemical, biochemical or biologic agents to target species in a zoo.<sup>4</sup> Migratory birds using the zoo grounds as a resting stop can be used as biologic or mechanical vectors. Arthropods such as ticks and mosquitoes can also be used as mechanical or biologic vectors.<sup>5</sup> Bioterrorists can also infiltrate the zoo staff and covertly expose target species to a combination of chemical or bio-pathogen agents.<sup>12</sup> Kites, balloons or remote controlled vehicles flown over a zoological park can be used to remotely release aerosols, powders or smoke containing bio-pathogens or toxic chemicals. How can these varied modes be foiled?

### **Counter Measures**

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From the perspective of the art of warfare, each attack, including the best attack has weaknesses or faults. These are weaknesses or faults which the defender can exploit to nullify the effectiveness of the attack. A robust surveillance program at a zoo can detect an increase in the rodent population and unusual pathogens transmitted by the rodents.<sup>11</sup> Maintaining an effective prophylactic program, including an optimal plane of nutrition for all animals can act as a buffer against the stress induced in an ecological niche due to the introduction of new pathogens or physical and chemical agents. Adequate preparation and an alert, highly trained technical zoo staff can take away the element of surprise used by many bioterrorists.

The role of each member of the team countering the potential bio-warfare attack begins prior to any emergency event. A preventive plan is developed and implemented. Drills and bio-war games are periodically conducted and the skills of the participating teams are honed.<sup>10</sup>

The toxicologist and risk assessor identifies, characterizes and analyzes the risk of the bioterrorist attack. Risk management advisories are also prepared. Dose-response curves and models of chronic and acute effects are also examined. The toxicologist and risk assessor along with the veterinary clinical bioinformatician and zoo veterinarian can develop or use software on medical decision-making to reduce error in diagnosis and therapeutic management.<sup>9</sup> The wildlife forensic pathologist can provide in-depth analyses and interpretations on risk reduction and pathogen detection.<sup>8</sup> The expert on bio-warfare can play a role in the development of counter tactics and strategies. The bio-warfare expert should be able to assess the combative skills and potentials of the bioterrorists and the impacts of their attacks on the health of animals in the zoo and animals and humans in neighboring communities.

## **Discussion and Conclusions**

A bioterrorist attack on a zoo has the potential for a bio-hazard or chemical hazard at a state or a national level. It can be conducted by a group of highly skilled, highly motivated, cunning and fanatic individuals. Counter measures to foil a bio-warfare attack must be robust from a military and medical sciences perspective. Lack of funding or poor funding along with ineffective communication by a variety of experts and/or specialists can be weak links in a coordinated effort to neutralize a bioterrorist attack on a zoo.<sup>14</sup>

Habitat destruction and over-population have been some factors contributing to the increased importance of zoos. There has also been a merging of the theatres of asymmetric warfare, in which all ecosystems are part of the theatre. In the current era of Info-medicine, the veterinary and human medical community must cooperate in their effort to prevent and combat epidemics in which domestic and non-domestic animals are abused as biochemical factories for deadly pathogens.<sup>6</sup>

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## REMOTE DELIVERY DART FOR HORMONAL IMPLANTS IN RHINOCEROS

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<sup>3</sup>*University of Veterinary Medicine, Institute for Biochemistry, Veterinaerplatz 1, 1210 Vienna, Austria*

### Abstract

Approximately 60% of female captive white rhinoceroses (*Ceratotherium s. simum*) are acyclic.<sup>3</sup> Recent ultrasound examinations, in combination with endocrine monitoring, have revealed ovarian pathologies such as cysts, micro-corpora lutea and inactive ovaries all of which appear to be associated with lack of reproductive activity.<sup>1</sup> In an attempt to treat these uterine and ovarian pathologies, long and short-acting GnRH agonist implants (Deslorelin Implant<sup>®</sup>, Peptech Animal Health, Sydney, Australia, Ovuplant<sup>™</sup> Deslorelin; Fort Dodge Animal Health, IA 50501, USA) have been applied.<sup>2</sup> As most rhinos examined in this study had not been trained to accept implant placement without anesthesia we developed a remote delivery method using a dart originally developed by Telinject<sup>®</sup> (Veterinaer Spezialgeraete GmbH, 67352 Roemerberg, Germany) for the remote delivery of microchips.

The dart consists of two distinct sections (Fig. 1 and 2): A front delivery system with a pin that pushes the implant through the needle and an air chamber which on impact slides into the front part. The implant is placed in a needle normally used for microchip implantation. This needle is attached to the front of the dart. On impact, the pressurized 10 ml air in the air chamber is released as the valve (B) is impaled on pin (C). The air rushes through sieve (D) to push plunger and pin (E) forward into the needle. The pin pushes the implant out of the needle and it is deposited in the subcutaneous tissue.

