# ANESTHETIC COMPLICATIONS AND CLINICAL INTERVENTION IN OPIOID-ANESTHETIZED CAPTIVE ELEPHANTS

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#### Abstract

#### Introduction

Providing safe and effective anesthesia for captive African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants pose a significant clinical challenge to the zoo veterinarian. <sup>2,3,5,8,9</sup> Challenges include unique anatomy, physiology and behavior, which require specialized equipment, facilities and trained personnel. Since captive elephants are prone to certain medical disorders (dental, tusk, foot, nail problems), anesthesia, sometimes prolonged, will be necessary to provide the required veterinary care. In captive elephants, this may be an infrequent event, therefore, it is difficult for zoo veterinarians to gain experience and confidence in elephant anesthesia. Furthermore, elephants are of great institutional and ecologic importance, which adds to the growing list of formidable challenges. This latter consideration should not change the principles or risks associated with anesthesia, but may add to the apprehension when deciding to anesthetize these charismatic animals. Hopefully, these challenges do not lead to delayed medical intervention and care.

Over the past 15 yr, the authors have been involved in over 100 prolonged anesthetic events in captive and free-ranging elephants. This represents no claim to be experts but only reflects a privileged and valued experience worth sharing. Complications observed during these events include non-compliant patients, respiratory acidosis, lactic acidemia, hypoxia, hypoventilation, hypercapnia, hypertension, hypotension, ventilation-perfusion mismatch, endobronchial intubation, kinked endotracheal tubes, neuropraxia, prolonged recumbency, improper substrate, inability to stand, prolonged induction and recovery, inappropriate depth of anesthesia, bloat and inadequate equipment and facilities. Although certain complications are difficult to avoid, we will discuss preventative measures and clinical interventions proven to be effective in minimizing physiologic impact on the anesthetized patient.

# **Complications and Clinical Interventions During Elephant Anesthesia**

Patient Positioning and Controlled Recumbency

A successful elephant anesthesia begins with proper positioning of the recumbent patient with adequate space to perform the procedure. Due to space limitations in some facilities, maintaining control of recumbency is critical to ensure the proper position for the intended procedure. This can be accomplished in both 'free' and 'protected' contact management systems. In protected contact, prior training of the patient is imperative to accept placement of

'tack and rigging' such as anklets and ropes. Sedation and cautious free contact with experienced personnel may be necessary in some cases and should be discussed ahead of time.

The procedure area must be of adequate size to allow access with heavy machinery and be equipped with appropriate mechanical advantage devices such as an overhead hoist or crane; sturdy posts; and appropriately placed 'dead man' anchors. A method to lift the animal back to its feet is extremely important and must be discussed prior to the procedure. During recovery, it is important that elephants have adequate space to rock back and forth and extend its legs to gain sternal position prior to standing.

A key component in the 'rigging' process is to have the patient pre-trained to accept anklets and tethers on all four legs to assist in the controlled 'pull-down' to recumbency as the anesthetic takes effect. Methods for rigging an elephant have been previously described. A non-tightening, one inch diameter rope is preplaced around the patients neck. This loop prevents the 'pull-down' rope from slipping over the back of the elephant during controlled recumbency. The next step is to place two belly straps (8-inch wide) around the animal's body, which will be used to lift the animal into a standing position if it cannot get up on at the end of the procedure. The pull-down rope is attached to the anklet of the intended down side rear leg, directed under the abdomen and toward the opposite side shoulder, thru the dorsal aspect of the neck loop and pulled in the direction of the intended recumbent side of the elephant.

As the animal becomes unsteady on its feet, tension is placed on the pull down rope with manpower or heavy machinery to guide the elephant into recumbency. Specialized devices such as 'block and tackle' and 'snatch block' pulley systems are necessary to redirect and offer mechanical advantage over the great weight of the animal, which at this time would prefer to remain standing. Strong tension, applied perpendicular to the long axis of the body, must be maintained on the pull down rope or the animal may lean against the exerted force and go down on the wrong side or in an improper position.

To prevent nerve damage during recumbency and neuropraxia during recovery, adequate padding must be provided under the patient. This may include deep sand or hay, specialized air bags, twin bed mattresses or combinations. Two mattresses are tied together and placed under the shoulder and hip for an average size adult cow. Ropes are attached and used to guide the mattresses under the animal just before recumbency. Once down, it is unlikely to re-position this padding. The head and legs can be lifted to add padding such as large tire inner tubes, however. To reposition the patient, the previously placed belly straps can be used with strategically placed pulleys and mechanical devices to perform minor adjustments.

Tethers, anklets and neck ropes are removed prior to administration of the anesthetic reversal drugs. In uncomplicated recoveries, belly straps should fall off as the animal stands. If some control of the animal is necessary on recovery, a long tether can remain on one of the front legs. Also, prior to recovery, evaluate the area to ensure it is suitable for the animal to rock up to a sternal position before standing. If the patient cannot gain sternal recumbency, the anterior belly strap and mechanical devices may be needed to pull on the patient's shoulders for assistance to the sternal position. All four legs must then be positioned under the animal to obtain the normal kneeling position prior to standing.

If the patient is unable to stand, lifting with a hoist or crane will be necessary before the animal exhausts itself to the point where standing is no longer possible. Working on the dorsal aspect of the animal, the preplaced belly straps are attached to a 'spreader bar' to better distribute the weight of the patient during lifting with the hoist or crane. The animal should be kept calm and comforted while in the sling mechanism and the hoist is relaxed as the patient gains stability on its feet. This may take minutes to hours. During assisted recoveries, experienced personnel may need to work in close proximity of the animal and this should be discussed ahead of time so it is not a surprise to management.

# Choice of Drugs and Drug Combinations

Drugs and dosages used in elephant anesthesia are well described in the literature. <sup>2,3,5</sup> Special consideration must be made in certain elephant cases for the type and timing of drugs to match the patient's behavior, training, quality of facility and intended procedure. An untrained, noncompliant elephant in a deficient barn will likely need a different anesthetic protocol than a well-trained patient in a quality facility. Sedation may be necessary to station the animal in a stall or elephant restraint device so ropes, straps and anklets can be pre-placed to assist in patient positioning during induction.

The anesthetic induction dose for elephants often contains a potent opioid and a sedative delivered by dart. Commonly, etorphine is provided at 2-4 ug/kg and is sufficient to keep the patient at a safe depth of anesthesia for approximately 60 min, in the author's experience. Administration of high induction doses may pose a problem early in anesthesia since this is when the patient is often being moved into position and monitoring becomes difficult. Therefore, maintenance anesthetic agents may be needed to complete lengthy medical procedures. Constant rate infusion (CRI) of etorphine has been used in numerous physical status I and II, captive and free ranging elephants (J.R. Zuba, pers. comm.) while offering titratable, predictable and reversible effects. This author recommends a starting CRI rate of approximately 20% of a proper induction dose of etorphine administered i.v. per hour. This is approximately 0.4-0.6 ug/kg/hr of etorphine and should be started at approximately 60 min following induction for prolonged anesthesia. Anesthetic depth should be evaluated prior to initiation of CRI, of course. The authors do not use gas anesthesia due to the inability to quickly and completely reverse effects, if needed.

# **Clinical Monitoring**

Standard monitors include temperature, manual pulse and respirations, pulse oximetry, capnography, electrocardiogram, direct and indirect blood pressure and blood gas analysis. In the authors experience direct blood pressure, ECG, capnography and blood gas offers the most reliable physiologic information on elephant patient status. The ear is routinely used for oximetry measurements in elephants but can be unreliable in authors experience due to movement, skin thickness and low perfusion. A newly released pulse oximeter with improved technology (Radical-7, Masimo Corporation, 40 Parker Irvine, CA, USA) showed superior comparability with blood gas values than standard oximetry in a recent clinical case by the

authors. This technology is new to the veterinary market and shows promise during motion and low perfusion conditions as well as the thick and colored skin of the elephant ear.

# Control of Respiration

Since potent opioids (i.e., etorphine, carfentanil and thiafentanil) are key induction agents used to anesthetize elephants, some degree of respiratory depression is expected in prolonged procedures. Anesthetized elephants are routinely placed in lateral recumbency, which may further complicate the breathing ability of the patient over time. In the author's experience, some degree of hypoventilation will occur in opioid-anesthetized elephants in lateral recumbency in procedures as early as 30 min - and certainly in cases lasting 45 min or more. Therefore, it is extremely important to have the ability to access the airway and assist ventilation as it is in other animals to maintain pH, PaCO<sub>2</sub> and PaO<sub>2</sub> in normal range.

This, of course, will require the need to intubate and ventilate. The authors have developed endotracheal tubes (ETT) of various sizes (35, 45 and 52 mm I.D. and lengths of 1.6-1.8 m) for elephants ranging from 1000-6000 kg. These tubes will be available to others, soon (J. R. Zuba, pers. comm.). Intubation using the stylet technique is usually simple and quick since induction doses typically produce a patient with a lack of jaw tone. The mouth is opened with a strap around the lower jaw tethered to a 4 m rope routed between the front legs of the patient and pulled caudally to open the mouth. The intubator's gloved arm and hand is introduced between the narrow dental arcade; the soft palate is elevated to gain access to the tracheal opening; fingers are placed within rim of the glottis and a 1 cm dia., 2 meter long polypropylene stylet is advanced 15-20 cm into the tracheal lumen. The free end of the stylet is placed through the ETT's 'Murphy eye' and the tube is advanced into the trachea using the stylet as a guide. The tube is secured to the tusk or trunk.

Large animal ventilators are easily adapted for use in smaller elephants, whereas two coupled LA ventilators may be necessary in larger patients. However, this may be cumbersome in small elephant barns or under field conditions. A full-sized elephant ventilator is available (Mallard Medical, Inc., Redding, CA) but it may be too large for small areas or field conditions. Horne et al.,<sup>5</sup> published a report on a handmade portable ventilator to provide IPPV in elephants under field conditions using 100% compressed oxygen. A novel portable, manually triggered, compressed oxygen-powered, venturi-ventilator<sup>9</sup> has been developed and tested in captive and free ranging elephants (J.R. Zuba, pers. comm.) and is near production. This device is more powerful than other portable demand ventilators and is capable of assisting ventilation in elephants, or other megavertebrates, up to 6500 kg.

#### Control of Blood Pressure

Normal blood pressure values for captive and free ranging elephants have been reported.<sup>4,7</sup> Free-ranging African elephants are routinely given azaperone with the etorphine induction dart as a hypotensive/sedation agent to protect against hypertension and pulmonary bleeding (pink foam syndrome).<sup>5</sup> Recently, other investigators (G.J. Fleming and J.R. Zuba, pers. comm.) have given 10 mg i.v. boluses of azaperone, as needed, to control elevated blood pressures under similar field conditions.

Interestingly, hypotension seems to predominate captive, opioid-anesthetized elephants – especially during procedures lasting over 60 min. Unfortunately, there is a lack of information on how to properly manage critically low blood pressures in elephants. Equine doses of vasoactive drugs found in veterinary literature<sup>6</sup> have been used successfully by this author (JRZ) as a guide for the treatment of hypotension in opioid-anesthetized elephants. Ephedrine, 30-50 ug/kg i.v., as needed, has resulted in 15-50% increase in mean arterial pressures within 3-4 min in hypotensive elephants. Duration of action is approximately 15-20 min. Dobutamine boluses or by constant rate infusion have also been used at equine doses with similar success by this author. Further research is necessary to better understand the pharmacokinetics and pharmacodynamics of these drugs in elephants.

Although the number of cases is limited, the authors recommend high-flow fluid pumps (Masterflex L/S, digital peristaltic pump, Cole-Parmer Co., Vernon Hills, Illinois) for the rapid infusion of maintenance volumes that cannot be matched by gravity alone. In a non-scientific, bench top test by this author, this pump delivered 70 L/hr of saline through a 10-ga catheter into a collection vessel. This volume and rate has not been tested on an elephant patient and infusion trauma to a single catheterized peripheral vein needs to be considered. These pumps can also be set to provide fluids by multiple lines to several catheter sites.

# **Case Reports**

#### Case One

- Signalment: Male, 22 yo, 4774 kg, African elephant, protected contact, tusk fracture with exposed pulp, very suspicious/anxious but fairly well trained patient
- Procedure: Partial pulpotomy, with expected anesthesia time of 3 hr
- Anesthesia: Single dart of 15 mg etorphine and 25 mg medetomidine for induction; intubate with 45 mm ETT and ventilate; CRI of 4.3 mg etorphine over 94 min for maintenance; 1000 mg naltrexone and 120 mg atipamezole for reversal
- Complications: Anxious and suspicious during induction, recumbent in improper position for procedure, bloat, hypotension, hypoventilation, respiratory acidosis, lactic acidemia, neuropraxia
- Interventions: Use of heavy machinery, mechanical advantage equipment, straps and ropes to move animal into proper position; i.v. boluses of 150 mg ephedrine provided four times for hypotension (MAP<80 mm Hg) with positive results; IV fluids at 20 L/hr for vascular support; the rate of IPPV with the portable ventilator is increased in response to bloat-induced decreased tidal volumes and hypercapnia; keepers are able to verbally assist in control of anxious patient during recovery and while the neuropraxia of the dependent left rear limb resolves.

#### Case Two

- Signalment: Female, 42 yo, 2516 kg, Asian elephant, protected contact, reflux of fluid of unknown origin, calm but debilitated patient
- Procedure: Esophagoscopy, gastroscopy, with expected anesthesia time of 2-3 hr
- Anesthesia: Sedation with 35 mg detomidine and 70 mg azaperone; 7 mg etorphine induction; intubate with 35 mm ETT and ventilate; maintenance with 0.5-1.0 mg i.v. boluses of etorphine, as needed; reverse with 800 mg naltrexone and 260 mg yohimbine
- Complications: Geriatric, debilitated, physical status IV, prolonged anesthesia, mild hypo- and hypertension, respiratory acidosis, hypoventilation, lactic acidemia, prolonged recumbency, unable to stand
- Interventions: Pre-procedural discussion with management of poor physical status and anesthetic risk; use of overhead hoist is anticipated; pre-placement of body straps to assist in standing if needed; bed mattresses under all pressure points; blood pressure fluctuates during anesthesia but no vasoactive drugs provided; assisted ventilation with portable ventilator; use of overhead hoist and straps for 1.5 hr to assist a debilitated patient to stand on its own

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# COMPARE AND CONTRAST TWO SUCCESSFUL ANESTHETIC PROTOCOLS IN THE NILE HIPPOPOTOMUS (Hippopotamus amphibius spp.)

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#### **Abstract**

Historical immobilization of the Nile hippopotamus has resulted in apnea, cyanosis, bradycardia and fatalities in up to 1/3 of the cases<sup>1</sup> and only 2 of 16 successful anesthesia's (16%) resulted in surgical anesthesia.<sup>2</sup> Recently two successful anesthetic protocols have been developed for the Nile hippopotamus using medetomidine (60-80 mcg/kg) and ketamine (1 mg kg) i.m. (MK) in captive settings, while a second combination utilizing butorphanol (0.12 mg/kg), azaparone (0.05-0.1 mg/kg), and medetomidine 0.04 i.m (BAM) has been used in both captive and free ranging settings (Table 1).<sup>3</sup>

Over 30 anesthetic events were recovered between the two protocols. Induction times varied with BAM having faster induction of  $8 \pm 5$  min vrs  $27\pm11.8$  min in MK protocol. Working times of 60-97 min with the MK group receiving additional ketamine in boluses equaling  $0.007 \pm 0.002$  mg kg min. While recovery was faster with the MK protocol ( $4.8 \pm 2.86$  min) with atipamezole (65% i.v./35% i.m.) compared to the BAM protocol with ( $10 \pm 5$ min) with i.m. administration of naltrexone (0.2 mg/kg i.m.) and atipamezole (0.1 mg/kg i.m.).

Transient apnea was seen in both combinations resulting in self-limiting breath holding for 4-7 min, which resolved over time and SpO<sub>2</sub> levels re-bounded once respirations resumed. Heart rates remained constant in both protocols (35-55 bpm) while metabolic acidosis was evident in blood gas analysis.

In conclusion both protocols provide effective immobilization of the Nile hippopotamus; however the collection of additional physiologic data may assist with developing safer and more effective anesthetic techniques.

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**Table 1.** Anesthetic protocols for Nile hippopotamus.

Drug	Dose mg/kg	Induction Time	Working Time	Recovery Time
Butorphanol	0.01-0.12 i.m	$8 \pm 5 \text{ min}$	$60 \pm 6 \text{ min}$	$10 \pm 5$
Azaparone	0.08-0.10 i.m			
Medetomidine	0.04-0.05 i.m			
Atipamezole	2 x med i.m			
Naltrexone	2 x but i.m			
Medetomidine	0.06-0.08 i.m	$27 \pm 11.8$	97 min	$4.8 \pm 2.86$
Ketamine	1.0 i.m			
Atipamezole	0.34 i.v./i.m.			

UTILIZATION OF CONTINOUS RATE INFUSION, MANUALLY CONTROLLED INFUSION, AND TOTAL INTRAVEOUS INFUSION FOR ANESTHESIA AND ANALGESIA IN ZOOLOGICAL COLLECTIONS

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#### **Abstract**

A balanced approach to anesthesia and analgesia in zoo species should be considered utilizing multiple drug administration modalities and classes. Constant rate infusion (CRI), manually controlled infusion (MCI), or total intravenous anesthesia (TIVA) of anesthetic/ analgesic agents are effective tools to level the plane of anesthesia, address pain, improve recovery times, and decrease drug volumes to be used. Various formulations have been published, mostly in domestic species. <sup>1-20</sup> Individual and species variation will exist for these various modalities. Choosing protocols must take into account species, immobilization conditions, comparative published data, and the level of anesthesia desired. Because calculating CRI dosages can be a mental quagmire, math is often the only limitation to using these valuable tools; therefore, a 'cheat sheet' or computer program can be utilized and is recommended.

## Overview

CRI, MCI, and TIVA modalities are based on the principle that a plasma drug concentration needed to produce anesthesia and analgesia has to be reached quickly and maintained over the planned event time. The steady flow of drug eliminates the "peak and valley" effect that can occur with other supplementary anesthetic protocols.<sup>5</sup> This can still be a risk of MCI where boluses are administered reactively or at specific intervals.; however, this regimen can still offer the advantage of providing the patient with consistent, effective maintenance of anesthesia and analgesia.<sup>5,13</sup> These infusion protocols are best utilized for field work, imaging or radiation procedures when an anesthetic machine is not available, surgical procedures that involve the upper airway (when placement of an endotracheal tube will interfere with surgery), bronchoscopic evaluation in smaller patients, and anesthesia for patients with intracranial hypertension concerns (inhalants increase blood flow to the brain while IV agents like propofol reduces the cerebral blood flow). 14 These regimens can decrease the impact that pain and rousal can have on physiologic parameters during maintenance of anesthesia. The eventual result is a lower drug dosage delivered steadily over time and overall reduced dose amounts during anesthesia thus reducing cost and the incidence of dose-related side effects. CRI allows for better control over drug administration with real time ease to change doses. The dose delivered during CRI can easily be decreased or increased based on patient need by adjusting the rate of the flow. Additionally, the use of CRI has been reported to lower gas anesthetic needs since these volatile agents are some of the most cardiac depressant drugs used in veterinary anesthesia. Studies have

shown that the mean alveolar concentrations of inhalant anesthetics are lower with the use of a CRI. Contraindications and side effects of CRI vary depending on the drug that is used. <sup>5, 13,14</sup> IV induction regimens are unlikely in zoological species, but once patient access is established for i.v. catheterization, CRI and other TIVA regimens can be used effectively to maintain smooth planes of anesthesia and reduce or eliminate the need for inhalant anesthetics. Loading doses are typically given initially to achieve initial therapeutic blood levels and are based on the volume of distribution as well as the initial plasma based on pharmacokinetic studies in domestic animals. <sup>13,14,18</sup> A drug in the same class as the agent to be used in the CRI is often utilized in the loading dose. Loading doses are needed prior to the initiation of the CRI in order to achieve initial therapeutic blood levels since these initial doses are typically both redistributed to tissues and eliminated. <sup>13,18</sup> Therefore, to maintain the desired plasma drug concentration, a CRI is initiated. The infusion rate is determined by the clearance of the drug and the drug concentration in plasma based on pharmacokinetic studies. <sup>14</sup>

Syringe and fluid pumps are ideal for CRI drug administration but gravity flow can be used as well. Programmable syringe pumps are cost effective and minimize error of drip rate and mathematic calculations making CRIs an inexpensive tool in your anesthetic arsenal. For example, Stein reports that an 8-hr mid-dose rate morphine/lidocaine/ketamine CRI for a 20 kg patient can cost a small animal practice less than \$1.50. As with any drug use, the suitability of a given drug infusion should be based on a sound understanding of that drug's use in an individual, patient health status, and pharmacokinetic data in that or comparative species. Fleming reported that by moving to a MCI dose of (0.4 mg per 10 min i.v. of M99) in over 100 elephant translocations reduced the total mg amount of M99 by50% when switching to a uniform MCI vs. topping off i.v. when the elephant was showing signs of arousal.