This dart system has been used successfully in numerous rhinoceroses to place deslorelin implants without the use of restraint or anesthesia. Long-term administration of GnRH agonist implants substantially improved the reproductive health status in aged female white rhinoceroses. This dart system constitutes an important additional tool in our remote delivery repertoire.

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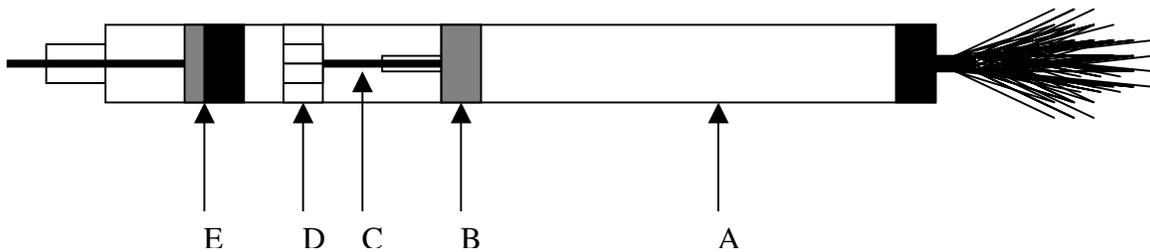
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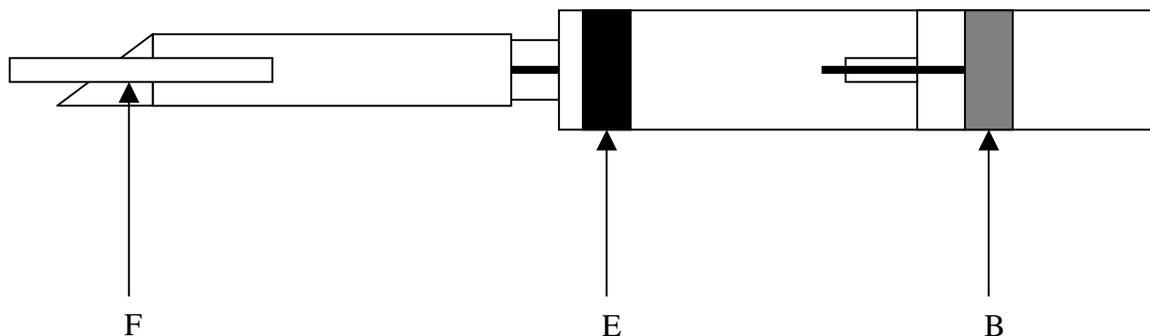
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**Figure 1.** On impact, air chamber (A) is pushed onto pin (C) opening rubber valve (B) and air rushes through sieve (D) to push plunger and pin (E) into needle and expulsing implant (see Fig. 2).



**Figure 2.** The air has pushed plunger (E) forward moving the pin into the needle and expulsing implant (F) depositing it in the subcutaneous tissue.



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## NESTED PCR AMPLIFICATION AND SEQUENCING OF A REPTILE REOVIRUS ASSOCIATED WITH DISEASE IN MOJAVE RATTLESNAKES (*Crotalus scutulatus*)

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### Abstract

Reoviruses have been shown to cause fatal pneumonia and subacute tracheitis in reptiles.<sup>1</sup> Diagnosis is challenging, as there are no inclusions, and histologic lesions resemble paramyxoviral disease. Serologic diagnosis has been limited to virus neutralization,<sup>2</sup> which is labor intensive and may not cross-react between strains. As reptile reoviruses do not hemagglutinate, hemagglutination inhibition is not an option. No published sequence for reptile reoviruses has been available. RNA-dependent RNA polymerase sequences from mammalian orthoreoviruses and piscine aquareoviruses were aligned. Degenerate primers were designed targeting conserved regions. These primers were used in a nested PCR to amplify sequences from a reovirus isolate associated with an outbreak of disease in Mojave Rattlesnakes (*Crotalus scutulatus*). Nucleotide sequencing of the PCR products showed that the reoviral sequences from these snakes were novel. Comparative sequence analysis shows that these viruses are probable members of the genus *Orthoreovirus*. These primers may be of use for obtaining initial sequence data from novel reoviruses. Sequence data will enable design of diagnostic PCR testing for specific viruses.