# **CRI Drug Classes/ Agents**

When considering CRI, MCI, or TIVA, one must examine the properties of the drugs to be used. The drugs utilized should be water-soluble to minimize toxicity associated with the solvent, stable in solution, and possess minimal risk of perivascular sloughing if extravasated. An ideal drug can be given as a concentrated solution to avoid fluid overloading, should not be absorbed by plastics and should not promote bacterial growth. Other desirable characteristics include rapid onset of action, rapid clearance from the body for quick recovery, no adverse side effects, good potency, lipid-solubility, relatively inexpensive, and chemically compatible with other drugs. There is no single agent that possesses all these properties, but these characteristics are important considerations when making drug choices. <sup>14</sup>

Propofol, a hypnotic agent, is the most commonly used agent for TIVA, CRI, and MCI. It has a higher elimination clearance and a shorter elimination half-life compared with other injectable agents. The clearance rate of propofol is faster than the liver blood flow.<sup>14</sup>

Opioids are often used in CRI alone or in combination with other classes. For domestic small animals, morphine, hydromorphone, and fentanyl are the most commonly used opioids. These drugs have good analgesic effects with mild to moderate sedation and offer the benefit of reversibility. CRI of concentrated narcotics can be used in megavertebrate anesthesia to reduce overall drug use during anesthetic events. Published and anecdotal doses are listed in Table 1.

Most of the side effects of opioids are dose dependent, including respiratory depression, bradycardia, vomiting, nausea, and occasional dysphoria. This class of drugs should be used with caution in felid species, starting at the low end of the dosing spectrum; higher rates may induce dysphoria, mydriasis, and excitation. Full agonists are the most commonly used opioids in domestic small animals but butorphanol, an agonist-antagonist, offers more sedation than excitement in cats. <sup>5,13,18</sup>

Benzodiazepines can be used for CRI and MCI as well. Midazolam is water-soluble; therefore, it should not precipitate, as diazepam will, when combined with other drugs. Benzodiazepines do not have analgesic effects of their own but do have excellent sedative and muscle-relaxing effects and are best utilized synergistically with an opioid resulting in an opioid sparing effect. Studies have shown benefits, including a reduction in the use of opiates and gas anesthetic mean alveolar concentration when midazolam was used as a CRI. Published and anecdotal doses are listed in Table 1. This class does also offer the addition benefit of antagonistic drugs to improve recovery time if indicated.<sup>5,13</sup>

Dissociatives in the NMDA- receptor antagonist class are often used in CRI. The use of drugs such as ketamine, which keeps the NMDA receptors from being overstimulated, can be very helpful in preventing central hypersensitization of the spinal cord when analgesia is needed during surgery. Additionally, studies suggest that antagonizing these receptors improves opioid receptor sensitivity, reduces opioid tolerance and minimizes the development of rebound hyperalgesia (the phenomenon of markedly increased pain when opioids are withdrawn). Although very beneficial, that mediation does not provide true analgesia, thus, these drugs must be administered in conjunction with true analgesic drugs (eg, opioids or NSAIDs) when pain control is a concern. <sup>5, 13,18</sup>

Local anesthetics such as lidocaine can be useful in CRI combinations, but be cautious that felid species can be sensitive to this class of drugs. Monitor closely and consider lower dosing when used in felids. Lidocaine has the additional antioxidant and anti-inflammatory modulation effects. It has also been reported to prevent ileus in small animals but its effect in domestic large animals is unknown. Lidocaine is light sensitive and should be kept covered if long-term use is planned <sup>13, 18</sup>

Alpha 2 agonists are used effectively for both sedation and analgesia in CRI and the effects are reversible. Human studies have shown that medetomidine significantly reduces the need for benzodiazepine and opioid use and does not seriously impair cardiovascular parameters (e.g., respiratory function).<sup>13</sup>

Synergistic combinations are commonly used in domestic small and large animal medicine and surgery and have great benefit for reducing inhalant anesthesia and improving cardiovascular function during anesthesia. The most commonly used combination is morphine and ketamine with or without lidocaine. Large animal literature reports guafensin, ketamine +/- alpha 2 combinations as well. These combinations are listed in Table 1. This table was formulated from a domestic and exotic literature review with a collection of clinically applied dosages in select zoological species. It is not intended to be all- inclusive but rather common comparative

regimens from the domestic industry that can be clinically applied to the variety of zoo taxa. Many of the dosages in the exotic species are, as typical of our field, based on empirical data, observations, and experience.

### **Calculation and Preparation of CRI**

Generally, dosing tables or individualized spread sheets should be used for constant rate infusions if accessible. They can prove more efficient for initiation of CRIs and reduce the risk of mathematic errors. Easy to use calculators are available online at:

http://www.vasg.org/resources\_and\_support\_material.htm

http://www.vasg.org/drug\_delivery\_calculators.htm

http://www.vasg.org/drug\_dose\_charts.htm

http://www.vasg.org/forms\_and\_text\_resources.htm

These resources allow you to vary the IV bag size, fluid delivery rate, and drug dose rates to satisfy any combination. <sup>18</sup>

CRI dosages can also be calculated using the formula: A x B x C x 60 / D x E x 1000 = mls of drug to add to diluent . A = desired dose in ug/kg/min , B = body wt in kg , C = diluent volume in mls , D = desired fluid rate in mls/hr , and E = drug concentration in mg/ml.  $^{13}$ 

Remember, any time a large volume of drug is added to a fluid bag for a CRI, an equal volume of fluid should be removed before adding the drug to keep the dose and volume accurate. The drugs should be added to the bag and the bag agitated to mix them before priming the fluid line and delivering the CRI to the patient. 13 18

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**Table 1.** Published and anecdotal CRI, MCI, and TIVA combinations used in domestic and exotic and/or zoological species. <sup>1-20</sup>

Agent	Dosage	Taxa And Comments	
Alphaxalone/ alphadolone	10 mg/kg/hr	primate dosing for anesthesia; loading/ induction dose required (other agents or 5-10 mg/kg i.v. $^{1}$	
Alfentanil	1.0 ug/kg/min	dog/cat dosing for anesthesia; loading dose 5 $\mu g/kg~i.v.^{14}$	
Butorphanol	0.1-0.2 mg/kg/hr	cat dosing for anesthesia/analgesia; loading dose 0.1 mg/kg i.v.;ceiling effect reported; recommend multimodal protocol <sup>5</sup>	
Detomidine Butorphanol (B)	(D)/ 30-50 μg/kg/15-20min (D) 30-50 μg/kg/15-20min (B)	elephant dosing for standing sedation; induction D 20 ug/kg + B 20 $\mu g/kg$ i.m.	
Diazepam	0.5-1 mg/kg/hr	ferret dosing for seizure control, use caution with mixing in line with other $\mathrm{drugs}^1$	
	0.2-0.5 mg/kg/hr	dog and cat dosing, use with opioid, loading dose of 0.1-0.25 mg/kg i.v. $^{\rm 14}$	
Etorphine	0.5 μg/kg/hr (MCI i.v. every 15-20 min in absence of mechanical pump)		
	0.5- 0.6 μg/kg/hr CRI 0.08-0.1 μg/kg/10-12 min MCI in absence of mechanical pump	elephant MCI (field/captive dosing) for anesthesia maintenance of anesthesia; multi-drug induction (Detomidine 20 $\mu g/kg$ , Butorphanol 20 $\mu g/kg$ i.m.,premed + Etorphine 1 $\mu g/kg$ , Azaperone 10-15 $\mu g/kg$ i.m. or Etorphine 2 $\mu g/kg$ , azaperone 20 $\mu g/kg$ i.m. for immobilization	
Fentanyl	0.0012 to 0.0036 mg/kg/hr	dog and cat dosing for analgesia; loading dose 0.003 mg/kg i.m.; shorter duration of action (30 min) than morphine, possible bradyarrhythmias; can cause respiratory depression and increased ETCO2 during anesthesia 5.13,18	
	0.2-2 μg/kg/min	dog/cat dosing for anesthesia, loading dose 5-10 $\mu g/kg$ i.v. $^{14}$	
	10-25 μg/kg/hr	primate dosing for anesthesia/analgesia; induction agents	
	0.1-0.4 µg/kg/min	required, use with Isoflurane as anesthesia sparing combo (isoflurane MAC 1.53 +/-0.07%) 1	
	1-5 µg/kg/hr	sumatran orangutan dosing (in combo with vecuronium 1-3 mg/kg/hr, midazolam 0.05-1 mg/kg/hr) <sup>1</sup>	
	0.2 μg/kg/min	capuchin monkey for anesthesia maintenance and analgesia (ketamine/ valium induction, loading dose of 10 $\mu g/kg$ i.v. prior to $CRI)^{20}$	
	5 μg/kg/hr	ovine dosing surgical anesthesia/ analgesia, multi-drug regimen (midazolam/methadone pre-medboth at 0.1 mg/kg i.v. pre- med, propofol 2-4 mg/kg i.v. induction), CRI combination with propofol 5-7 mg/kg/hr) <sup>17</sup>	
Guaifenesin (G)/ Ketamine (K)	0.5-1 ml/kg/hr	giraffe for anesthesia maintenance, multi-drug induction regimen (Detomidine 35 ug/kg + Butorphanol 35 µg/kg i.m. pre-medication, Thiafentanil 10 µg/kg + Ketamine 0.75- 1 mg/kg i.v. for immobilization), Mix G 5% (50 mg/ml) + K (0.5-1 mg/ml), titrate dosing for depth/physiologic effects	

**Table 1.** Published and anecdotal CRI, MCI, and TIVA combinations used in domestic and exotic and/or zoological species. <sup>1-20</sup>

Agent	Dosage	Taxa And Comments
	0.25- 1 ml/kg/hr 1-2ml/kg/hr	black rhino for anesthesia maintenance, multi-drug induction regimen (Thiafentanil 2.7 μg/kg, azaperone 60 μg/kg i.m.+ loading dose of Ketamine 0.2 mg/kg i.v. or Butorphanol 60 μg/kg, azaperone 40 μg/kg, medetomidine 25 μg/kg i.m. + ketamine loading dose 0.1-0.2 mg/kg i.v.) Mix G 5% (50 mg/ml) + K (0.5-1 mg/ml), titrate dosing for depth/ physiologic effects white rhino for anesthesia maintenance, multi-drug induction regimen (Butorphanol 60 μg/kg, azaperone 40 μg/kg, medetomidine 20 μg/kg i.m., ketamine loading dose 0.1-0.2 mg/kg i.v.) Mix G 5% (50 mg/ml) + K (1-2 mg/ml), titrate dosing for depth/ physiologic effects
Guaifenesin Ketamine Xylazine (X)	(G)/ (K)/ 4-4.5ml/kg/hr	equine dose for surgical anesthesia; pre-med (X 1.1 mg/kg i.m.) + induction 1.1 ml/kg of infusion), Mix G 5% (50 mg/ml) +K (1-2 mg/ml)+X (0.5 mg/ml), best at K 2 mg/ml) $^9$
	1.1ml/kg/hr	cattle dosing study (calves), pre-med (X 0.3 mg/kg i.m.) + induction with 1.1 ml/kg infusion i.v., mix G 5% (50 mg/ml) + K (1 mg/ml) + X (0.1 mg/ml), lidocaine extradural anesthesia/ intercocygeal space (lidocaine 2% 0.18 ml/kg) given, negative cardiovascular effects from X pre-med, resolved with oxygen 15
	2.2ml/kg/hr	suid dosing for anesthesia; Mix G 5% (50 mg/ml)+K (1-2 mg/ml)+X (1 mg/ml); Induction required (0.5-1 ml/kg i.v.) $^{1}$
Hydromorphone	0.012mg/kg/hr	cat dosing for anesthesia/analgesia; loading dose of 0.05 mg/kg i.v.; may cause hyperthermia <sup>5</sup>
Ketamine	0.12 to 1.2 mg/kg/hr	dog/cat dosing for sedation/analgesia (not anesthesia), loading dose of 0.25 to 0.5 mg/kg i.m./i.v.; combine with opioids, monitor for dysphoric effects upon recovery <sup>5,13,18</sup>
	1.5mg/kg/hr	horse dosing study for analgesia; no sedative/ anesthesia effects noted; benefit of analgesia/anti-inflammatory <sup>8</sup> horse dosing study for analgesia; no sedative/ anesthesia
	0.4-0.8mg/kg/hr 1.2mg/kg/hr	effects noted <sup>3</sup> horse dosing study for analgesia, no sedative/ anesthesia effects, loading dose 0.55 mg/kg i.v. over 15 min, slowed GI transit time noted <sup>2</sup>
	0.2-0.4mg/kg/hr	ankole dosing case for intraoperative anesthesia/ analgesia; no loading dose; can use in combo with Lidocaine (25 µg/kg/min; loading dose 1 mg/kg over 10 min)
Lidocaine	10 to 50 μg/kg/min (0.6 to 3.0 mg/kg/hr)	dog dosing for analgesia, loading dose of 1 mg/kg i.v. <sup>13,18</sup>
	10 to 50 μg/kg/min	cat dosing for analgesia, loading dose 0.25 to 1.0 mg/kg i.v., possible side effects= cardiac depression/ CNS excitation <sup>5,13,18</sup>
	50ug/kg/min	cattle dosing study in calves, pre-med (Xylazine 0.1 mg/kg i.m.) + induction (Ketamine 4 mg/kg i.v.); loading dose 2 mg/kg i.v., reduced isoflurane use in study vs.

**Table 1.** Published and anecdotal CRI, MCI, and TIVA combinations used in domestic and exotic and/or zoological species. <sup>1-20</sup>

Agent	Dosage	Taxa And Comments	
		control <sup>19</sup>	
	25ug/kg/min	ankole dosing for intraoperative pain control, loading dose 1 mg/kg over 10 min, used in combo with Ketamine at $0.2\text{-}0.4$ mg/kg/hr	
	50 μg/kg/min	horse dosing study, induction (xylazine 0.7 mg/kg + ketamine 2 mg/kg + diazepam 0.02 mg/kg i.v.); loading dose 1.3 mg/kg, i.v. over 15 min; decrease sevoflurane $MAC^{16}$	
Medetomidine (M)/ Dexmedetomidine (D)	1- 6 μg/kg/hr (M) 1-4 ug/kg/hr (D)	dog/cat dosing for sedation/analgesia; loading dose 1-6 $\mu$ g/kg i.v./i.m. (M), 1-2 $\mu$ g/kg i.v./i.m. (D) <sup>5,13,18</sup>	
	0.2-12 ug/kg/hr (M)	dose dependant study on hemodynamic effects in beagles (Induction/ loading dose range 0.2-12 $\mu$ g/kg), low dose range proved to have minimal effects (0.2- 1.7 $\mu$ g/kg/min) <sup>6</sup>	
	1.5 μg/kg/hr (M)	dog dosing for anesthesia maintenance; isoflurar induction; reduced cardiac index/ heart rate/tissroxygenation, not recommend for prolonge administration alone or in combo with fentanyl due negative cardiovascular effects <sup>5</sup>	
Midazolam	0.2 to 0.4 mg/kg/hr	dog/ cat dosing for sedation/ anesthesia (not analgesia); loading dose 0.2 to 0.4 mg/kg i.v./i.m. $^{13}$	
	0.2-0.5mg/kg/hr	dog/cat dosing for an esthesia; combo with opioid; loading dose 0.1-0.2 mg/kg i.v. $^{14}$	
	0.05-1mg/kg/hr	sumatran orangutan dosing; combo with vecuronium 1-3 mg/kg/hr, fentanyl 1-5 $\mu g/kg/hr^l$	
Midazolam (Md)/ Fentanyl (F)	8ug/kg/min (Md)/ 0.8-2ug/kg/min (F)	dog/ cat dosing for anesthesia; loading dose 0.2 mg/kg (Md) + 10 $\mu g/kg$ (F) $i.v.^{14}$	
Morphine 0.12 to 1.2 mg/kg/hr (dog/ cat) 0.03mg/kg/hr (cat)		dog/ cat dosing for analgesia (not anesthesia); loading dose 0.5 mg/kg i.m.; dilute with saline/ give slowly to avoid histamine release; protect from light 13,18	
1 ml/kg/hr (pump set at patient's weight [kg]= deliver 1 ml/kg/hr, rate increase up to 3 ml/kg/hr)			
Morphine/ Ketamine/ Lidocaine	1 ml/kg/hr (pump set at patient's weight [kg]= deliver 1 ml/kg/hr, rate increase up to 3 ml/kg/hr)	dog/cat dosing for sedation/analgesia; Ketamine 600 mg + Morphine 60 mg + Lidocaine 500 mg in 500 ml fluids (Ketamine 1200 mg + Morphine 120 mg + Lidocaine 1000 mg in 1L fluids) <sup>13</sup>	
Propofol	0.2-0.5mg/kg/min	dog/ cat dosing for an esthesia; loading dose 1-4 mg/kg i.v. $^{\!\!^{14}}$	
	0.25mg/kg/min	lizard/snake dosing for an esthesia; loading dose 10 $\rm mg/kg$ i.v.,i.o. $^{\rm l}$	
	1 mg/kg/min	chelonian dosing for an esthesia; loading dose 5-10 $\rm mg/kg$ i.v., i.o. $^{\rm l}$	

**Table 1.** Published and anecdotal CRI, MCI, and TIVA combinations used in domestic and exotic and/or zoological species. <sup>1-20</sup>

Agent	Dosage	Taxa And Comments
	0.4-1 mg/kg/min	avian dosing- some species variation, Loading dose 3-5 mg/kg i.v.,i.o. <sup>1</sup>
	0.5-1.2mg/kg/min	chicken study for anesthesia; induction 5-10 mg/kg i.v.; arrhythmias/ respiratory/cardiovascular depression 10
	0.8mg/kg/min	canvasback study for anesthesia; induction 15 mg/kg i.v.; mortality during study, low therapeutic index? <sup>11</sup>
	0.8-0.9 mg/kg/min	mute swan study for an esthesia, loading dose 8 mg/kg $\mathrm{i.v.}^{12}$
	0.2mg/kg/min	ostrich study for anesthesia, induction (medetomidine (80 $\mu$ g/kg) + ketamine (2 mg/kg) i.m.; loading dose 1- 3 mg/kg i.v. <sup>7</sup>
	0.05- 0.1 mg/kg/min	ostrich dosing for anesthesia maintenance; reduce/eliminate inhalant use, induction (medetomidine 20 µg/kg +Telazol 1-1.5 mg/kg i.v.)
	4mg/kg/hr	lagomorph dosing, loading dose 2-6 mg/kg i.v. <sup>1</sup>
	0.4-0.6mg/kg/min	primate dosing; loading dose 2-5 mg/kg i.v. <sup>1</sup>
	5-7mg/kg/hr	ovine dosing for surgical anesthesia/analgesia, multi-drug regimen (pre-med midazolam/ methadone both 0.1 mg/kg i.v., induction propofol 2-4 mg/kg i.v.); CRI combo with fentanyl 5 ug/kg/hr <sup>17</sup>
Remifentanil	0.2-2.0 ug/kg/min	dog/ cat dosing for anesthesia, loading dose 5-10 $\mu$ g/kg i.v. <sup>14</sup>
Sufentanil	0.1-0.2 ug/kg/min	dog/ cat dosing for anesthesia, loading dose 2-5 $\mu g/kg$ i.v.
Thiafentanil	5ug/kg/hr	giraffe anesthesia maintenance, multi-drug regimen (Detomidine 35 ug/kg + Butorphanol 35 $\mu$ g/kg i.m. premed, Thiafentanil 10 ug/kg + Ketamine 0.75- 1 mg/kg i.v. induction), +/- loading dose of Thiafentanil 3-4 $\mu$ g/kg i.v.
Thiopental	15-17mg/kg/hr	primate dosing (general); loading dose 10-15 mg/kg i.v. <sup>1</sup>

# CHALLENGES WITH ELASMOBRANCH CAPTURE AND ANESTHESIA IN LARGE AQUARIUMS

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#### Abstract

Immobilization of large elasmobranchs in multimillion gallon aquatic systems provides many technical difficulties and requires special skill sets. Prior to embarking on an anesthetic event with large elasmobranchs, it is highly recommended that a pre-procedure briefing occurs with clear goals and assigned staff roles, that safety issues are recognized and that an emergency protocol is in place in the event of a human safety incident (on land and underwater). The procedure itself should be as quick as possible without comprising animal or human safety.

#### **Immediate Areas of Concern**

- 1) Human safety (below)
- 2) Target animal safety
  - a. potential risk of anesthesia
  - b. attention must be paid to conspecifics that may be aggressive and damage the sedate individual
  - c. focal damage from darts or darting devices and equipment or net damage
  - d. the larger the animal the more possible it is to inflict damage to the animal itself (e.g. spinal damage) and organ damage (flipping large rays can result in hepatic fracture if not done carefully).
- 3) "Collateral damage"
  - a. non-target animals
    - i. exposure to anesthetic drugs
    - ii. traumatizing/stressing them due to proximity or presence
  - b. exhibit space damage
    - i. glass or plexiglass cracks or scratches by anesthesia or restraint equipment
    - ii. 'furniture' in the exhibit can be broken, such as coral heads.

When thinking about human safety, handlers should be experienced with elasmobranchs; if they are divers, they should be physically fit and outfitted with proper protective gear. Whether they are on land or underwater, constant attention must be paid to the location of the oral cavity of the animal, to the skin of the sharks and to the barbs of stingrays (even if trimmed). Protective items can include poles or baffles to keep animals at a distance, Kevlar <sup>TM</sup> or chain mail gloves or clothing where indicated, protective tubing over barbs/tail protrusions (especially with freshwater rays), and nose devices for sawfishes. There should also be a plan in place for accidental exposure of staff members to anesthetic drugs (as with zoo hoofstock). Ultrapotent narcotics are not typically used, however, alpha 2 agonists at dosages used for large elasmobranchs are a significant concern.

#### Removal from Enclosure

How do you remove a single animal from a mixed species large aquarium? Some basic categories for approaching these animals are:

- 1) Training and removal from the aquarium
  - a. the animal is trained into a device which can be removed from the water (a cage, a sling, a net)
- 2) Training animals into smaller areas for delivery of anesthetic drugs or for manual restraint. The animal is
  - a. targeted into a device or smaller space (a medical pool) for net or manual restraint
  - b. cornered or baffled and anesthetic drugs are delivered
  - c. is moved into an even smaller device (a swimming pool) for use of immersion drugs
- 3) Surprise catch
  - a. Scoop method: the animals are manually caught in a net or other restraint device by catching them off the surface during a feed or patterned swim movement.
  - b. Catching the animals under water without sedation: manually or with nets.
    - i. In some cases, a net across the entire tank that is capable of moving toward a wall and can be used to 'push' animals and isolate the target animal or group
      - 1. This requires a great deal of dive staff, depending on the size of the enclosure, maneuverability around the exhibit and you can get many non-target species
      - 2. This can be a very effective and rapid technique with a skilled team
- 4) Anesthetic presedation or tranquilization and then restraint.
  - a. Drugs
    - i. Oral (fed out): often requires very high dosages and many classic mammalian drugs have little or no overt effect (needs more research)
    - ii. Injectable: see section below
    - iii. Immersion: smaller area, animal separated (e.g. swimming pool), versus whole tank exposure.
      - 1. Obvious difficulties depending on total animal numbers and volume of water
      - 2. Very good at moving an entire collection of animals if appropriate drugs are used
      - 3. Can require a lot of staff
      - 4. Need to consider effluents
      - 5. Drugs:
        - a. Tricaine methane sulfonate (MS-222)
          - i. Varied doses
          - ii. Graded anesthesia (high induction, lower maintenance)
        - b. 2-Phenoxyethanol

- c. Eugenol
- iv. Over the gill: high doses of immersive drugs, most typically tricaine methane sulfonate delivered focally over the gills or through the mouth for induction
- b. Restraint devices
  - i. Sling
  - ii. Sock
  - iii. Box
  - iv. Box net
  - v. Hoop net, large
  - vi. Manual restraint

## **Injections-Technical Aspects**

When a procedure requires injection of the animal underwater while it is free swimming then a number of additional considerations must be had. What tools are available?

- 1) Hand syringe
  - a. Results in very close contact with the animal
  - b. Must use proper angle and strength
- 2) Pole syringe
  - a. Must use proper angle and strength; need to be quick
- 3) Dart guns (e.g. laser aimed or other similar underwater gun)
  - a. Practical range of 8 feet
  - b. Compression of air at depth alters discharge
  - c. Usually used at the surface or in shallow water
  - d. Pushing dart through the water is harder than it seems, difficult to gauge pressures
  - e. Cost of procuring or producing an underwater dart gun
- 4) Hawaiian sling/other similar (e.g. speargun)
  - a. Very difficult to judge projectile strength
  - b. Recovery procedure versus euthanasia

What are some of the technical difficulties with injections into elasmobranchs?

- 1) Thickness of skin, very abrasive skin, dulls needles
- 2) Potentially large volumes of drugs
  - a. concentrated drugs are recommended
  - b. fish muscles are not elastic and cannot hold as much as mammal muscles
- 3) Location of injection
  - a. Intramuscular
    - i. red muscle (aerobic) is best but is less likely for injection than white muscle (anaerobic)
    - ii. location on the body: the 'saddle' is the reputed best spot
  - b. Intravenous
    - i. Intended for

- 1. conditioned animals (stingrays) or slow moving larger elasmobranchs (whale sharks)
- 2. manual restraint
- 4) Leakage of drug
  - a. the dart should stay in animal for several minutes to allow sufficient discharge and to prevent flowback (out of the injection site)
    - i. the animal's response to the dart being removed is a useful indicator of the level of induction.
  - b. leakage post injection can be as much as 20% (pers. comm. M. Andrew Stamper)
- 5) Darts not going off at depth
- 6) Human limitations
  - a. assumption of distance underwater is challenging
  - b. compensation for the angle of refraction of the mask

# Some Successful Intramuscular Drug Combinations

What is successful? The expectations of sedation are important. Most reports of using injectable anesthetics have resulted in highly varied results ranging from no sedation to sedation that lasted for several days and in some cases, death. Mostly however, the general result is an animal that is notably affected by the drug (slow, less likely to respond to a human and mildly ataxic) and can be led into a safer restraint device. Some drug combinations that have been successful (see references for details on dosages and responses):

- 1) Ketamine and xylazine +/- midazolam
- 2) Ketamine and medetomidine (or dexmedetomidine)
- 3) Etomidate
- 4) Butorphanol and medetomidine
- 5) Medetomidine or dexmedetomidine alone
- 6) Alphaxalone-alphadolone (Saffan) (no longer available)

#### **Considerations for Induction**

Regardless of the methods chosen, efficiency and speed are key, particularly with pelagic species. Elasmobranchs follow three general lifestyles: benthic, intermediate and pelagic. The more pelagic the species, the more physiologic stress that animal will endure with an ensuing lactic acidosis. However, consideration for how much struggling even a benthic animal does during the initial capture and or induction is also an important consideration. Habituated or trained animals tend to handle stressors better than newly caught or naïve animals. Obligate ram ventilators must have a continual stream of water pumping over the gills, which may mean that underwater pumps must be available to dive staff. The pelagic animals (e.g. *Carcharhinus limbatus* and *C. acronotus*) are strict ram ventilators and must be ventilated as soon as possible as they are very susceptible to hypoxia and capture stresses. These animals are not good candidates for underwater injections or long capture attempts. The intermediate species, such as *C. plumbeus*, *C. melanopterus and C. taurus* tolerate handling and short periods of poor ventilation well. Benthic species such as *Ginglymostoma cirratum* are highly tolerant. Stingrays

in general, even pelagic rays, are seemingly more tolerant of handling stresses. Exertional rhabdomyolysis can occur, though the more immediate and life threatening blood gas fluctuations are more common. The lifestyle of the animal should guide how to best immobilize elasmobranchs.

Induction quality and length of time vary with the choice of drugs and modalities of capture. Injectable anesthetics can take as long as an hour for induction. Typically these animals begin to have an awkward gait and start bumping into exhibit materials. Where safe and possible, guiding the animal away from such objects is warranted. Under some circumstances, there need to be divers prepared with baffles and restrictive devices to keep other animals away from the animal that is being induced. Naturally curious or well trained animals may prove to be a nuisance under these circumstances. Animals, like sawfish, can be hazardous to divers or to large nets and must be kept away from the focus animal and anesthetic equipment.

#### **Anesthetic Maintenance**

Once animals are induced and considered safe to handle, following other basic principles of fish and elasmobranch anesthesia are prudent and can be found in the below references. For elasmobranchs, key elements for stable anesthesias will include continued ventilation (from beginning to full recovery), good water quality, and periodic blood gas evaluation (getting a baseline is fundamental). Ultrasound evaluation of cardiac contractility with skilled eyes offers another method to determine how well animal is performing under anesthesia.

## **Recovery**

As above, safety is key and all the above points bear weight during recovery. It is preferable to recover animals in an isolated area, if not a medical pool then a penned area. If this is not possible, then retaining the animal until it is capable of evading tankmates is necessary. It is a judgment call of when to release an animal, as they are rarely ready at the first notice of voluntary motion. This can be difficult with pelagic species as they need continual ventilation until the last moment. A common issue with fish handlers, is the desire to "walk" animals or to place their oral cavity into a outflow of water. This is not an efficient method of gas exchange and can be detrimental during the recovery phase of anesthesia. The most effective method of ventilation (as evidenced by blood gasses) is with a low flow pump that is directed over both gill arches. This becomes a human safety risk as the hands are near the oral cavity at this timeframe and sudden movements from the animals can result in trauma to the handler, therefore proper protective gear is important. Rays and certain sharks have crushing plates that can inflict serious injury and the shark mouth is itself a dangerous area even for an incidental scratch by casual contact with the teeth. Once the animal is released, it is not uncommon for them to fall to the bottom of the enclosure; depending on the stage of recovery, it may be appropriate to leave the animal. However, if the animal is a ram ventilator or still does not have the ability to propel itself well in the water column, it must be retrieved and further supported until it is ready to swim. If possible, the animal can be positioned in the direct path of a water inlet pipe or with a water pump to ensure steady flow of water over the gills during the recovery phase.

#### **ACKNOWLEDGMENTS**

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# GROUND-BASED DARTING OF BIGHORN SHEEP (Ovis canadensis) WITH MEDETOMIDINE-KETAMINE: EFFICACY AND SAFETY

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#### Abstract

Twenty-eight bighorn sheep were captured between September 2009 and December 2011 in Kananaskis Provincial Park, Alberta, Canada. Sheep were approached and darted on foot, or from a vehicle. A laser range finder was used to assess distance, and a dart rifle was used to deliver either a 3 or 5 ml dart containing medetomidine {0.16+/-0.04 mg/kg (mean+/-SD)} combined with ketamine (4. 2+/-1.6 mg/kg). Induction time (9.8+/-9.4 min) was taken as the time from first dart placement to becoming recumbent. The animal was maintained in sternal recumbency, as much as possible. An arterial blood gas sample was obtained from the femoral or auricular artery, to determine oxygenation, ventilation and acid-base status. Samples were corrected for body temperature and analyzed immediately with a portable clinical analyzer. After 77+/-25 min, atipamezole (0.8+/-0.2 mg/kg) was administered intramuscularly. Time from atipamezole to standing was 3.4+/-1.7 min. Induction was smooth and controlled. Recovery was complete, with animals being able to negotiate steep terrain. The major side effect was hypoxemia, supplemental inspired oxygen is recommended.

**Table 1.** Arterial blood gas analysis, and vital signs in bighorn sheep anesthetized with medetomidine-ketamine. Data reported as mean+/-standard deviation, and range.

medetonname ketamme. Data reported as mean // standard deviation, and range.				
pН	PaCO <sub>2</sub>	$PaO_2$	HCO <sub>3</sub>	BE
	(mmHg)	(mmHg)	(mmol/L)	(mmol/L)
N=28	N=28	N=28	N=28	N=28
7.40+/-0.03	55.0+/-5.7	39.4+/-8.8	33.1+/-3.8	7.9+/-3.4
7.34-7.46	40.8-65.9	23.0-55.0	23.3-40.2	-1-15
Lactate	Rectal temp	Respiratory rate	Heart rate	Mean arterial
(mmol/L)	(Celsius)	(breaths/min)	(beats/min)	pressure(mmHg)
N=27	N=28	N=28	N=27	N=4
1.0 + / -0.5	39.3+/-0.6	86+/-28	56+/-16	142+/-19
0.3-2.3	37.9-40.8	24-150	30-123	125-169

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#### BLOOD GAS ANALYSIS IN ZOO AND WILDLIFE MEDICINE

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#### **Abstract**

With the widespread availability of point-of-care analyzers, blood gas analysis can easily be performed in zoo collections and in the field. Deleterious changes in blood pH, oxygenation and ventilation can be rapidly diagnosed and treated. This presentation will discuss commonly available equipment and interpretation of arterial and venous blood gases. We will cover common sampling errors and artifacts, as well as species specific nuances in blood gas interpretation for non-mammalian species.

#### Introduction

Point-of-care blood gas analyzers are ideally suited for use in field and zoological settings. The most commonly used analyzers are battery operated and require only a small volume of blood (0.2-0.5mL). This allows them to be used in remote settings and with small patients. Additionally, results are generated in 2-3 min, which can satisfy the needs of many impatient zoo clinicians. Most analyzers can provide immediate information about blood oxygenation, ventilation, blood pH, glucose and electrolytes. Immediate information also allows rapid adjustment of anesthetic management. This lecture will focus on clinical scenarios and how the use of a blood gas analyzer can affect management.

Maintenance of normal blood pH (approximately 7.4 for most mammals) is crucial for homeostatic function. As such, respiratory and metabolic processes have evolved with multiple redundancies to preserve physiologic pH. Most ectothermic species and some mammals and birds (especially diving animals) have adaptations which allow for a much wider range of acceptable blood pH. While this provides an additional safety buffer for these animals, it does complicate interpretation of blood gasses and determining what is normal and what requires correction. Hypoxemia, hypoventilation and poor perfusion are some of the most significant anesthetic complications experienced in the anesthesia of captive and free ranging wildlife. With routine use of blood gas analyzers many of these complications can be detected early and potentially corrected. In addition to perianesthetic management, there is a role for blood gas analyzers in the management of many intensive medical cases.

What can a blood gas tell you that you did not already know? Both pulse oximetery and capnography provide useful, non-invasive second to second, assessments of ventilation and oxygenation. But both methodologies can have some drawbacks that compromise their accuracy. Direct blood gas analysis can identify abnormalities in species for which non-invasive

methodology is not validated. A capnograph can provide a non-invasive assessment of expired  $CO_2$ , which should be similar to arterial  $CO_2$ . Without blood gas analysis, it is possible that an increase in physiologic dead space (causes listed below) could compromise the accuracy of the capnograph. In species capable of pulmonary shunting, such as reptiles, the expired  $CO_2$  may not represent the arterial  $CO_2$  at all.

# **Technical Aspects of Blood Gas Analysis**

Sample Collection

Blood should be collected anaerobically into a heparinized syringe. Excessive heparin can affect parameters including hematocrit and ionized calcium.<sup>1</sup> Samples should be analyzed as quickly as possible and care should be taken to not introduce air bubbles into the sample. In reality, small air bubbles may not affect the accuracy of the sample significantly, but can cause the analyzer to malfunction. Excessive room air contamination can cause a decrease in the measured CO<sub>2</sub> and an increase or decrease in PO<sub>2</sub> depending on the percentage of inspired oxygen.

**Arterial Collection Sites** 

Auricular artery: elephants, rhino, most ruminants. Facial, transverse facial artery: equids, some ruminants.

Radial artery: Apes.

Dorsal pedal artery: large carnivores.

Femoral artery: small carnivores, small primates.

Superficial ulnar artery: birds.

**Venous Collection Sites** 

Jugular, auricular or lingual veins: Jugular and auricular sites provide the easiest sampling and jugular sampling also provides the closest approximation of a true mixed venous sample. Lingual samples provide a close approximation of arterial gas tensions.

Arterial vs. Venous Comparison

Arterial samples are crucial for assessing pulmonary performance, especially oxygenation. A venous blood gas can provide pH, CO<sub>2</sub> and a crude estimate of body oxygen demand. The oxygen content of venous blood varies greatly depending on the sampling site, and level of metabolism and should not be used to approximate arterial oxygen content. A central mixed venous sample can provide very useful information about oxygen consumption and anaerobic metabolism, but requires an arterial sample for comparison.

# **Reported Values**

A blood gas report contains multiple values, some of which are directly measured and some are calculated from the measured parameters based on algorithms validated for human use. Measured parameters include pH, partial pressure of CO<sub>2</sub> (PCO<sub>2</sub>), Partial pressure of oxygen (PO<sub>2</sub>), hematocrit and lactate. Most analyzers will also measure certain electrolytes, including Na, Cl, K and ionized Ca. From these measurements, the machine will calculate hemoglobin, bicarbonate (HCO<sub>3</sub>), base excess and oxygen saturation (SO<sub>2</sub>). Formulae for calculated parameters are given in the appendix. Oxygen saturation is calculated assuming a "normal" adult human hemoglobin oxygen dissociation curve. Some machines will correct for changes electrolyte concentrations based on changes in blood pH

# **Correcting Blood Gases for Temperature**

All blood gas analyzers measure dissolved gasses at a standard temperature (usually, 37°C). When the patient's body temperature differs significantly from 37°C it may be useful to "correct" the sample data to the body temperature. Temperature correction of blood gasses is fairly controversial, even in human medicine. In short, the concern is that in a hypothermic patient, reported values (measured at 37°C) may not represent the true values in the patient. At the same time, corrected values are not applicable to any known reference ranges. Human reference ranges are designed to be used with a blood temperature of 37°C. For near normothermic mammals, temperature correction does not make a significant difference and the difference is not usually a reason to change the course of treatment. For ectotherms and severely hypothermic mammals, temperature correction may be more valuable. Most analyzers use a built-in algorithm for temperature correction. There are multiple published references detailing correction formulae for ectotherms.<sup>2</sup>

The i-STAT<sup>®</sup> portable analyzer only operates at an ambient temperature of 16 to 30°C. When working outside of these temperatures, it is critical to control the temperature of the analyzer with extra heat packs or ice packs in an insulated cooler. The analyzer has an internal thermometer and will report its temperature and if there is an ambient temperature error.