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## PERICARDIAL MESOTHELIOMA IN AN ARABIAN CAMEL (*Camelus dromedarius*)

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### Abstract

An 18-yr-old female Arabian camel (*Camelus dromedarius*) presented with an acute onset of anorexia and cough. Physical examination findings revealed cyanotic mucous membranes. Despite treatment the animal's condition continued to deteriorate over the next 3 wk, and euthanasia was elected.

At necropsy, the pericardial sac contained approximately 2 L of serosanguineous fluid. Approximately 30% of the epicardial surface was covered by 0.5-2.0 cm diameter soft, red, pedunculated masses that were most numerous in the atrial and apex regions. Several 0.3-0.5 cm diameter, firm, pink-red, raised masses were present on the visceral pericardial surface. Histologically, the masses were comprised of variably dense fibrous connective tissue cores and branching stalks covered by polygonal to cuboidal cells. The cells were of moderate cellular and nuclear heterogeneity with a moderate to high mitotic index. Many areas contained lymphoplasmacytic infiltrates, hemorrhage, fibrin accumulation, and hemosiderosis. Other significant necropsy findings included ventral subcutaneous edema, ascites, and hepatic changes consistent with chronic-passive congestion.

Gross and histologic findings in this case were consistent with mesothelioma of the pericardium and epicardial surfaces.<sup>2,3</sup> The presence of these masses led to an accumulation of pericardial fluid, which likely resulted in heart failure.<sup>1</sup> Additional findings of ascites, dependent subcutaneous edema, and chronic passive congestion within the liver are supportive of congestive heart failure as the cause of this camel's clinical signs. To the best of the author's knowledge, this is the first reported case of mesothelioma in a camelid.

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## THE EFFECT OF GENTAMICIN TOXICITY ON NOVEL RENAL DISEASE MARKERS IN THE PIGEON (*Columba livia*)

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### Abstract

Diagnosis of mild to moderate acute or active renal disease remains clinically challenging in avian species, yet early intervention can provide the best outcome. Available markers of avian renal dysfunction principally consist of those that accumulate in the blood due to reduced clearance, and those released into the urine as a result of renal tubular epithelial injury. Previous work in the pigeon and other avian species has suggested the potential value of BUN and uric acid for the detection of renal injury.<sup>1</sup> However, both of these markers are variously influenced by diet, hydration status, and metabolic state. Likewise, the sensitivity of these markers is limited when significant normal renal tissue exists, as often occurs early in the disease process. Preliminary work suggested that gamma glutamyl transferase (GGT) and N-acetyl- $\beta$ -D-glucosaminidase (NAG) increased in the urine in response to an acute renal insult; however, the former compound was relatively labile (Wimsatt, et al., 1995, unpublished data). Creatine is the preponderant metabolite of the energy substrate creatine phosphate excreted from avian muscle. Thus, depending on its distribution in the body, and on its renal disposition, it could serve as a renal disease marker similar to creatinine in mammals. The objective of the present study was to characterize the histopathologic changes associated with gentamicin toxicity in the pigeon, and correlate these pathologic changes with blood plasma levels of uric acid, NAG and creatine, and urine levels of NAG and creatine.

### Methods

All procedures were approved by the institutional animal care and use committee. Nineteen culled breeder homing pigeons (n = 19; 5 controls and 14 treated) in excellent health were donated to the project, and were placed on a specially formulated semi-liquid 8% protein enteral diet. The prepared enteral formula provided complete maintenance nutrition and hydration (dietary water) when fed on a body weight basis, and was delivered in divided twice daily oral tube feedings. Prior to the study, pigeons were fed on this diet 1 wk, while their body weight, and stool/urate volume and consistency, blood parameters and overall demeanor and health were

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monitored. During the experimental period, 50 mg/kg gentamicin (Schering, 50 mg/ml) was intramuscularly delivered in twice daily doses. Birds were blood sampled and plasma (Na heparin) was collected before gentamicin treatment started, and on the last study day of gentamicin treatment, prior to humane euthanasia. Fixed tissues were submitted to treatment-blinded histopathologic examination by a specialty boarded pathologist for renal histopathologic tissue scoring. Tissue scores were as follows: 0=normal tissue, 1=mild changes, 2=moderate changes, and 3=severe changes. Parameters scored consisted of: proximal tubule (PT) degeneration, PT inflammation, PT necrosis, distal tubule (DT) degeneration, DT inflammation, DT necrosis, and (intraluminal) urate deposition.

For plasma and urine analyses, an original Boehringer-Mannheim creatinine procedure was modified and validated to measure creatine using a Hitachi 917 clinical chemistry autoanalyzer for high throughput, as previously reported.<sup>2</sup> NAG and uric acid (UA) were assayed using standard commercial kits (Roche) validated for avian species, and run on the same analyzer. Statistical analysis was performed using a Kruskal-Wallis nonparametric ANOVA (tissue scores; this method ranks, and does not require equidistant scoring intervals) and Student's independent sample T Tests, employing the Bonferroni correction (plasma and urine markers), employing SPSS version 11.0 for Windows XP. In all cases,  $\alpha = 0.05$ .