## **Blood Gas Interpretation**

Basic interpretation should be a straightforward, step-by-step process taking 30-60 seconds. While there are more advanced ways of interpreting acid-base changes in human and veterinary critical care, they will not be covered here.

#### 6-Step Interpretation

1) What is the pH? Normal pH for most mammals is 7.35-7.45. Is the patient's pH low (acidemia) or high (alkelemia)?

pH: pH only gives us the direction and extent of the derangement, but does not tell us the source of the problem. It does help narrow down the differentials for the primary problem

and the list of actions that need to be done to correct the problem. Carnivores tend to have slightly more acidic pH, while herbivores and omnivores with high carbohydrate diets tend to have more alkaline blood pH.

2) What is the PO<sub>2</sub> (arterial sample)? Normal arterial PO<sub>2</sub> 100mmHg, while breathing room air. Is the patient hypoxemic? Is the patient's oxygen tension appropriate for the fraction of oxygen that it is breathing?

 $PaO_2$ : Normal  $PaO_2$  (arterial partial pressure of oxygen) is 100mmHg when breathing room air and 400-500mmHg when breathing 100% oxygen. Hypoxemia is defined as a  $PaO_2$  <80mmHg. Calculating an Alveolar-to-arterial oxygen gradient (A-a gradient) provides useful information about the cause of the hypoxemia. In addition, calculating a  $PaO_2/FiO_2$  (partial pressure to fraction of inspired oxygen) ratio provides a very easy means of assessing pulmonary function. The A-a gradient is most accurate when the patient is breathing room air (21% oxygen) while the  $PaO_2/FiO_2$  ratio can be done with any  $FiO_2$ .

A measured hypoxemia is typically the result of one of five problems:

- Hypoventilation: i.e. patient is not breathing frequently or deeply enough. In cases of hypoventilation, the PaO2 is low but there is a normal A-a gradient.
- Low FiO2: The inspired percentage of oxygen is too low. This is rare as most anesthetized animals are breathing an enriched oxygen mixture. The animal is hypoxemic with a normal P/F ratio
- Ventilation/perfusion mismatching: This is quite common and is likely the main source of hypoxemia in anesthetized large animals. It is often associated with atelectasis and can be exacerbated by poor cardiac output and poor pulmonary perfusion.
- Diffusion impairment: Rare, will not be discussed
- Anatomic right to left shunt: Rare, will not be discussed
  - 3) What is the PCO<sub>2</sub>? Normal PCO<sub>2</sub> is 35-45 mmHg. PCO<sub>2</sub> represents the respiratory component of the acid base derangement. Changes in PCO<sub>2</sub> result in respiratory acidosis and alkalosis.

PCO<sub>2</sub>: Carbon dioxide tension quantifies the balance between cellular metabolism and alveolar ventilation. Hypercapnea typically results from a decrease in ventilation, but can be a result of increased metabolism (exertion). Hypocapnea could be from hyperventilation or decreased metabolic activity. PCO<sub>2</sub> can also be compared to end-tidal CO<sub>2</sub> to determine if there is an increase in physiologic dead space. End-tidal CO<sub>2</sub> should slightly underestimate arterial CO<sub>2</sub> by 5mmHg. An increase in this difference indicates that there are areas of lung that are ventilated, but not perfused. This occurs with pulmonary thromboembolism and decreased pulmonary perfusion secondary to decreased cardiac output.

4) What is the metabolic component ( $HCO_3$  and base excess)? Are the changes appropriate for the changes in  $PCO_2$  or is there a metabolic acidosis or alkalosis?

Bicarbonate and base excess: Both of these calculated parameters provide information about metabolic alkalosis or acidosis. These are indirect measures as both are derived from the

measured  $CO_2$  on the blood gas (formulas given above). A typical reference range for  $HCO_3$  is 19 to 24mEq/L. To account for the effect of  $CO_2$  on  $HCO_3$  calculation, base excess (BE) can be used. Base excess determines the amount of bicarbonate that needs to be added to blood to bring the pH back to 7.4, when  $PCO_2$  is set at 40. Essentially, base excess factors in the effect of body buffer systems and factors out the effect of  $CO_2$  on bicarbonate to determine the metabolic contribution to acid-base balance. Reference interval for bicarbonate is -4 to 4 mEq/L. While not a perfect system, BE provides a rapid way of determining the metabolic disturbance. If BE is high, there is a metabolic alkalosis and if it is low there is a metabolic acidosis, regardless of the respiratory disturbance.

5) What is the level of compensation? Derangements of either respiratory or metabolic acid-base balance often result in compensatory change from the other system, i.e. metabolic acidosis often results in a compensatory respiratory alkalosis (hyperventilation).

Compensation: Before evaluating compensation, look back at the pH. If the pH is low, the primary process is an acidosis and the compensatory process (if present) is an alkalosis. Compensation rarely brings the patient back to a normal pH and never overcompensates. A primary chronic respiratory acidosis (hypoventilation) will lead to a compensatory metabolic alkalosis, but pH will not return to normal and will not become alkalotic. Methods of compensation include chemical buffers (few seconds), respiratory (few minutes) and metabolic compensation (few days).<sup>3</sup>

6) Are there abnormalities in electrolytes or lactate?

Lactate and electrolytes: Blood lactate is a by-product and indicator of anaerobic metabolism. Increases in blood lactate typically accompany decreases in tissue perfusion. This could include ischemic muscle from a positional or exertional myopathy in which metabolic oxygen demand has outstripped the available oxygen delivery. Focal ischemia (strangulated intestine, compromised blood flow to a limb after trauma) can also increase lactate production, and in some cases the hyperlactatemia will only be seen after perfusion is reestablished. Electrolyte interpretation is similar to routine chemistry interpretation.

This step-by-step process should lead to an assessment of oxygenation and acid-base disturbance. Acid-base disturbances are described by the pH change (acidosis or alkalosis) and the source of the disturbance (metabolic or respiratory). In many cases, there is a mixed metabolic and respiratory disturbance. A few causes of the four main acid-base disturbances in animals are listed:

*Metabolic Acidosis:* Gastrointestinal bicarbonate loss (diarrhea), renal bicarbonate loss, Lactic acidosis secondary to hypoperfusion.

Metabolic Alkalosis: Pyloric outflow obstruction, excessive exogenous bicarbonate therapy.

Respiratory Acidosis: Hypoventilation due to anesthesia, muscle relaxation, central nervous system (especially medullary or cervical) disease, airway obstruction, excessive dead space ventilation or hyperthermia.

*Respiratory Alkalosis:* Hyperventilation due to hypoxemia, pain, anxiety, inappropriate ventilator settings.

#### **Effect of Capture on Blood Gases**

Typically changes seen during capture include hypoxemia, hypercapnea, lactic acidosis and hyperkalemia from acidosis and myocyte rupture. Strenuous capture can result in metabolic acidosis from increased production of lactate. Hypoxemia can exacerbate lactate production and hypercapnea from increased metabolic production of  $CO_2$  during exertion can lead to worsening of acidosis. Similar changes in blood oxygenation,  $CO_2$  and lactate production can be result from body positioning during anesthesia. For example, moving a rhinoceros from lateral to sternal recumbency may improve its ventilation and oxygenation while compromising muscle perfusion and increasing lactate build-up.<sup>4</sup>

#### **Ectotherm Blood Gases**

There are a number of published reports on the use of blood gases for evaluating reptiles, fish and invertebrates.<sup>2</sup> Important aspects to keep under consideration include the role of temperature compensation and the wide range of acceptable blood pH in most ectotherms. The majority of the referenced studies use a taxon specific formula for correcting pH and dissolved gases for body temperature. In many cases, body temperature is assumed to be the ambient temperature. In some instances, clinically significant abnormalities were not noted unless temperature correction was performed. While the correction formulas may be valid, it is important to remember that corrected values should not be compared to standard reference ranges. Species and temperature specific ranges should be established.

## **Appendix**

Formulae used by blood gas analyzers for calculated parameters:

- 1.  $HCO_3$ :  $log HCO_3 = pH + log (PCO_2 7.608)$
- 2. Base excess =  $HCO_3$ -24.8+16.2(pH-7.4)
- 3. Anion Gap: ([Na<sup>+</sup>]+[K<sup>+</sup>])-([Cl<sup>-</sup>]+[HCO<sub>3</sub><sup>-</sup>])
- 4. A-a gradient=PAO<sub>2</sub>-PaO<sub>2</sub>= (FiO<sub>2</sub>x(P<sub>bar</sub>-PH<sub>2</sub>O) -PaCO<sub>2</sub>/0.8)-PaO<sub>2</sub>
  If performed at standard atmospheric pressure (760mmHg) and room air (21% oxygen) the formula is simplified to: 150-1.2(PaCO<sub>2</sub>)-PaO<sub>2</sub>. Normal A-a gradient is 10-15mmHg
- 5. Physiologic dead space: Vd/Vt=(Pa CO<sub>2</sub>-Et CO<sub>2</sub>)/Pa CO<sub>2</sub>. Normal dead space is 0.3-0.5.

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#### GIRAFFE CARDIOVASCULAR PHYSIOLOGY: IMPLICATIONS FOR ANESTHESIA

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#### **Abstract**

Being the tallest animal on earth, the giraffe has an arterial blood pressure twice that of other mammals, and its cardiovascular anatomy and physiology has been subject to ample speculation and myths.

Using state-of-the-art methodology we performed hemodynamic measurements in 24 anesthetized giraffes, and studied the cardiovascular anatomy of 35 freshly dead giraffes.

Relative heart mass resembles that of most other mammals ( $\approx$ 0.5% of BW), but the heart can generate high pressures because of smaller inner ventricular radii and a thickened left ventricular wall. As a consequence, stroke volume and cardiac output are lower than in similar-sized mammals ( $\approx$ 34 ml/(min·kg BW)). Blood volume is unusually low ( $\approx$ 5.6% of BW) as is the compliance of the vascular system.

When the head of the anesthetized giraffe is lowered, blood pressure at head-level peaks, before returning to much lower values. The lowering of the pressures coincides with pooling of blood in the compliant jugular veins, leading to a decreased cardiac preload and consequently lower systemic blood pressure. Similarly, even a small volume depletion causes an immediate and marked reduction in blood pressure. As a consequence of this mechanism, the arterial pressure at head level is maintained at or near 100 mmHg, and the central blood pressure is directly proportional to the position of the head relative to the heart.

Considerable ventilation/perfusion mismatch prevails when the giraffe is placed in lateral recumbency, but not when suspended upright.

This data confirms the conventional wisdom that the anesthetized giraffe should be placed as sternally as possible with the head elevated.

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# THE USE OF CONTROLLED INDUCTION TO MINIMIZE TRAUMATIC INJURY TO RETICULATED GIRAFFE (Giraffa camelopardalis reticulata) USING THIAFENTANIL, MEDETOMIDINE, AND KETAMINE

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#### Abstract

In the last 5 yr, thirteen full immobilizations of eight reticulated giraffe (*Giraffa camelopardalis reticulata*) (340-914 kg) were performed, for either castrations or significant corrective hoof trimming. All giraffe were immobilized with thiafentanil (0.44-1.18 µg/kg), medetomidine (0.9-2.4 µg/kg), and ketamine (0.55-1.62 mg/kg) administered in a single dart. Occasional intraprocedural supplementation with guaifenesin (0.4% in NaCl) was used, by continuous-rate intravenous infusion administered to effect. In eleven of these immobilizations, giraffe were darted while confined in a pen with chain-link fence walls and decomposed granite substrate and allowed to become recumbent without intervention. Inductions were generally rapid and uneventful. However, two juvenile males suffered mandibular fractures after colliding with the fence or ground while becoming recumbent.

An in-path Giraffe Restraint Device was subsequently utilized for controlled induction of two juvenile male giraffe. The Dallas Zoo restraint device is positioned within a pathway that the giraffe must traverse to exit the barn. It has elevated catwalks on the sides, a manual locking push-wall, and a hinged opposite wall that swings out to a 90-degree position, allowing manual manipulation of the recumbent giraffe out of the device. In each case the giraffe was darted after entering the restraint device and being closed within it by closing a sliding door. A halter, eye cover, and ear plugs were placed on the head as early as the giraffe would tolerate it. A lead rope was passed over an overhead-mounted pulley, which aided control of the giraffe's head as the giraffe dropped into sternal position. Once the giraffe was able to be safely manipulated, the side wall was opened while maintaining head control, and personnel entered to support and manipulate the giraffe. An endotracheal tube was inserted, ropes and straps were placed under and around the body, and the giraffe was moved out through the open side wall of the device into an adjacent open stall to complete the procedure.

The risks of giraffe immobilization have been well-documented and include both induction hazards and anesthetic complications. <sup>1-8</sup> Most potential induction period problems involve self-trauma while becoming recumbent. If the facility design, management will, and staff expertise allow, the use of a restraint device for controlled induction is recommended to minimize these complications. <sup>2,5,6</sup>

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2012 PROCEEDINGS AAZV CONFERENCE 35

# ECOIMMUNOLOGY: Chaunus marinus AS A CASE STUDY TO ILLUSTRATE THIS NEW DISCIPLINE

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#### Abstract

Ecoimmunology is an emerging field that studies the immune investment strategies of wild organisms and the causes and consequences of that investment. 1-3 This topic has caught the attention of the National Science Foundation, which has funded a Research Collaborative Network to foster collaborations and progress the science (Ecoimmunology.org). We believe that this field and the techniques it offers will become increasingly important for wildlife disease specialists and the evaluation of health in wildlife populations. Utilizing ecoimmunology as the framework, we investigated the relationship between immune function of the native marine toad (Chaunus marinus) and two habitats: organic and traditional rice fields, in the Puntarenas province of Costa Rica. The health and immune function status was assessed through body condition measures, corticosterone levels, response to phytohemagglutinin (PHA), as well as lungworm, tick and gastrointestinal parasite diversity and abundance. Based on body condition scores, fat body measurements and paratoid gland size, Chaunus marinus have significantly lower condition scores in conventional rice fields. Interestingly, females are generally more heavily affected than males. However, lungworm (*Rhabdias* spp.) and adult trematode loads are higher in organic rice farms than in conventional rice, likely due to the effects of pesticides on intermediate hosts or free-living lifestages of these parasites. In contrast, gastrointestinal nematode abundance was higher in fields treated with pesticide, which may indicate immunosuppression. This data suggests that pesticide use negatively impacts the condition of amphibians living in rice fields, outweighing a release from parasite pressure, which may translate into a loss of fitness.

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SNOW LEOPARD (*Uncia uncia*) FUNCTIONAL GENOMICS INITIATIVE: INTEGRATING GENOMICS INTO THE MANAGEMENT OF CAPTIVE ENDANGERED SPECIES

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#### **Abstract**

Using current technology, it is feasible to sequence the genome of virtually any species; however, application of genomics information to population management or to the prediction of individual traits is more difficult. The goal of the Snow Leopard Functional Genomics Initiative (SLFGI) is to develop genomics-based tools for use by population managers to address problems encountered in conservation of small captive populations of endangered species. Although the snow leopard is the focus of the initial work in this project, the models developed will be broadly applicable to other small populations of endangered species, with the ultimate goal being to maintain species diversity and robustness in these populations.

Supported by an Institute of Museum ands Library Services National Leadership Planning Grant, SLFGI has built the foundation for a proof-of-principle model for integration of genomics into captive population management plans. The initial step was to convene a workshop, held in January 2010, to bring together potential project partners and consultants including geneticists, immunologists, and members of the North American Snow Leopard Species Survival Plan. Based on workshop discussions and continued interaction with participants after the workshop, we identified key concepts, requirements, needs and concerns that must be considered when devising a strategy for using genomics information in endangered species conservation. In addition, we have established a bank of blood and tissue samples from more than 60 captive snow leopards, constructed a draft of the snow leopard genome, developed a PCR-based saliva assay for papillomavirus infection, and begun analysis of polymorphisms in specific genes associated with immune function.

# **ACKNOWLEDGMENTS**

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SNOW LEOPARD (*Uncia uncia*) PAPILLOMAVIRUS INFECTION, VACCINE DEVELOPMENT, SEROLOGIC TESTING, VACCINATION AND TREATMENT OPTIONS

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#### **Abstract**

Snow leopards (*Uncia uncia*) develop papillomavirus (PV) lesions in the mouth and on the skin. <sup>6,9</sup> Skin lesions develop on the forelimbs, neck and face and occur as dark black, slightly thickened, irregular, raised areas, 2-15 mm in diameter, which cannot be scrapped off. <sup>6</sup> Oral lesions are raised, pale pink plaques, often confluent, 1-100 mm on the edge of the tongue or around the frenulum. Histopathology and immunohistochemistry (IHC) are confirmatory. <sup>6</sup> Transformation of lesions to squamous cell carcinomas (SCC) can take up to 9 yr. <sup>6</sup> Early diagnosis of SSC is difficult but once confirmed, the prognosis is poor, since metastasis is common. <sup>6</sup>

Snow leopards should be examined opportunistically (ideally annually) for evidence of papillomas or SCC.<sup>6</sup> Lesions must be excised surgically as laser surgery, cautery and/or cryosurgery cause the PVs to seed surrounding tissues.<sup>2</sup> Non-healing wounds on the forelegs, neck or head, and oral swellings should be biopsied to rule out SCC.<sup>6</sup> Raised thickened pigmented skin lesions that are easily scraped off, leaving an ulcer, should increase one's suspicion of the possibility of an early SCC.<sup>6</sup>

A snow leopard oral papillomavirus (UuPV1)<sup>7</sup> vaccine has been produced using previously described methods. <sup>3-5,8</sup> Vaccination safety testing in domestic cats is underway.

Using previously described methods, <sup>1,4,5,8,10</sup> the UuPV1-virus-like particles produced in this process, were used as immunologic reagents to detect anti-UuPV1 antibodies, and hence determine the seroprevalence of papillomavirus infection in captive North American snow leopards. Both UuPV1 seropositive snow leopards with lesions and others with no history of having lesions have been identified.

# **ACKNOWLEDGMENTS**

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# EFFICACY OF HEMATOPHAGOUS ARTHROPODS IN SCREENING ZOO ANIMALS FOR TUBERCULOSIS AND BLUETONGUE VIRUS

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# **Abstract**

Hematophagous triatomine bugs of the family Reduviidae were used to sample blood from four different species of zoo animals for the screening of bluetongue virus (BTV) and tuberculosis (TB). These large blood-sucking insects constitute a useful tool for stress-free blood sampling of zoo and wild animal species that would otherwise need to be immobilized.

Hematophagous insects have been successfully used in xenodiagnosis<sup>5,6</sup> in humans, in investigations of energy balance, water budget and hormone levels studies in small mammals,<sup>3,4,10-12</sup> primates,<sup>9</sup> birds,<sup>1,2</sup> for rabies serology<sup>13</sup> as well as in many zoo species for haematology and blood chemistry examination.<sup>7,8</sup>

To validate the efficacy of blood-sucking bugs for standard serologic and molecular tests, the results of intravenous-drawn blood samples were compared to blood collected by the bugs. Sterilly hatched and fifth instar stage nymphs of *Dipetalogaster maxima* were used to collect up to 1.1 ml of blood. The blood was immediately extracted from the distended stomach of the bugs with a syringe and placed in a Li-Heparin vial following which individuals were decapitated.