## Results

Controls and treated birds were not significantly different in regards to body weight change over the study period ( $P = 0.299$ ). Renal histopathology revealed statistically significant changes (in renal tubule scores) in treated birds as compared to controls for the following microscopic lesions: PT degeneration ( $P = 0.002$ ), DT degeneration ( $P = 0.002$ ), DT inflammation ( $P = 0.041$ ), and intraluminal urate deposition ( $P = 0.001$ ) in the tubules. PT necrosis ( $P = 0.115$ ), PT inflammation ( $P = 1.0$ ), and DT necrosis ( $P = 0.136$ ) were not statistically different between the treatment and control groups. Inducing gentamicin toxicity caused no significant effect on body weight in treated as compared to control birds. Changes in levels of urine NAG (88.1 fold,  $P \leq 0.001$ ) and creatine (5.7 fold;  $P \leq 0.001$ ) from baseline were both significantly elevated in treated birds, as compared to controls. Changes in plasma NAG (6.3 fold;  $P = 0.019$ ) and uric acid (54.9 fold;  $P = 0.033$ ) were significantly elevated in treated as compared to control birds, whereas, creatine while elevated, was not significantly so, apparently, due to large variances (22.5 fold;  $P = 0.387$ ).

## Discussion

Proximal tubular damage is commonly reported in a range of species in response to gentamicin toxicity. Distal tubular damage observed could reflect the dose, secondary hypoxia, or some as yet unexplained effect of gentamicin. Of the three markers evaluated in plasma, NAG and uric acid were elevated, but the former exhibited less variability in response to gentamicin exposure. In urine, creatine and NAG were significantly elevated, while uric acid is typically precipitated

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and is not reliably measured. The observation that creatine was elevated in the urine but not in the plasma needs to be more closely evaluated. This observation may suggest rapid renal clearance, a small volume of distribution, and/or variation in the time course of creatine released from muscle in response to gentamicin injections. For example, assessing the degree of muscle necrosis, (e.g., CPK) may allow creatine elevations to be better interpreted during renal disease assessment.

Although preliminary, these studies suggest the potential to noninvasively screen urine samples for renal dysfunction in the future, and hopefully will allow clinicians to better detect disease and prevent serious renal disease outcomes. The potential to identify acute disease, and to sample either blood or urine to identify a nascent renal toxic insult as reported here, if supported by further studies in a range of species, could improve the outcomes for captive avians with renal disease in the future. These noninvasive methods might also be adapted for use during wild bird health surveys; for example, to assess the impact of lead at wildlife refuges, and in response to other nephrotoxic exposures in the wild.

#### **ACKNOWLEDGMENTS**

The authors would like to thank Dr. M. Fettman for several helpful suggestions.

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## UPPER RESPIRATORY DISEASE ASSOCIATED WITH A NOVEL *Mycoplasma* ISOLATE FROM EASTERN BOX TURTLES (*Terrepepe carolina carolina*)

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### Abstract

A new upper respiratory disease syndrome similar to upper respiratory tract disease (URTD) affecting American tortoises<sup>4,5</sup> was identified in wild Eastern box turtles (*Terrepepe carolina carolina*) from Virginia. Coincident with this discovery, turtles submitted to both the University of Virginia and the Wildlife Center of Virginia presented with tympanic bulging, oral lesions, swollen eyelids or mucopurulent nasal discharge typical of upper respiratory disease, commonly attributed to vitamin A deficiency<sup>1</sup> and organochlorine exposure.<sup>6,10</sup> The present preliminary study was undertaken to determine the geographic distribution of the URTD-like disease in Virginia, to assess its prevalence in animals exhibiting the latter somewhat similar upper respiratory disease syndrome(URS), and to characterize the phylogenetic relationship of this *Mycoplasma* isolate to other pathogenic members of the genus.<sup>2</sup>

### Results

Clinically, affected animals exhibited varied signs including: unilateral to bilateral serous to mucopurulent nasal discharge, epiphora, and conjunctival injection and edema. All animals were collected from May through July, in 2001-3. Generic and agent-specific PCR detected *Mycoplasma* positive nasal swabs from seven of 24 animals sampled, representing six of eleven Virginia counties where animals originated. Three specific *Mycoplasma* PCR positive animals had aural bulging or oral lesions typical of URS, while four did not. A single animal without any upper respiratory signs that underwent a saline nasal flush was PCR positive, while 13 others without any upper respiratory signs, likewise flushed and screened were PCR negative. Sequence analysis based on the 16s ribosomal region from three clinical cases and a presumed carrier not exhibiting signs, representing isolates from four different counties, revealed a single unique agent. The new isolate was distinct from *M. agassizii*, but closely (98% 16s sequence

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agreement) related. Nasal swab samples from all the animals were negative for chelonian herpesviruses<sup>11,12</sup> and invertebrate<sup>7</sup> and vertebrate (including FV3)<sup>8,9</sup> iridoviruses by PCR.