For BTV antibody and antigen screening the blood from white-lipped deer ( $Cervus\ albirostris$ ) (n = 12) and domestic sheep ( $Ovis\ aries\ domesticus$ ) (n = 4) was tested using enzyme-linked immunosorbent assay and polymerase chain reaction.

To test for TB the Chembio TB STAT-PAK assay was used in Malayan tapirs ( $Tapirus\ indicus$ ) (n= 5) and South American sea lions ( $Otaria\ flavescens$ ) (n = 5).

Positive and negative results were found for BTV and TB, and both blood sampling techniques yielded identical results.

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# INCORPORATION OF COMPUTED TOMOGRAPHY (CT) TECHNOLOGY INTO ROUTINE ZOOLOGICAL MEDICINE: HOW IN-HOUSE EQUIPMENT CAN ENHANCE QUALITY OF CARE

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#### Abstract

Over the past 20 yr, the use of computed tomography (CT) in veterinary medicine has become more widespread, representing advancement in the standard of care. Dedicated equipment in veterinary colleges and referral centers has led to substantial use in domestic animals, but use of CT in zoological medicine remains fairly limited, restricted primarily to challenging clinical cases, high-profile specimens, and clinical research. Although many zoos have established a relationship with a local CT facility, its use is invariably limited due to issues of convenient access, animal/staff safety, and logistics with animal transport. There is really no substitute for immediate in-house access to CT technology, but the associated costs are substantial. Modernization of a hospital room, equipment purchase, and utility (electrical and ventilation) upgrades all represent significant investments. Service contracts and replacement parts are considerable ongoing expenses, as well as the necessary investments in training and education for staff to become proficient with CT unit operation and image interpretation.

In 2009 the Chicago Zoological Society (CZS) made the financial commitment to provide inhouse CT imaging and installed a GE Medical Systems HiSpeed Advantage CT scanner in the veterinary hospital, making it one of only three zoos in the world with CT technology on site. Immediate, unlimited access to the scanner has provided numerous enhancements in the level of veterinary care that can be provided at the zoo. Scans are completed quickly and efficiently, without the need for off-site transport, drastically decreasing anesthetic times and eliminating many logistic and safety-related challenges. New examination findings that indicate a need for CT can be addressed immediately, precluding the need for an additional anesthetic event, a particularly great benefit for patients with high anesthetic risks or that require complicated immobilizations.

The daily challenges of zoological medicine also provide many new prospects for CT use and the opportunity to incorporate CT imaging into many routine procedures. Routine use of CT can provide diagnostic benefits not available with other imaging modalities and may accelerate reaching a diagnosis in many cases. CT imaging with 3D reconstruction is a valuable tool for assessing skeletal morphology, organ position, and surgical approaches in species where detailed anatomic information is sparse. Routine CT use for dental evaluation is a valuable tool in species where adequate oral visualization is challenging (e.g., aardvarks, macropods, rodents). At CZS, CT scans are becoming standard practice in certain species during quarantine and preventative health examinations to evaluate potential concerns and provide a 'baseline' for future comparison. In certain species, whole body scans are performed during regular exams to begin establishing a database of normal CT anatomy. Interventional procedures (e.g. CT assisted

aspirates and biopsies) are also greatly facilitated by immediate on-site CT access. Such procedures can have significant diagnostic and therapeutic benefit and represent another emerging use in zoological medicine.

There is a clear benefit in having CT available for difficult clinical cases, but we are only beginning to recognize the advantages of routine use and the full spectrum of potential applications in zoological medicine. As costs continue to decrease and use becomes more widespread, CT will certainly become a standard in the practice of zoological medicine.

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EVALUATION OF NON-INVASIVE BLOOD PRESSURE MEASUREMENT TECHNIQUES VIA THE COCCYGEAL ARTERY IN ANESTHETIZED CHEETAHS (Acinonyx jubatus)

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### Abstract

Captive cheetah populations are affected by hypertension-related diseases, and accurate measurement of blood pressure can be a vital tool for detection, monitoring response to treatment, and tracking disease progression. 1-3 Indirect blood pressure measurement by Doppler sphygmomanometry and oscillometry at the ventral coccygeal artery was compared to simultaneous direct blood pressure measurement at the dorsal pedal artery in captive anesthetized cheetahs (Acinonyx jubatus). Systolic arterial pressure (SAP) obtained via Doppler sphygmomanometry and mean arterial pressure (MAP) obtained via oscillometry had the greatest agreement with simultaneous direct SAP and MAP measurements. Systolic and diastolic arterial pressure (DAP) measurements obtained via oscillometry had less agreement with simultaneous direct SAP and DAP measurements. Both indirect techniques exhibited trends that correlated with the trends of direct blood pressure measurements over a wide interval of arterial pressures. In a clinical setting, indirect blood pressure measurement via the ventral coccygeal artery may be useful for assessing trends in a cheetah patient, but caution should be taken when interpreting individual values. A cheetah's medical history, current clinical condition, and anesthetic protocol should be considered to determine whether indirect or direct blood pressure monitoring techniques are most appropriate.

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# AVIAN BORNAVIRUS INFECTION IN FREE-RANGING WATERFOWL: A RETROSPECTIVE AND PROSPECTIVE STUDY

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# **Abstract**

A new strain of avian bornavirus (ABV) has been recently identified as a cause of neurologic disease and mortality in free-ranging Canada geese (Branta canadensis) and trumpeter swans (Cygnus buccinator) in Southern Ontario. A retrospective evaluation of pathology cases from wild waterfowl euthanatized or found dead on the Toronto Zoo site or submitted to the Canadian Cooperative Wildlife Health Centre, Ontario (1992-2011) was carried out. The presence of virus in tissues as assessed by immunohistochemistry and qRT-PCR was highly correlated with histologic lesions resembling those described in parrots affected with proventricular dilation disease. RT-PCR products were sequenced and their nucleotide sequences were 100% identical amongst themselves. Although ABV has been identified in apparently healthy geese, our study confirmed that ABV can also cause disease (clinical signs and pathologic lesions) in wild waterfowl species.<sup>2</sup> In addition, cloacal swabs and blood samples were collected from 600 freeranging Canada geese, trumpeter swans, mute swans (Cygnus olor) and mallard ducks (Anas platyrhynchos) to estimate the prevalence of ABV infection in Ontario. We found a 14% prevalence of fecal shedding (qRT-PCR) in geese caught on the Toronto Zoo site compared to a 0% prevalence in geese sampled at three other locations in Ontario. The prevalences of shedding of ABV in mute swans and trumpeter swans were 9% and 0%, respectively, despite the fact that these species commingle. The reason for these differences among species and locations is currently unknown. The waterfowl strain of ABV appears broadly distributed with the ranges of the susceptible species and has likely been endemic within North America for a substantial period of time.

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46 2012 PROCEEDINGS AAZV CONFERENCE

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# PSITTACINE CIRCOVIRAL INFECTION IN JUVENILE AFRICAN GREY PARROTS (Psittacus erithacus)

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# **Abstract**

Psittacine circovirus, the causative agent of psittacine beak and feather disease (PBFD), is typically characterized by symmetric feather loss, abnormally shaped feathers, and beak abnormalities. The disease has been associated with immunosuppression, and most affected birds eventually succumb to secondary infections. Juvenile African grey parrots (*Psittacus erithacus*) can develop a peracute to acute form of the disease leading to death without feather abnormalities. Current literature on this syndrome is limited, and there are no detailed descriptions of cases within the United States, despite anecdotal reports of its presence.

A 5-mo-old male Congo African grey presented for a 2-day history of anorexia and lethargy. Hepatomegaly and yellow urates were noted on physical exam, and diagnostics revealed severe leukopenia and anemia, hepatomegaly, and an opacity in the right caudal lung. Despite aggressive supportive therapy, the bird died 2 days after presentation. Histopathology revealed profound lymphoid depletion of the cloacal bursa and botryoid intracytoplasmic viral inclusions. In addition, acute multifocal hepatic necrosis, and a large pulmonary fungal granuloma were noted. Electron microscopy, in situ hybridization of the cloacal bursa, and whole genome amplification and sequencing confirmed the presence of psittacine circovirus strain J.<sup>4</sup> Review of an avian pathology database revealed fifteen similar cases in juvenile African grey parrots, with characteristic intracytoplasmic inclusions within the bursa. Circoviral infection was confirmed with PCR and *in situ* hybridization. Peracute circoviral infection in juvenile African grey parrots causes severe leukopenia, liver necrosis, immunosuppression, and opportunistic infections but lacks the characteristic feather and beak abnormalities.

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# GASTROINTESTINAL TORSION AND INTUSSUSCEPTION IN NORTHERN KOALAS (*Phascolarctos cinereus*) AT THE SAN DIEGO ZOO, 1976-2012

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### Abstract

This case series describes gastrointestinal torsion and intussusception in five northern koalas (Phascolarctos cinereus) aged 2-11 yr at the San Diego Zoo from 1976 – 2012. Three of the individuals were males and two were females. Two of the animals died shortly after presenting. Diagnosis of an ileocecal intussusception resulting from enteritis in one of these peracute cases and cecal torsion in the other was made at necropsy. Two small intestinal mesenteric torsion and one proximal colon mesenteric torsion case were successfully surgically corrected. The colonic mesenteric torsion case had recurrent clinical signs two weeks later and a second surgery requiring resection and anastomosis of ischemic jejunum was performed, with the koala dying shortly afterwards. One of the small intestinal torsion cases had a recurrence of the torsion 22 mo later and consequently died. The second small intestinal torsion case remains alive 5 mo postsurgical correction. All five koalas presented with signs of colic that included anorexia, lethargy, depression, acute abdominal distension, abdominal stretching, decreased fecal output, and/or open-mouth gasping. Abdominal radiographs in cases of this type may show stacked gastrointestinal linear gas patterns and contrast stasis.<sup>1,2</sup> Clinical signs and radiographic changes are indicators that surgical intervention is required. High mortality in koalas with gastrointestinal torsion and intussusception emphasizes the importance of timely recognition and surgical correction. 1-4

#### **ACKNOWLEDGMENTS**

The authors thank the veterinary, nutrition, and pathology departments along with the koala keepers at the San Diego Zoo for their dedicated care and support of the koala collection. A special thanks to the entire veterinary department and support staff for the student education they have all so graciously provided.

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# VALIDATION OF TRANSTHORACIC ANATOMIC M-MODE ECHOCARDIOGRAPHY IN THE BOTTLENOSE DOLPHIN (Tursiops truncatus)

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# **Abstract**

The use of transthoracic echocardiography to evaluate the dolphin heart has been limited so far because of technical and anatomic specificities. Anatomic M-mode (AMM) is an echocardiographic technique capable of generating M-mode studies from two-dimensional (2D) cine loops independently of the ultrasound beam orientation. The aim of the present study was to determine the within-day (repeatability) and the between-day (reproducibility) variability of AMM in awake healthy bottlenose dolphins (BN, *Tursiops truncatus*).

Four healthy BN (9-31yr; 180-250 kg) trained to lie in left lateral recumbency were included. A total of 96 echocardiographic examinations were performed using a Vivid i system (GE Medical System, Waukesha, WI, USA) equipped with a 3.5 MHz phased-array transducer by the same trained observer on 4 different days over a 2-week period with 4 dolphins examined 6 times/day. Video clips of 2D left parasternal long-axis views showing the left ventricle (LV) ventrally and the aortic root dorsally were recorded at each examination and analyzed on the same day for AMM measurements. A general linear model was used to determine the within-day and between-day coefficients of variation (CV).

All examinations were interpretable allowing calculation of 10 AMM variables (i.e. end-diastolic and end-systolic ventral and dorsal LV myocardial wall thicknesses, LV and aortic diameters, mean aortic diameter, and LV shortening fraction). Most within- and between-day CV values (16/20) were <10%, the lowest being observed for the end-diastolic LV diameter (1.6%).

#### Conclusion

AMM provides a simple non-invasive evaluation of left heart morphology and function in BN with good repeatability and reproducibility.

#### **ACKNOWLEDGMENTS**

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# UNDERSTANDING THE SPREAD OF JOHNE'S DISEASE IN ZOO ANIMALS: WHO SHOULD WE WORRY ABOUT?

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# **Abstract**

Johne's disease (caused by Mycobacterium avium subsp. paratuberculosis; MAP) is a chronic, progressive bacterial enteritis of ruminants which can cause serious losses of both livestock and exotic species. Disease risk in exotic ruminants has been shown to be associated with maternal infection status, but the effect of other herdmates on risk of infection is not well understood.<sup>2</sup> A retrospective epidemiologic study was conducted to evaluate the association between Johne's infection status and early-life exposure to infected herdmates. The study population included 1599 individuals representing 52 species housed within the San Diego Zoo facilities between 1991 and 2010. Pre- and post-mortem disease surveillance records were reviewed to identify the infection status of all individuals in the population. Early-life (< 180 days) exposure was considered to have occurred when individuals were contemporaneously housed with infected herdmates. Herdmate infection status was further classified according to stage of infection, age, and whether diagnostic lesions were ultimately found at necropsy. Conditional maximum likelihood methods were used to estimate the effect of contact with infected herdmates while controlling for maternal exposure, differences in species susceptibility, and herd management. Herdmate contact was significantly associated with disease within some of the evaluated scenarios, but was less important as a disease risk than maternal infection status. Disease odds declined by approximately 20% per year during the study period, reflecting the effectiveness of the MAP control program. These findings may be used to improve the efficiency and effectiveness of MAP surveillance and control in zoo animals.

#### ACKNOWLEDGMENTS

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TWO CASES OF SUSPECTED POLIOENCEPHALOMALACIA SECONDARY TO THIAMINE DEFICIENCY IN WHITE-FRONTED MARMOSETS (Callithrix geoffroyi) EXHIBITING CONCURRENT CLINICAL SIGNS OF WASTING MARMOSET SYNDROME

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#### Abstract

Two white-fronted marmosets (*Callithrix geoffroyi*) with a history of diarrhea and weight loss were presented with acute neurologic abnormalities, primarily consisting of a stuporous mentation and blindness. Initial physical examination and blood test abnormalities in both animals fit the typical description of Wasting Marmoset Syndrome. The first case, a 7-yr-old female, was euthanatized after one week of empirical treatment due to lack of clinical response. Histopathology findings included chronic enterocolitis and a laminar pattern of microgliosis and astrogliosis in the cerebral cortex (considered to be a reactive response to polioencephalomalacia.) The second case, a 13-yr-old male, received treatment with thiamine and steroids after presentation; his neurologic abnormalities resolved within 48 hr. Clinical improvement continued for 3 mo while he was treated with prednisolone, metronidazole, dietary changes, and B vitamins. The animal was euthanatized in March of 2012 due to a rapid decline in condition of unknown etiology; histologically evaluated brain tissue was unremarkable.

Based on these cases, it was theorized that animals exhibiting signs of Wasting Marmoset Syndrome are susceptible to developing clinical thiamine deficiencies. Blood from healthy marmosets is currently being collected for thiamine level evaluation through use of high-performance liquid chromatography (Bio-Center Laboratory, Wichita KS 67219 USA). Initial results suggest that *Callithrix geoffroyi* has a thiamine level approximately four times greater than is expected in humans. While further work is needed to establish species specific reference ranges to aide in management of diseased individuals, parenteral vitamin B supplementation should be considered in cases with neurologic impairment or suspected malabsorptive disease.

# **ACKNOWLEDGMENTS**

The authors thank the hospital staff and primate keepers at The Phoenix Zoo for their dedication to the care of these animals. Additionally the authors thank Dr. Donna Iallegio, and Barbara Toddes, Nutrition Director at The Philadelphia Zoo, for their time, knowledge, and willingness to offer nutrition and treatment recommendations during the management of these cases.

DEVELOPMENT OF A CUTANEOUS WOUND HEALING MODEL FOR EVALUATION OF PLATELET-DERIVED GROWTH FACTOR (REGRANEX®) IN THE BEARDED DRAGON (Pogona vitticeps)

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#### **Abstract**

Wounds in reptiles are a common cause for presentation to a veterinarian, <sup>2,4</sup> however, published information regarding therapy for wound healing is limited. <sup>1,5</sup> A cutaneous wound healing model with bilateral circular wounds over the dorsal scapular region was developed in the bearded dragon utilizing the splinted wound healing model developed in mice. <sup>3</sup> A treatment group (n=5) was administered a topical synthetic platelet-derived growth factor, becaplermin (Regranex®, Healthpoint Biotherapeutics, Fort Worth, TX, USA), on one wound and vehicle (methylcellulose) on the other. A control group (n=5) received vehicle on one wound and saline on the other. The wounds were imaged using a Nikon digital SLR fitted with a macro lens at each treatment session. Wounds were treated daily for days 0-17, then every second day until 80% wound healing was achieved. Image analysis software was used to calculate wound area by manually tracing the advancing epithelial front as well as the border of the dermal wound margin (to quantify wound contraction). Day 0 and day 15 wound areas were compared to calculate percentage wound closure. A Mann-Whitney test was used to compare each of the four treatments. Becaplermin significantly accelerated (p<0.016) wound closure compared to vehicle. No significant differences were found between other treatment groups. This wound healing model may be used to evaluate other topical products and reptile wound healing physiology.

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# ZOO ETHICS IN THE VETERINARY SCHOOL CURRICULUM: TEACHING THE YOUNG DOGS OLD TRICKS

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#### **Abstract**

Ethics is arguably one of the most important and difficult disciplines to teach in a professional curriculum. It is likely that a student's ethical mindset is primarily developed at an early age, long before the student enters college; yet new tools <u>can</u> be acquired and new information <u>can</u> be received throughout life. With this in mind, Michigan State University College of Veterinary Medicine has developed a course to expose veterinary students to ethical dilemmas they may face in professional practice and challenge them in an interactive environment with their peers and experienced faculty. One of the many areas of discourse covered is the trade-off encountered in zoo practice between individual animal welfare and the needs of an endangered population. In practice, this discussion often occurs in a very public forum, making decision-making even more difficult.

Multiple fictitious scenarios were devised for the students based on real experiences to simulate the kinds of choices that veterinarians must make at zoos, such as:

- 1) The choice to take in a rescued American black bear from a humane society, thus displacing a breeding pair of spectacled bears.
- 2) The choice of taking in a USFWS confiscation of 10,000 assorted reptiles and amphibians into a closed herpetologic collection.
- 3) The choice of assisting local law enforcement as they confiscate declawed tigers from "crack houses" in an urban environment.

# Students were then asked to assess:

- 1) Who/what are the stakeholders in this dilemma?
- 2) What are the possible choices of action?
- 3) Who are the 'losers' and 'winners' of each choice?
- 4) Can anything be done to mitigate the damage to the 'losers'? Is this ethical?
- 5) What would be your best professional recommendation to the zoo administration?

These discussions have value not only for veterinary students destined for a zoo medicine career, but for all veterinarians who often become leaders in their communities. In conclusion, this

course has provided excellent opportunities to educate students about the complexities of zoo practice and the relevance of these issues to all citizens of the world.

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# BREED AND CULL: LET'S TALK ABOUT A TABOO

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#### Abstract

Population management in zoos and protected areas is a reality. Zoo veterinarians routinely apply reversible and irreversible contraception and methods are continuously updated. However, contraception excludes the animals from all aspects of reproductive behaviour (courtship, pairbonding, mating, pregnancy, rearing offspring, mother-infant bonding, playing, and other socialization of the young by the adults and vice versa), and therefore also from its unique enrichment potential. We also need reproduction of zoo animals for longterm preservation of the widest possible genetic variety of endangered species. The application of contraception as the only method for population control in zoos is therefore controversial with regard to animal welfare and conservation, and the culling of surplus animals has to be considered a valuable alternative.<sup>3,4</sup> Zoo's attract the public (and raise its awareness for conservation issues) with animal babies which makes consequent killing of offspring a major emotional dilemma. Furthermore, cultural and legal aspects also need to be taken into account as well as the way of dealing with staff, public awareness and the media. The actual decision of culling an animal can only be made by a person who possesses the specific expertise and is familiar with the situation (e.g., the zoo veterinarian).<sup>2</sup> Killing of surplus animals and information has to be done in a professional way. Zoo Basel sacrifies zoo animals and performs whole carcass feeding of Artiodactyla to carnivores. The procedures are supervised and controlled by the zoo vet. Body cavities are opened and meat is inspected by the zoo vet to ensure best quality and hygiene. The public is informed about it both actively and passively. The purpose of this paper is to encourage the discussion of this issue within the zoo community.

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# IMPROVING WELFARE OF CAPTIVE WILDLIFE IN CHINA: PROMOTING INTEGRATED VETERINARY AND BEHAVIORAL MANAGEMENT

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#### Abstract

Animals Asia Foundation is an international animal welfare organization committed to ending bear bile farming and addressing numerous animal welfare issues. Since 2000, Animals Asia has rescued 381 bears from bear bile farms, providing extensive veterinary care and behavioral management at our two Moon Bear Rescue Centres in China and Vietnam. While welfare of captive wildlife remains an ongoing development worldwide, there exist unique challenges in China. Animals Asia has initiated comprehensive investigations and assessments of zoological facilities, safari parks, and veterinary hospitals throughout China. Through participation in local veterinary and zoo conferences and the development of positive collaborative relationships with local veterinary associations and the China Association of Zoological Gardens (CAZG), Animals Asia has gained valuable insight into the current standards of veterinary training and captive animal facilities in China. Challenges include the lack of both animal welfare legislation and standardized animal management and veterinary training. Animals Asia hosted two workshops at our Moon Bear Rescue Centre in Sichuan Province, inviting animal caretakers, managers and zoo veterinarians from zoological parks across China, promoting an integrated approach to management of captive wildlife by using our rescue centre as a model. Emphasis was placed on concepts of animal welfare, preventative medicine and the importance of minimizing stress in captivity including the importance of environmental enrichment and the need to integrate veterinary care and behavioral management. The successes of such workshops highlight the willingness of the CAZG to collaborate and accept constructive feedback and advice to improve facilities and care of captive wildlife in China. In addition, notable progress was made in October 2010 when the CAZG backed a directive by the Ministry of Housing and Urban-Rural Development which included a ban on animal performances in zoos (Ministry of Housing and Urban-Rural Development of the Peoples' Republic of China. 2010. The guidance on further zoos. No. strengthening regulation of [2010] 172. www.mohurd.gov.cn; http://www.animalsasia.org/index.php?UID=GRQ69AW36ZC). In addition, a draft of an animal protection law has been backed by Chinese lawyers (Prevention of cruelty to animals law of the (Experts' Draft Proposal). http://www.china.com.cn/news/law/2010-03/17/content 19623441.htm; http://www.animalsasia.org/index.php?UID=YX79ZJEGRF7) and in 2011, delegates from the CAZG were invited to the UK by Animals Asia to participate in international training. Currently draft zoo management guidelines are being developed by the CAZG, addressing all aspects of animal management including veterinary care and animal management training. Animals Asia remains hopeful that with continued collaborations, positive

60 2012 PROCEEDINGS AAZV CONFERENCE

progress will lead to further improvements in the integrated veterinary and behavioral care of captive wildlife in China.

# THE ROLE OF THE ZOO VETERINARIAN IN THE REGULATION OF AVIAN WELFARE UNDER THE ANIMAL WELFARE ACT

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#### Abstract

The United States Department of Agriculture regulates animal welfare through inspections of covered animals in breeding, research, transportation, and exhibition facilities. Although birds, other than those bred for use in research, have been covered under the Animal Welfare Act since it was amended in 2002,<sup>2</sup> they are not currently inspected because regulations have not yet been published. Timing for publication of the proposed regulations for comment hinges on multiple factors, including budgetary allowances and political influences. Because the proposed regulations are in departmental clearance at the time of this writing, contents of the draft cannot be shared. However, approaches to regulation of minimum standards of care for birds will likely not stray far from how mammals are currently regulated, thus an understanding of that process is helpful.<sup>3,4</sup> In lieu of governmental minimum standards of compliance for avian welfare, the question for who should and can take the lead on the creation of standards of care for birds remains unanswered. Captive avian welfare remains a challenge as zoos focus on avian conservation in the wild, the aviculture industry decreases in size and scope, exotic animal veterinary associations realign, and sanctuaries expand. Given the often dichotomous opinions on avian care between these various stakeholders and the need for scientifically based approaches to welfare guidelines, the potential for the role the zoo veterinarian can play in promoting and protecting avian welfare is one that should be explored further.

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# BEWARE OF YOUR RACOONS AND OPOSSUMS! SALMONELLA SURVEILLANCE OF WILDLIFE REVEALS UNEXPECTED RESULTS

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#### Abstract

Due to the increased morbidity and mortality associated with salmonellosis in children, most studies have focused on reptiles, where there is a clear relationship between ownership of certain pets and infection. Surveys have documented the presence of Salmonella in a variety of wildlife species; 1-6 however, their role as reservoirs remains unknown. We investigated Salmonella prevalence and the geographic/temporal variation of Salmonella serovars from water and mesomammals in two watersheds in Georgia, one of which (South) has a history of high cases of human salmonellosis. Monthly water and quarterly mammal samples were collected from 3 stations in each watershed for 12 mo. The prevalence of Salmonella recovered from surface waters from the Southern stations was 48%, while the prevalence in the North was 60%. The prevalence of infection in opossums was 41% (95% CI: 0.29, 0.55; n=65) and 61% (95% CI: 0.47, 0.73; n=63) in the Northern and Southern watersheds respectively; and that of raccoons was 43% (95% CI: 0.24, 0.62; n=38) and 50% (95% CI: 0.31, 0.69; n=25). In both species, the highest prevalence occurred during the summer months (p = 0.012). Of particular significance, the Salmonella serotypes recovered from raccoons and opossums were diverse (n=13), but their Pulse Field Gel Electrophoresis (PFGE) patterns matched those serotypes recovered from water and from human cases in the CDC PulseNet database.<sup>7</sup> Due to readily-accessible food and habitat, anthropogenic areas, including zoos, often attract raccoons and opossums, which we consider to be asymptomatic transporters of environmental Salmonella serotypes responsible for human salmonellosis cases.

#### **ACKNOWLEDGMENTS**

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EVALUATION OF PORTABLE URIC ACID/GLUCOMETER AND HEALTH ASSESSMENT IN FREE RANGING CAPE VULTURES (Gyps coprotheres) IN SOUTH AFRICA

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#### Abstract

Thirty free-ranging cape vultures (Gyps coprotheres) were examined and sampled to establish standard values for species-specific morphometric measurements, complete blood cell counts (CBCC), blood chemistry values, genetic speciation, and mercury levels in feathers. All were juvenile or adult birds, of unconfirmed sex. No mercury levels suggesting toxicity exposure were noted. Blood chemistry values showed no significant variations compared to most avian species, while the CBCC did show a trend for a white blood cell count more elevated than most avian species, though that might have been attributable to overnight confinement in pen. Uric acid values using a human portable machine were compared to values from a commercial laboratory. Values from the portable unit were consistently lower than values from the commercial laboratory, varying from 14-222% lower. No uric acid values were considered elevated compared to other *Gyps* spp. values, so further testing for comparison of clinically relevant (elevated) values should be considered. With the known risk of renal failure from feeding on livestock carcasses of animals administered non-steroidal anti-inflammatory diclofenac welldocumented in Asian Gyps vultures, validation of the use of an inexpensive, simple portable field unit for triaging uric acid levels in debilitated birds may be useful. Genetic testing was also performed that confirmed that the Cape Vulture is a distinct species from the white-backed vulture (Gyps africanus) and such information may help to avoid using hybrid individuals in reintroduction efforts.

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AGE-RELATED CHANGES IN HEMATOLOGY, PLASMA BIOCHEMISTRY, AND URINALYSIS VALUES IN ENDANGERED, WILD RING-TAILED LEMURS (Lemur catta) AT THE BEZA MAHAFALY SPECIAL RESERVE, MADAGASCAR

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# **Abstract**

In 2011, forty wild ring-tailed lemurs were captured using Telazol<sup>®</sup> (tiletamine/zolazepam) administered via blow dart. Lemurs were divided into three age classes: <5 yr old (n=8), 5-9 yr old (n=17), and  $\geq$ 10 yr old (n=15). Whole blood was collected from a femoral vein and used to perform hematology (white blood cell counts and differentials, hematocrit, total protein) and plasma biochemistry profiles (sodium, potassium, chloride, ionized calcium, glucose, blood urea nitrogen, creatinine, and hemoglobin) at the Beza Mahafaly Special Rerserve (BMSR) field laboratory. Hematology profiles were performed manually, and plasma biochemistry profiles were obtained using an i-STAT<sup>®</sup> portable chemistry machine. Urine samples were collected via manual expression of the bladder from 37 of the 40 lemurs: <5 yr old (n=8), 5-9 yr old (n=16), and  $\geq$ 10 yr old (n=13). For each urine sample, biochemical values and specific gravity were determined and the sediment was evaluated at the BMSR field laboratory.

Younger lemurs (<5 yr old) had higher average hematocrit; higher average plasma total protein, potassium, and glucose; lower average plasma ionized calcium; higher average urine pH; and more frequent low-level proteinuria and glucosuria than middle-aged and older lemurs. Older lemurs ( $\ge 10$  yr old) had higher average blood urea nitrogen values and lower average white blood cell counts than middle-aged and younger lemurs.

Identifying age-related changes in hematology, plasma biochemistry, and urinalysis values in apparently healthy wild ring-tailed lemurs will aid in proper clinical diagnosis and treatment of captive lemurs, which is especially relevant for management of geriatric animals in zoo populations.

# **ACKNOWLEDGMENTS**

The authors thank Abaxis Animal Health, Union City, CA for their generous lending of an i-STAT® chemistry analyzer for use in Madagascar.

EPIDEMIOLOGIC INVESTIGATION OF CANINE DISTEMPER VIRUS IN DOMESTIC DOGS AND JAGUARS (Panthera onca) IN THE SURROUNDINGS OF THE CALAKMUL BIOSPHERE RESERVE IN SOUTHERN MEXICO

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# **Abstract**

The transmission of infectious diseases between domestic animals and wildlife is a conservation concern. 1,3 Domestic dogs often act as reservoir species that can maintain infectious diseases in their populations and may transmit these to wildlife.<sup>2</sup> The goals of this study were to examine the exposure to canine distemper virus (CDV) in domestic dogs and free-ranging jaguars (Pantera onca) near the Calakmul Biosphere Reserve in Southern Mexico and determine the risk factors associated with CDV seropositivity. We conducted a cross-sectional household questionnaire survey to obtain information on vaccination status and demographic data of dog populations in three villages surrounding Calakmul. We used a microneutralisation test to determine serum antibodies to CDV in 93 domestic dogs. Serum samples from 13 jaguars captured in the reserve in previous years will be tested at Cornell University Veterinary Diagnostic Laboratory, Ithaca, New York. Dog population sizes and levels of exposure to CDV varied between the villages with a high prevalence and large dog population size in the largest village, Caobas. More than 90% of all dogs sampled had never been vaccinated against CDV and opportunities for direct contact with wildlife were demonstrated due to dog hunting activities and predation of domestic animals. Jaguar CDV results will provide baseline information on disease exposure critical for monitoring the population health of this endangered felid. Our results demonstrate that domestic dogs may play an important role in CDV spillover to wild carnivores in the Calakmul region.

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The authors thank the villages of Caobas, Narciso Mendoza and 20 de Noviembre and Mr. Francisco Zavala for their participation and support to perform this study.

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# USE OF DISTRACTION OSTEOGENESIS IN TWO WILD RAPTOR SPECIES

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# **Abstract**

Distraction osteogenesis was used in two wild raptor patients at The Raptor Center. The first case was a hatch year female peregrine falcon (*Falco peregrinus*) that was admitted with an open oblique distal tibiotarsus fracture of the right leg. The fracture was surgically managed using the external skeletal fixator intramedullary pin tie-in technique (ESF-IM pin tie-in),<sup>3</sup> and it healed as expected. The bone healed with significant limb shortening and consequently developed pododermatitis. The second case was an adult female great-horned owl (*Bubo virginianus*) that was admitted with a partially healed over-riding tibiotarsus fracture. The fracture was too old to fix surgically using the technique mentioned above and the limb was significantly shortened. In order to solve the limb shortening problem, both patients were fitted with a ring fixator device and distraction osteogenesis was performed over a period of time until the length of the limb was appropriate. This process led to complete recovery and release back to the wild in both cases.

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# FIELD ELECTRONARCOTIZATION, ANESTHESIA AND SONIC TRANSMITTER IMPLANTATION OF FREE-RANGING ROBUST REDHORSE (Moxostoma robustum) IN THE BROAD RIVER SYSTEM OF GEORGIA

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#### Abstract

The robust redhorse (Moxostoma robustum) is an imperiled catostomid species which was rediscovered in 1980 and is only found in three Atlantic slope drainages in Georgia, South Carolina, and North Carolina. Many studies have shown that these fish migrate upstream to spawn and river dams can block migratory routes and alter water flow leading to loss of spawning habitats. Between 1995 and 1998, 39,000 robust redhorse juveniles were reintroduced into the Broad River system, Georgia, which is currently a population above the fall line, the remaining wild population occurs downsteam.<sup>2</sup> The purpose of this study was to assess the movement and survival of free ranging fish following electronarcosis, chemical anesthesia and surgical transmitter implantation of sonic transmitters and microchips. Twenty robust redhorse (15 males and 5 females) were anesthetized using buffered MS-222 at 150 ppm and surgically implanted with transmitters over a 1-yr period. Each transmitter was 3.5 cm long and weighed 11g. Pre-operative medications included meloxicam (0.2mg/kg IM) and ceftazidime (22mg/kg IM). The fish ranged from 439-555mm standard length and from 1890-3434 grams. The first 6 fish were tagged 25 mo ago and the second group (14 fish) were implanted 13 mo ago. Underwater receivers have recorded 90,000+ sonic detects within the reservoir and river. One signal has been stationary since 2 weeks post surgery and one transmitter was not detected again after 2 mo post surgery. This field anesthesia and surgical implantation procedure appears safe and of value in tracking the movements of fish for needed data on population status, distribution, spawning, and habitat use.

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# DIAGNOSIS AND MANAGEMENT OF TB IN A COLONY OF ORANGUTANS IN THE REAL WORLD: COMPLEX BUT NOT COMPLICATED

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### Abstract

Due to the insidious nature, severity and zoonotic potential, *Mycobacteria tuberculosis* complex (MTB) is uniquely difficult to diagnose in great apes and especially in orangutans. While there are numerous detailed plans and guidelines to combat and manage tuberculosis in captive nonhuman primates, these are often poorly adapted to field or in-situ scenarios. Due to the poor performance of the various unspecific diagnostic tests (e.g., clinical examination, chest x-rays and blood work) and inadequate and non-validated sensitivity and specificity of indirect and direct tests (e.g., inter-alia: comparative skin tests, interferon-gamma release tests, Ziehl-Nielsen staining) the clinician and manager is necessarily faced with a confusing array of results. We demonstrate in a colony of orangutans in East Kalimantan how the attempt to adhere to complex guidelines in a diagnostic-training-constrained environment is inherently rife with uncertainties. Integrating risk analysis and defining acceptable risk from the onset appears key to moving forwards in addressing this complex multi-faceted problem. When knowledge is uncertain and predictive values are weak, this is often used as an argument to obstruct problem transformation and resolution. We show that finding a transparent forward-thinking approach in dealing with uncertainties is the key to addressing complex problems such as TB management in orangutans.

# CATARACT REMOVAL IN AN AFRICAN ELEPHANT (Loxodonta africana)

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# **Abstract**

Bilateral cataracts were diagnosed in a 38-yr-old male African elephant. Within 6 mo, eyesight deteriorated such that the animal could no longer navigate the exhibit, necessitating confinement to a holding area. Over the next several months, behavioral depression, lethargy, and dramatic muscle mass loss were noted.

Eighteen months after presentation, the cataract in the left eye was removed via phacoemulsification. Prior to surgery, the eye was imaged via spectral-domain optical coherence tomography (Envisu R2300, Bioptigen, Research Triangle Park, North Carolina 27709 USA). The second cataract was removed 6 mo later. Despite easy removal of cataracts, lens capsule damage prohibited installation of prosthetic lenses. Though ocular discomfort was not evident and visible inflammation was minimal, post-surgical treatments included the following medications: oral flunixin meglumine (1500 mg; Banamine paste, Schering-Plough Animal Health Corp., Union, New Jersey 07083 USA) and topical prednisone acetate (1%, Pacific Pharma, Irving, California 92612 USA), nepafenac (Nevanac ophthalmic suspension, 0.1%, Alcon Laboratories Inc., Fort Worth, Texas 76134 USA), tropicamide (1%, Bausch & Lomb Inc., Tampa, Florida 33637 USA), moxifloxacin (Vigamox 0.5%, Alcon Lboratories, inc., Fort Worth, Texas 76134 USA) and ciprofloxacin (0.3%, Pack Pharmaceuticals, LLC., Buffalo Grove, Illinois 60089 USA).

Vision improved incrementally post-procedure. The elephant remains aphakic and far-sighted, but easily navigates its enclosures and locates food. Quality of life and body condition have improved dramatically since access to exhibit. Contact lenses (Acrivet, Neuendorfstabe 20a, Hennigsdorf 16761, Germany) have been fabricated in an attempt to further improve eyesight; placement will not be considered until late 2012.

#### ACKNOWLEDGMENTS

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# GASTRIC PHYTOBEZOARS CAUSED BY INGESTION OF PERSIMMON IN SLENDER TAILED MEERKATS (Suricata suricatta)

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# **Abstract**

Two meerkats (*Suricata suricatta*) died acutely and gastric bezoars were found on postmortem examination. Full diagnostic examinations were performed on the remaining eight animals in the group and gastric bezoars were found radiographically in four additional meerkats. The gastric bezoars completely filled the stomach and were firm, black in color, comprised of fibrous material and measured approximately 6.5 cm by 4 cm. The bezoars were removed surgically via gastrotomy from all four meerkats. All four meerkats recovered uneventfully after gastrotomy to remove the bezoars. Histologic examination of the gastric bezoars was consistent with persimmon fruit. Persimmon ingestion has been reported to cause phytobezoar formation in humans and horses. <sup>1,3</sup> Tannins found in ripe persimmons are known to coagulate in the presence of gastric acid and the resultant phytobezoars can lead to gastrointestinal obstructions. <sup>2</sup> It is suspected that a diet reduction in the group due to obesity may have led to food aggression and uncharacteristic consumption of persimmons produced by a tree in the exhibit. The tree was immediately removed from the exhibit and dietary modifications, including slight increase in amount offered and increase in number of feed stations were instituted. No further cases have been identified.