## Discussion

These preliminary findings suggest the emergence of a new URTD-like syndrome in Eastern box turtles from Virginia. Clinically, this disease was indistinguishable from *M. agassizii*-induced disease in tortoises, except for a differing species predilection.<sup>4,5</sup> Although preliminary, no relationship was noted between *Mycoplasma* PCR positive tests, and URS signs common to wild turtles from this region. Similar to the description of tortoise URTD,<sup>3</sup> preliminary observations suggest that animals may harbor the agent, but exhibit only intermittent clinical signs, and clinical signs appeared to increase the likelihood of disease detection by nasal swab/conjunctival PCR testing as in URTD (Wimsatt, unpublished data). To our knowledge, this is the first report of a *Mycoplasma* recovered from multiple clinically ill wild box turtles from the mid-Atlantic region of the US, a purportedly *M. agassizii*-free area. Further work is required to confirm that this unique *Mycoplasma* isolate is the causative agent of the observed disease syndrome, to map and assess the agent's distribution and impact on wild turtle populations, to identify cofactors facilitating disease progression, and to better classify the organism biochemically and taxonomically.

## ACKNOWLEDGMENTS

We would like to thank Elliott Jacobson for helpful suggestions, and Joe Mitchell and Jean Marie Richards for their valuable contributions. Samples were collected under permits from the Virginia Department of Game and Inland Fisheries (Scientific Collection Permit number 022992), and federal permit FRSP-2003-SCI-0002 from the National Park Service.

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**ASSESSING LIVER COPPER LEVELS IN CHRONIC WASTING DISEASE TEST-POSITIVE AND NEGATIVE MULE DEER FROM NORTHERN COLORADO**

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**Abstract**

Copper deficiency has been implicated as having a causative or contributory role in chronic wasting disease (CWD) of cervids. In this study, we measured select trace mineral levels in liver tissue from mule deer culled and harvested from free-ranging populations in northern Colorado where CWD disease has been found. We compared copper, molybdenum, and manganese levels between CWD-infected (n = 46) and apparently uninfected (n = 171) deer. Preliminary analyses revealed a wide range of concentrations of all three minerals (Cu: 5.6–393 ppm; Mo: 0.71–4 ppm; Mn: 0.05–25 ppm). However, no differences in trace mineral levels between infected and uninfected deer were evident.

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## OPTIMIZATION OF THE ANTIGEN 85 IMMUNOASSAY FOR DIAGNOSING JOHNE'S DISEASE IN ELK (*Cervus elaphus*)

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### Abstract

Johne's disease (JD) or paratuberculosis, caused by *Mycobacterium avium* ssp. *paratuberculosis* has previously been documented in a free-ranging herd of tule elk (*Cervus elaphus nannodes*) at the Point Reyes National Seashore. Due to the herd's proximity to ongoing dairy operations, the risk to other free-ranging cervid populations, and the possible risk to humans, the National Parks Service, in collaboration with the California Department of Fish and Game, has been attempting to control this infection through proactive test-cull processes. Disease assessment in this wildlife population, however, has been hampered by diagnostic test methods that are oftentimes difficult or impossible to utilize and interpret in an ante-mortem fashion and the near-total lack of validation, optimization and standardization of the available test methods in the species of interest.

Recently, researchers working with tuberculosis in humans have developed an immunoassay that detects a serum protein complex (the antigen 85, or Ag85, complex) produced by mycobacteria in the early stages of mycobacterial infections.<sup>1</sup> Previous work has shown that this method is a promising diagnostic tool in the evaluation of tuberculosis exposure in some captive hoofstock species.<sup>2</sup> In order to determine the applicability of this method for detecting JD in tule elk, optimization and validation of the immunoassay was attempted through analysis of serum from known infected and non-infected individuals and comparisons with other diagnostic methods. Preliminary results indicate that this method may be a valuable adjunct to other testing methods (including gamma interferon and multiple-antigen ELISA) to allow a better evaluation of true paratuberculosis status in this species.

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