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MINIMALLY INVASIVE TECHNIQUE FOR ADMINISTRATION OF CHEMOTHERAPEUTICS VIA A VASCULAR ACCESS PORT IN A MONGOOSE LEMUR (Eulemur mongoz)

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### **Abstract**

Vascular access ports (VAPs) have been used for many years to address challenges associated with chemotherapy in both laboratory and companion animals. 1,2,3 A 16-yr-old, female mongoose lemur (Eulemur mongoz) was diagnosed with a hepatocellular carcinoma on routine examination. Following tumor debulking surgery, a Le Port CompanionPort Vascular access port with 5 french catheter (Norfolk Vet Products, Skokie, Illinois 60076 USA) was placed over the dorsum between the shoulder blades, and routed by catheter to the right jugular vein to facilitate weekly follow-up chemotherapy. Chemotherapeutics Gemcitabine (2 mg/kg i.v., Eli Lilly, Indianapolis, Indiana 46285 USA) and Carboplatin (10 mg/kg i.v., Hospira, Lake Forest, Ilinois, 60045 USA) were instituted once every seven days for two weeks followed by a recovery week for six cycles. The combination of the VAP, manual restraint, and operant conditioning facilitated administration of all drugs without complication and without the need for chemical immobilization. The VAP was used without complication for 6 mo and will remain as a permanent implant. Currently, the lemur remains free of any adverse signs related to the therapy and the carcinoma remains in clinical remission. Further, use of the VAP facilitated therapeutic monitoring allowing serial blood sampling throughout the course of therapy. This case illustrates how placement of VAPs can be a valuable tool in the management of serial treatments in zoo species, providing an increased ease of drug administration, while minimizing the risk to the patient through repeated immobilizations.

## **ACKNOWLEDGMENTS**

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# MANAGEMENT OF SEVERE BILATERAL CHRONIC SUPERFICIAL KERATITIS (PANNUS) IN AN AFRICAN WILD DOG (Lycaon pictus)

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### Abstract

A 5-yr-old, intact male African wild dog (*Lycaon pictus*) developed progressive ocular lesions and blindness caused by bilateral severe ulcerative chronic superficial keratitis (pannus). Oral prednisone (1 mg/kg p.o. b.i.d.) and cyclosporine (3.5 mg/kg p.o. s.i.d.) resulted in partial improvement, including return of vision. Topical medications were not feasible due to temperament. The patient was later anesthetized for insertion of subconjunctival sustained-release cyclosporine implants (10% cyclosporine/silicone matrix, Lux Biosciences, Inc., Jersey City, New Jersey 07302 USA) o.u. in the dorsal bulbar conjunctiva. Oral medications were discontinued following surgery. The patient remained visual and comfortable after implant placement. However, a 6-mo follow-up examination revealed that the o.d. cyclosporine implant was migrating out of the conjunctival pocket. It was repositioned in a new, more lateral pocket. The o.s. implant remained in place, but the cornea was diffusely pigmented and fibrotic. Neither eye was actively inflamed, but the cyclosporine implants alone were no longer controlling disease. Oral prednisone was reinitiated (0.5 mg/kg p.o. b.i.d.), and a cyclosporine misting spray (20 mg/ml, Civic Center Pharmacy, Scottsdale, Arizona 85251 USA) was compounded for topical administration (o.u. b.i.d.).

The use of sustained-release cyclosporine implants has been described previously for treatment of keratoconjunctivitis sicca in a red wolf (*Canis rufus*). Pannus has not been previously described in African wild dogs, and the species' aggressive temperament makes management challenging. A multimodal therapy including cyclosporine implants, oral medications, and topical drugs may be required.

#### ACKNOWLEDGMENTS

The authors wish to thank the Carnivore-Primate staff at the Phoenix Zoo for their care and diligence in treating this patient, and the veterinarians and staff at Eye Care for Animals for donating their valuable expertise, time, and equipment to this case and other patients at the Phoenix Zoo.

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# KIRICEPHALUS COARCTATUS IN AN EASTERN INDIGO SNAKE (Drymarchon couperi); ENDOSCOPIC REMOVAL, IDENTIFICATION, AND PHYLOGENY

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## Abstract

A wild, adult, male Eastern Indigo (Drymarchon couperi) snake presented for placement of an intracoelomic radio transmitter. The patient was in good body condition and physical exam was unremarkable. Five months later, the snake re-presented with 24% weight loss from initial presentation and field biologists reported it to have had intermittent respiratory discharge on two occasions. Complete blood count revealed heterophilia (12.2x10<sup>3</sup>/µL) and monocytosis (6.6x10<sup>3</sup>/μL). A pulmonary wash was performed by flushing 4mls of sterile saline through a red rubber catheter inserted into the trachea and aspirating. A second sample was collected as the snake was held vertically so that remaining fluid drained out of the lungs and trachea and through the mouth and nares. Cytology on the first sample was unremarkable. However, the second sample revealed larvated and non-larvated eggs, as well as larvae consistent with pentastomid parasites. Seven adult worms were identified and removed via transcutaneous pulmonoscopy from the air sac distal to the lung using a combination of rigid<sup>a</sup> and flexible<sup>b</sup> endoscopy. Removal of male pentastomids was uncomplicated as they were freely movable within the air sac. Females were more difficult to remove as the anterior aspect of the pentastomid was embedded. Specimens were morphologically identified as Kiricephalus coarctatus. Polymerase chain reaction and sequencing was performed and compared to other genetic sequences from species within Pentastomida. Phylogenetic analysis of this data indicates that K. coarctatus forms a well-supported clade with Armillifer armillatus and Porocephalus *crotali*, two species capable of causing significant pathology in mammalian intermediate hosts. <sup>1,2</sup>

## ACKNOWLEDGMENTS

The authors thank the Orianne Society for their support in the diagnosis and treatment of this case.

<sup>&</sup>lt;sup>a</sup> Storz rigid endoscope 4.0 mm x 30 cm with 17.5 fr sheath, Karl Storz GmbH & Co. KG, Tuttlingen, Germany

<sup>&</sup>lt;sup>b</sup>Storz 4.9 mm x 85 cm fiberscope, Karl Storz GmbH & Co. KG, Tuttlingen, Germany

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# TWO CASES OF CANINE ADENOVIRUS TYPE 1 INFECTION IN MALAYAN SUNBEARS (Helarctos malayanus) AT THE OAKLAND ZOO, CALIFORNIA

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### Abstract

Two cases of canine adenovirus (CAV) -1 occurred 5 yr apart in two Malayan sunbears (*Helarctos malayanus*). Though the two individuals did not overlap in lifetime, each had contact with an asymptomatic conspecfic that shared the same exhibit. In the first case, a 23 yr old female presented acutely comatose. MRI revealed hypoperfusion of the head and brain; euthanasia was elected. Virus neutralization (VN) serology was negative for CAV-1 and CAV-2. Histopathology revealed disseminated endotheliotropic adenovirus infection. Amplified viral DNA from brain samples had 100% identity to CAV-1.

Five years later, a 3-yr-old female had one day of lethargy and anorexia with progression in 24 hr to a coma with vertical nystagmus. Sedation was maintained by diazepam CRI (0.25 – 0.5 mg/kg/hr i.v. in 0.9% saline) for 36 hr while supportive care was provided. Ganciclovir (250 mg i.v. b.i.d. for 2 days) and cidofovir (375 mg i.v. once) were administered. Complicating disseminated intravascular coagulopathy was managed with heparin (5 USP units/kg s.q. t.i.d. for 2 days). Recovery allowed release from the hospital 12 days after presentation. A significant rise in CAV antibody titer via VN, as well as, a positive PCR in whole blood were demonstrated on acute and convalescent samples (days 1 and 12).

Skunks and raccoons observed within/near the exhibit were suspected as the source of exposure. Improved wildlife exclusion methods were implemented; all conspecific sunbears were vaccinated intramuscularly twice 1 mo apart with a modified live multivalent vaccine containing CAV-2.

### **ACKNOWLEDGMENTS**

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SUCCESSFUL MANAGEMENT OF RECURRENT EOSINOPHILIC GRANULOMA WITH STEROIDS AND ANTIHISTAMINES IN A BLACK RHINOCEROS (Diceros bicornis)

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## **Abstract**

Skin diseases of the black rhinoceros (Diceros bicornis), including eosinophilic granuloma (EG), are well reported.<sup>2,3</sup> A 7-yr-old male captive-born male black rhinoceros presented with a hemorrhagic lesion on the mucosal surface of the upper lip. Histologic evaluation confirmed an EG with mixed cellular infiltrates and hyperplastic epithelium. Over the next 4 yr, the animal was anesthetized 14 times to treat nine episodes of EG, affecting the mucosa of the nasal and oral cavities, as well as, the skin of the prepuce. Symptomatic treatment consisted of cryotherapy, intralesional triamcinolone, or topical antimicrobial/steroid ointment, however, lesions continued to recur. Due to unrewarding results, significant behavioral changes, and the risks associated with repeated anesthesia, medical treatment was initiated using a tapering 12-day dose of oral corticosteroids (Dexamethasone; initial 0.1 mg/kg p.o., q 24 h x 3 days, then 0.075 mg/kg p.o., q 24 h x 3 days, then 0.05 mg/kg p.o., q 24 h x 3 days, then 0.025 mg/kg p.o., q 24 h x 3 days). The lesions dramatically improved within 1-2 days and completely resolved within one week, but would recur soon after treatment was discontinued. Continuous oral antihistamines (Hydroxyzine pamoate; 1 mg/kg p.o., q 12 h) were then provided as an immune modulator due to reported association between insect bite hypersensitivity and EG in horses. Treating medically with steroids and antihistamines has minimized anesthetic events and greatly reduced the incidence and severity of the lesions. An allergic etiology is suspected based upon the positive response to antihistamines.

# ACKNOWLEDGMENTS

The authors gratefully acknowledge the veterinary and animal care staff of the San Diego Zoo Safari Park.

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# TREATMENT OF DISKOSPONDYLITIS ASSOCIATED INTERVERTEBRAL DISK HERNIATION IN AN AARDVARK (*Orycteropus afer*): LESSONS ON ICU CARE AND REHABILITATION FOLLOWING A LUMBAR HEMILAMINECTOMY

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## Abstract

Progressive rear limb proprioceptive deficits and ataxia were noted in a 17-yr-old aardvark (*Orycteropus afer*). CT findings were consistent with intervertebral disk protrusion and diskospondylitis at L2-L3. MRI demonstrated disk rupture, spinal cord compression, and significant inflammation in the dorsal vertebral muscles. A hemilaminectomy was performed; anatomy limited removal of disk material, but adequate decompression was obtained. Culture of disk material and muscle aspirates yielded a multi-drug resistant *Enterococcus* sp.

Post-surgical intensive care (PICC line, urinary catheter, analgesia) and rehabilitation presented many species-specific challenges. Voluntary motor function was absent following surgery. Physical therapy and rehabilitation were initiated four days post-surgery. Fentanyl-ketamine (0.5-1.0 µg/kg/min, 5 µg/kg/min, respectively) CRI, midazolam (0.4 mg/kg), and hydromorphone (0.16-0.2 mg/kg) were titrated to effect for sedation and analgesia. Surgical dehiscence occurred after 3 weeks. Negative pressure wound therapy (vacuum-assisted closure) and silver-impregnated bandages aided wound healing. An oxazolidinone antibiotic, linezolid (12 mg/kg, q24 hr, p.o., 10 wk) was used to treat the diskospondylitis. Patient attitude, size, and anatomy led to challenges not encountered with companion animals, necessitating development of custom mechanical devices and individually-tailored therapies. Additional challenges encountered during 3 mo of rehabilitation included: aural hematomas, pressure sores, and urinary tract infections. Follow-up CT revealed a stable subluxation with disk space collapse at L2-L3 and apparent diskospondylitis resolution.

Frequent antibiotic use in this aardvark for common problems (e.g., dental disease, poor wound healing) is speculated to have promoted *Enterococcus* resistance. A cutaneous wound may have led to infection in the vertebral muscles and subsequent diskospondylitis.

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TREATMENT OF PROBABLE Coccidioides SPECIES MENINGOENCEPHALITIS WITH SECONDARY OBSTRUCTIVE HYDROCEPHALUS IN A BUFF-CHEEKED GIBBON (Nomascus gabriellae) THROUGH MEDICAL MANAGEMENT AND PLACEMENT OF A VENTRICULOPERITONEAL SHUNT

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### **Abstract**

An 8-yr-old intact male buff-cheeked gibbon (Nomascus gabriellae) had a four day history of vaguely abnormal behavior, a mildly decreased appetite, and a one day history of dull mentation. Mild generalized muscle wasting and weight loss were appreciated on physical examination. Routine immunodiffusion serology for *Coccidioides* spp. returned IgG and IgM positive at 1:64. Oral fluconazole was initiated at 20 mg/kg twice daily, however the condition of the gibbon markedly declined within 48 hr and he became stuporous. MRI brain sequences were consistent with an infectious meningoencephalitis and secondary obstructive hydrocephalus. A ventriculoperitoneal shunt (UNI-SHUNT with reservoir, Codman & Shurtleff, Inc., Raynham, Massachusetts 02767 USA) was placed to reduce the imminent risk of mortality from increased intracranial pressure. Post-operative treatment was centered on oral fluconazole (to be continued lifelong; 10 mg/kg p.o. b.i.d.) and a slowly tapered course of prednisolone (initial 0.5 mg/kg p.o. b.i.d.). Improvement of mentation, neurologic deficits, and strength was slow but consistent. Daily training sessions with his zoo keepers and enrichment items were utilized to both objectively monitor his progress and to aide in his rehabilitation. The gibbon was fit to be returned to exhibit eight weeks post shunt placement, with only slight residual behavior changes appreciated. This case of coccidioidomycosis in a non-human primate demonstrates the complications that can occur with dissemination to the central nervous system. In this particular case, placement of a ventriculoperitoneal shunt was a life-saving procedure and should be considered in other cases of obstructive hydrocephalus.

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## LONG-ACTING ANTIBIOTICS IN ZOO ANIMALS: WHAT DO WE KNOW?

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## **Abstract**

Zoo veterinarians deal with animal species wherein each single treatment event may imply logistic challenges and health hazards for the animals (e.g., remote injection, immobilization). Long-acting antibiotics meet the need of providing antibiotic cover in species that are difficult to medicate on a regular basis. For domestic animals, new long-acting antibiotics were developed recently, but the question is what can be used in zoological and wildlife medicine?

With cefovecin, the very long half-life in dogs and cats allows a dosing interval of 14 days. <sup>14,15</sup> However, species differences in pharmacokinetics are highly relevant and likely preclude the use of this antimicrobial agent in non-evaluated species. <sup>17</sup> For cattle, pigs, and horses, a sustained release ceftiofur suspension (ceftiofur crystalline free acid, CCFA,) was developed. Pharmacokinetic studies are underway for other species. In reptiles, other cephalosporins allow a long dosing interval (e.g., ceftazidime). <sup>13</sup> Tulathromycin is a long-acting macrolid antibiotic used in domestic animals with the potential of evaluation for zoo animals. Long-acting tetracyclines, and doxycyline formulations have been utilized in practice for a longer time. Other modes of administration may be employed so that antibiotics are administered at a less frequent interval (e.g., ballistic implants, impregnated beads).

In Table 1 we compile a list of long-acting antibiotics that may be useful for the zoo veterinarian. Examples of pharmacokinetic data of several long-acting antibiotics are included, as well as, examples wherein long-activity is not achieved.

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<b>Table 1.</b> Examples of pharmacokinetic data of long-acting antibiotics for different species	Table 1.	Examples of	pharmacokinetic	data of long-act	ing antibiotics	for different spe	cies.
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Antibiotic	Species (reference)	Dose;	Half-	Interval	Remarks
		Route	life (br)	recom-	
Amoxicillin,	Domestic goat <sup>10</sup>	2800mg	(hr) 130.03	mended	implant produced
controlled release	(Capra aegagrus hircus)	degradable	±39		by authors
controlled release		implant	±37		by authors
Cefovecin	Domestic cat <sup>15</sup>	8mg/kg	166	14 day	
	(Felis sylvestris catis)	s.c.	±18		
	Domestic dog <sup>14</sup>	8mg/kg	133	14 day	
	(Canis lupus familiaris)	s.c., i.v.			
	Squirrel monkey <sup>12</sup>	8mg/kg	2.6		not long-acting
	(Saimiri sciureus)	s.c.	±0.1		
	Cynomologus macaques <sup>12</sup>	8mg/kg	6.3		not long-acting
	(Macaca fascicularis)	s.c.	±1.8		
	Rhesus macaques <sup>12</sup>	8mg/kg	8.0		not long-acting
	(Macaca mulatta)	s.c.	±0.6		
	Rhesus macaque <sup>2</sup>	8mg/kg	6.6		
	(Macaca mulatta)	S.C.	±1.0		
	Scarlet ibis <sup>16</sup>	10mg/kg			not long acting,
	(Eudocimus ruber);	s.c.			preliminary stud
	African grey parrot <sup>16</sup>				
	(Psittacus erithracus); Blue-fronted Amazon <sup>16</sup>				
	(Amazona aestiva); Russian tortoise <sup>16</sup>				
	(Testudo horsfiedi);				
	Spur-thighed tortoise <sup>16</sup>				
	(Testudo graeca);				
	Russian ratsnake <sup>16</sup> (Elaphe				
	schrenckii);				
	Boa constrictor <sup>16</sup>				
	(Boa constrictor)				
	Chicken <sup>16</sup>	10mg/kg	0.9		not long-acting
	(Gallus domesticus)	s.c.	±0.3		not long atting
	Green iguana <sup>16</sup>	10mg/kg	3.9		not long-acting
	(Iguana iguana)	s.c.			
	Ring tailed lemur <sup>4</sup>	10mg/kg		>5 day	
	(Lemur catta)	2 2		,	
	Geoffroy's spider monkey <sup>4</sup>	10mg/kg		<48 hr	
	(Ateles geoffroyi)	- <del>-</del>			
	Domestic goat <sup>4</sup>	10mg/kg		<24 hr	not long-acting
	(Capra aegagrus hircus)				,
	Soemmering's gazelle <sup>4</sup>	10mg/kg		<24 hr	not long-acting
	(Nanger soemmerringii)				
	Rheem gazelle <sup>4</sup>	10mg/kg		<24 hr	not long-acting
	(Gazella subgutturosa				
	marica)	40 "		24.	
	Speke's gazelle <sup>4</sup>	10mg/kg		<24 hr	not long-acting
	(Gazella spekei)	10 "		. 7. 1	
	Domestic pig <sup>4</sup>	10mg/kg		>5 day	
C C '1'	(Sus scrofa)	20 "	20.50	70.1	
Ceftazidime	Loggerhead sea turtles <sup>13</sup>	20mg/kg	20.59	72 hr	
	(Caretta caretta)	i.v.	±3.24		

Antibiotic	Species (reference)	Dose; Route	Half- life (hr)	Interval recom- mended	Remarks
		20mg/kg i.m.	19.08 ±0.77	72 hr	
Ceftiofur, crystalline free acid	Domestic goat <sup>6</sup> (Capra aegagrus hircus)	6.6mg/kg s.c.	36.9		
	Alpaca <sup>5</sup> (Vicugna pacos)	6.6mg/kg s.c.	44.7		local reactions after multiple administrations
	Helmeted guineafowl <sup>18</sup> (Numida meleagris)	10 mg/kg i.m.	29.0 ±4.9	3 day	
	American black ducks <sup>9</sup> (Anas ribripes)	10 mg/kg i.m.	32	3 day	
	Ball python <sup>1</sup> (Phython regius)	15mg/kg i.m.	64.31 ±14.2	5 day	
Oxytetracycline	Loggerhead sea turtle <sup>7</sup> (Caretta caretta)	41-82 mg/kg then 21 mg/kg i.m.	61.9 then 66.1	72 hr	
Oxytetracycline, long-acting	Tammar wallaby <sup>11</sup> (Macropus eugenii)	20 mg/kg i.m.	19.35 ±11.07		long activity questioned
	American alligator <sup>8</sup> (Alligator mississippiensis)	10 mg/kg i.m.	131.23	5 day	
Tulathromycin	Domestic goat <sup>19</sup> (Capra aegagrus hircus)	2.5 mg/kg s.c.	110 ±19	once	
	Domestic pig <sup>3</sup> (Sus scrofa)	2.5 mg/kg i.m.	75.6	once	

# **CARPAL SURGERY IN A NEUTERED MALE Zalophus californianus**

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## **Abstract**

Chronic right front limb lameness was diagnosed prior to January 2010 in a 19-yr-old neutered male California sea lion (Zalophus californianus). Radiographs of the consistently swollen and warm right carpus revealed variable osteolysis of the osseous structures of the radio-carpal, intercarpal, and carpometacarpal joints with collapse of the joint spaces and instability. Various antibiotics and analgesics provided no resolution. Bilateral cataracts with anterior lens luxation o.d. were also present and prompted surgical attention. Bilateral cataract extraction and an exploratory arthrotomy of the radiocarpal/intercarpal and carpometacarpal joints were performed concurrently. Devitalized bone in each carpal joint was debrided and joints were flushed liberally. A pneumatic tourniquet was secured to the distal radius and an intraosseous catheter placed into the radius distal to the tourniquet. One gram of amikacin (Amiglyde-V<sup>®</sup>, Fort Dodge Animal Health, Overland Park, Kansas 66210 USA) was infused to complete a regional limb perfusion over 45min. Collagen sponges soaked with Bone Morphogenic Protein 6 (Infuse BMP-6<sup>®</sup>, Medtronic, Minneapolis, Minnesota 55432 USA) were packed into each joint space to promote osteogenesis and to increase joint stability. Histology of bone and synovial membrane revealed widespread severe lymphocytic-plasmacytic inflammation of the synovium and subsynovial connective tissue. Special stains did not reveal bacteria or fungi. All cultures were negative for bacterial growth. The patient recovered well with primary healing of all incisions. Sequential radiographs revealed improved bone density, stability and no further osteolysis. Clinically the patient shows no clinical signs, lameness is resolved, and the sea lion is back on exhibit.

## **ACKNOWLEDGMENTS**

The authors thank the staff of Birmingham Zoo, Inc.'s Animal Health Center and Predators Department for their hard work and dedication to this patient.

MANAGEMENT OF A CONCURRENT RANAVIRUS AND HERPESVIRUS EPIZOOTIC EVENT IN CAPTIVE EASTERN BOX TURTLES (Terrapene carolina carolina)

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### **Abstract**

Ranavirus is an emerging pathogen affecting captive and wild Eastern Box Turtles (Terrapene carolina carolina) in eastern North America. In July 2011, five Eastern Box Turtles from a group of 27 presented dead or moribund with fibrinonecrotic stomatitis and cloacitis. The remaining 22 animals were quarantined indoors and isolated into one of three groups based on clinical severity: no lesions, mild, or severe. Treatment included nutritional support, fluid therapy, antibiotics, and antiviral famciclovir (10, 20, or 30 mg/kg p.o. q 24 hr, randomly assigned). Treatment was discontinued at 34 days for the no lesions group and 10 days after clinical resolution for the others. Oral swabs from days 0, 10, 34, and 60 were tested for Ranavirus by quantitative real-time PCR and from day 0 for Herpesvirus by conventional PCR. On day 80, the surviving 14 turtles were returned to the outdoor exhibit for brumation. Overall, 77.3% tested positive for Ranavirus and 54.5% for Herpesvirus. Median duration of treatment for Ranavirus-positive survivors was 49 days (range 34 – 80 days). On days 0, 10, 34, and 60, Ranavirus prevalence was 72.7% (n=22; median viral copies (MVC) 7.06 x 10<sup>6</sup>), 50% (n=18;  $MVC 9.11 \times 10^7$ ), 31.3% (n=16;  $MVC 2.46 \times 10^6$ ), and 0% (n=14; MVC 0). The survival rate was 64.7% (n=11) among those that were *Ranavirus*-positive. Of the 17 *Ranavirus*-positive animals, 10 were concurrently *Herpesvirus*-positive. Survival was 57% among those that tested only Ranavirus-positive, and 70% among those that tested positive for Ranavirus and Herpesvirus. All 14 turtles survived brumation, showing no clinical signs 1 mo after emergence.

# AFLATOXICOSIS IN CAPTIVE REARED AMERICAN ALLIGATORS (Alligator mississippiensis)

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## **Abstract**

Since 2009, an increased number of liver pathology cases in captive reared alligators has been obnserved. Common gross findings include icterus (skin, sclera, mucus membranes, and greater vessels) with a nodular and fibrotic liver. Histopathologic evaluation of one case revealed diffused marked periportal and bridging hepatic fibrosis, biliary hyperplasia, and oval cell hyperplasia affecting up to 90% of the liver parenchyma. A second case revealed approximately 90% of the hepatic tissue was effaced and replaced by hyperplastic biliary ducts and edematous loose connective tissue with numerous heterophils, moderate numbers of lymphocytes and plasma cells, and multifocal hemorrhage. Portal areas were moderately expanded by fibrous and loose connective tissue and frequently seen severely thickened vessel walls by fibrosis. In both cases, liver tissue tested positive for aflatoxin M1.

The animals in the first case were identified as part of an ongoing alligator health surveillance program with the Louisiana Department of Wildlife and Fisheries. These animals were originally from the state of Georgia and part of the diet consisted of whole chickens. Those in the second case were identified at the time of slaughter. These animals were fed a commercial pelleted diet only. Due to the high turn around time of feed, we were unable to sample feed used before the diagnosis to confirm the presence of aflatoxins in the diet. One farmer did report problems with high humidity in the silo used for storing the feed. Feeding practices and feed storage are believed to be associated with the occurrence of disease.

### **ACKNOWLEDGMENTS**

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# OCULAR LESIONS IN 67 SNAKES SEEN AT A UNIVERSITY VETERINARY TEACHING HOSPITAL (1985-2010)

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## **Abstract**

The distribution and clinical course of snakes diagnosed with ocular disease at a veterinary medical teaching hospital were described (VMTH). Medical records of all snakes presented from 1April1985 to 1October2010 were reviewed. Signalment, duration, diagnosis, therapy, and response were recorded for all snakes with ocular disease. Ocular disease was detected in 67/508 (13%) of snakes examined. Affected snakes were of the Boidae, Pythonidae, Colubridae, and Viperidae families. No significant difference in distribution of taxonomic family (P = 0.14), age (P = 0.33), or sex (P = 0.76) was detected between snakes with and without ocular disease, but snakes of the genus *Epicrates* (Boidae family) with ocular disease were over-represented (P =0.0002). The most common diagnoses across families were retained spectacle (58%), pseudobuphthalmos/subspectacular abscessation (18%), trauma (11%), and cataracts (6%). Pseudobuphthalmos/subspectacular abscessation was more likely in Colubridae than non-Colubridae (P = 0.0056). Follow-up information for 25/41 snakes with retained spectacles revealed recurrence/relapses in nine; five of which had multiple recurrences. Follow-up information for 9/13 snakes with pseudobuphthalmos/subspectacular abscessation revealed that two never fully resolved and six improved immediately following surgery; however one had a recurrence and four had multiple recurrences/relapses. In conclusion, snakes of the genus Epicrates had a higher than expected frequency of ocular disease, and those of the family Colubridae had a higher than expected frequency of pseudobuphthalmos/subspectacular abscessation.

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# DESENSITIZATION AND OPERANT CONDITIONING OF REPTILES TO FACILITATE VETERINARY CARE: CURRENT EXAMPLES AND FUTURE APPLICATIONS

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### **Abstract**

Training for veterinary procedures has long been performed in mammalian zoo species; however, the application of behavioral training techniques remains underutilized in reptile collections. A survey was sent via behavioral and zoological list-serves to determine what behavioral training techniques are being employed with reptiles in zoological collections worldwide. Nineteen institutions provided examples of training techniques for lizards, snakes, turtles, and crocodiles. All 19 institutions (100%) used desensitization and/or operant conditioning techniques. Desensitization was used at 13/19 (68%) institutions in over 9 species to facilitate handling for examination, radiographs, ultrasounds, blood collection, and topical and ocular medications. Operant conditioning, referring to modifying behavior involving positive or negative reinforcement was most frequently used to have animals target, station, or shift for procedures. Operant conditioning was used at 18/19 (95%) institutions in over 20 species to facilitate obtaining weights, examinations, radiographs, ultrasounds, blood collection, and administering oral and parenteral medications. The examples of reptile training already being employed at zoos worldwide illustrate how zoological institutions can use techniques such as desensitization and operant conditioning to improve the veterinary care of reptiles.

## **ACKNOWLEDGMENTS**

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# EFFECTS OF INTRANASAL ADMINISTRATION OF DEX-MEDETOMIDINE AND KETAMINE ON THE YELLOW-BELLIED SLIDER (*Trachemys scripta scripta*)

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## **Abstract**

Freshwater turtles are popular research, exhibit, and companion animals. As a result, there is an increasing need for chemical restraint methods for routine physical examination, biologic sample collection, and therapeutic procedures.<sup>2</sup> However, anesthesia is often challenging because of their unique physiology, anatomy, and behavior. Intranasal anesthesia has been shown to be a reliable, effective, and easy method for the administration of anesthetic drugs in both human and veterinary medicine. 1,3,4,5 In this pilot study, we evaluated the safety and utility of dexmedetomidine (M) and ketamine (K) and the reversal with atipamezole administered intranasally to Trachemys scripta scripta. Eight adult, free-ranging healthy turtles received 10 mg/kg of ketamine (100 mg/ml, Fort Dodge Animal Health, Fort Dodge, IA, 50501) with 0.2 mg /kg of dexmedetomidine (0.5 mg/ml, Pfizer Animal Health, NY, NY, 10017) intranasally with the use of a micropipette. Heart rate, respiratory rate, body temperature, and a sedation scores were all evaluated. A sedation score was assigned by determining the level of consciousness ranging from 0-5 (0 = fully conscious with no detectable effects, 1 = mild sedation, 2 = moderatesedation, 3= heavy sedation, 4=light anesthesia, 5 = surgical anesthesia). Blood was collected 45 min post-induction from both the subcarapacial sinus and dorsal tail vein, followed by a 2 mg/kg intranasal atipamezole (5mg/ml, Pfizer Animal Health, NY, NY, 10017) administration. Heart rate, respiratory rate, and cloacal temperatures remained stable throughout the entire procedure with no adverse effects. The mean time to a sedation score of 1 was 21±8 min. The median sedation score was 2, a level of anesthesia deep enough to perform a thorough physical exam and minor clinical procedures. At 45 min post-induction, ketamine and dexmedetomide plasma levels were measured using a using liquid chromatography-tandem mass spectrometer at 1014.49  $\pm$  621.50 ng/ml/kg (K) and 17.28  $\pm$  8.57 ng/ml/kg (D) from the tail vein and 2390.63 $\pm$  2965.79 ng/ml/kg(K) and 24.59  $\pm$  23.93 ng/ml/kg(D) from the subcarapacial vein. After administration of atipamezole, turtles returned to pre-anesthetic activity in an average of 19±7 min. Results suggest that a combination of intranasal dexmedetomidine and ketamine should be considered as a reversible option for a moderate sedation for physical examination and blood collection in Trachemys turtles.

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# FACIAL DISFIGURATION SYNDROME IN FREE-RANGING SNAKES THROUGHOUT THE EASTERN US: AN EMERGING PATHOGEN ASSOCIATED WITH CHRYSOSPORIUM

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## **Abstract**

With the current rate of declines in global biodiversity, it is apparent that wildlife diseases are serving as additional threats to population declines and potentially species extinctions. Free-ranging Eastern massasaugas (*Sistrurus catenatus*) have been reported susceptible to numerous health threats, one of which is a fatal fungal dermatitis. The disease presents as facial disfiguration due to granulomatous dermatitis and osteomyeltits with intralesional fungi. The keratinophilic fungi *Chrysosporium* has been identified in multiple, but not all cases, and has resulted in 100% observed mortality. The prevalence of Chrysosporium has thus been investigated since 2008. The PCR prevalence in this population was 4.4%, 0%, 1.8%, 0%, and 2.3% in the years 2008 through 2011. In concurrent health assessments, no predictable pattern using hematology, plasma biochemistries, or heavy metal analysis has been observed.

Facial disfiguration without the identification of Chrysosporium has occurred within this population, and highlights the limitations of antemortem diagnosis of this pathogen. Additionally, collaborators have observed a similar disfiguration syndrome in timber rattlesnakes, black rat snakes, and yellow-bellied water snakes from across the eastern US, but have inconsistently identified Chrysosporium. However, a garter snake (*Thamnophis* spp.) from a separate location in Illinois with similar clinical signs was identified with a Chrysosporium with 100% sequence homology to the massasauga isolate. These additional cases from distinct locales designates that this syndrome is widespread or becoming widespread and should be considered a potential threat to ophidian biodiversity and future studies are needed to truly identify the causative agent or agents.

## ADVANCES IN REPTILE ANESTHESIA: BLOOD GASES AND BLOOD PRESSURE

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### Abstract

Blood pressure has long been advocated as an important cardiovascular parameter to measure and maintain in anesthetized patients because it represents quantification of blood flow and tissue perfusion. Despite the inherent value of measuring blood pressure, such methods are rarely used in reptile anesthesiology. 1,7,8 Blood pressure is typically under close autonomic regulation, is largely mediated by the baroreflex, and can be quantified by systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressure (MAP).<sup>5</sup> Despite previous recommendations by Lichtenberger,<sup>6</sup> other authors have considered indirect blood pressure monitoring to be inaccurate and imprecise in reptiles. 1,3,6 In green iguanas (*Iguana iguana*) indirect measurements suffered an 81% failure rate, while in boid snakes indirect measurements frequently over-estimated SAP, under-estimated DAP and MAP, and at SAP < 100 mmHg all three pressure variables were variably under-estimated.<sup>2,3</sup> Conversely, direct measurements of blood pressure in iguanids and boids have been found to be accurate and repeatable<sup>1,2</sup>; however, the need for surgical exposure and catheterization make such techniques impractical for most clinical work (Figures 1 and 2). Isoflurane has been shown to have profound effects on iguanid blood pressures, with isoflurane at 3% decreasing MAP from 65-85 to <40 mmHg. Despite previous comments that atropine has little effect in reptiles, atropine does significantly increase heart rate while maintaining MAP, while β1-antagonists like atenolol reduce heart rate, again while maintaining MAP.<sup>5</sup> Medetomidine, atipamezole, dopamine and phrenylephrine appear to have little to no effect in green iguanas; however, norepinephrine at 0.4 and 0.5 µg/kg/min significantly increased MAP from 27 to 66 mmHg (unpublished data).

Arterial blood gases are used to assess the adequacy of ventilation (PaCO<sub>2</sub>), blood oxygenation (PaO<sub>2</sub>), and acid-base status (pH, PaCO<sub>2</sub>). There are a variety of portable units in practice but most only directly measures pH, PCO<sub>2</sub>, and PO<sub>2</sub> with lactate, bicarbonate, TCO<sub>2</sub>, BE, and SaO<sub>2</sub> calculated using human algorithms. Unsurprisingly, some controversy and unique problems exist regarding the interpretation of blood gases in reptiles. Some argue that all reptile samples should be corrected to 37°C which results in decreases in values for pH, CO<sub>2</sub>, and PO<sub>2</sub>. Others have advocated correcting to the reptile's body temperature which would result in higher values for these same parameters. Carotid collection is preferred because it accurately reflects blood flow to brain. Intracardiac sampling is likely to result in a mixed arterial-venous sample. Venous samples can only indirectly reflect PaCO<sub>2</sub> and ventilation, and are even less likely to accurately reflect PaO<sub>2</sub>. Furthermore, venous PCO<sub>2</sub> can be elevated due to increased metabolism or impaired tissue perfusion. A recent study in green iguanas has indicated that there are significant circadian changes in arterial PO<sub>2</sub> and SaO<sub>2</sub>, and that pulse oximetry (SpO<sub>2</sub>) is an inaccurate measure for SaO<sub>2</sub> due to under-estimation (Table 1).<sup>5</sup> There were no circadian effects observed from venous

samples, but significant differences existed between arterial and venous results for PCO<sub>2</sub>, PO<sub>2</sub>, and SaO<sub>2</sub>/SvO<sub>2</sub>. There were no significant differences between arterial and venous results for lactate, bicarbonate, TCO<sub>2</sub>, and BE. To summarize, arterial samples are needed for PO<sub>2</sub> and SaO<sub>2</sub> evaluations, but venous samples are useful for other parameters incl pH, lactate, bicarbonate, TCO<sub>2</sub>, and BE.

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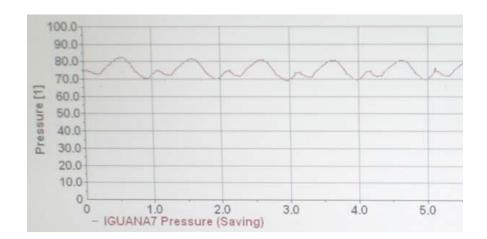
**Table 1.** Mean  $\pm$  SD arterial and venous blood gas values as determined at 37°C in 15 conscious green iguanas (*Iguana iguana*) breathing room air (Hernandez et al., 2011). a,b,c

Arterial			Venous		•
9 to 11 am	3 to 5 pm	P value <sup>d</sup>	9 to 11 am	3 to 5 pm	P value <sup>d</sup>
$7.29 \pm 0.11$	$7.29 \pm 0.11$	0.499	7.31 <sup>e</sup>	7.25 <sup>e</sup>	0.594
$(7.38 \pm 0.12)$	$(7.38 \pm 0.12)$	(0.500)	$(7.36 \pm 0.13)$	$(7.32 \pm 0.15)$	(0.729)
$42 \pm 9 (32 \pm 7)$	$46 \pm 10 (35 \pm 8)$	0.186 (0.187)	$49^{d} (36 \pm 7)$	$56^{d} (42 \pm 11)$	0.084
					(0.056)
81 ± 19†	$94 \pm 21 \dagger$	0.043	$46 \pm 23$	$37 \pm 15$	0.929
$(54 \pm 15)$	$(64 \pm 16)$	(0.044)	$(30 \pm 15)$	$(24 \pm 9)$	(0.878)
$2.7\pm1.1$	$4.2 \pm 3.4$	0.192	2.3 <sup>e</sup>	3.5 <sup>e</sup>	0.975
$20 \pm 4$	$22 \pm 4$	0.111	$22 \pm 5$	$24 \pm 4$	0.128
$22 \pm 4$	$24 \pm 5$	0.134	$24 \pm 5$	$25 \pm 4$	0.126
$-6.3 \pm 5.5$	$-4.3 \pm 5.3$	0.199	$-5 \pm 6$	$-4 \pm 6$	0.306
$92 \pm 6$	$95 \pm 3$	0.027	84 <sup>e</sup>	49 <sup>e</sup>	0.064
$86 \pm 6$	ND	ND	ND	ND	ND
	9 to 11 am  7.29 $\pm$ 0.11  (7.38 $\pm$ 0.12)  42 $\pm$ 9 (32 $\pm$ 7)  81 $\pm$ 19†  (54 $\pm$ 15)  2.7 $\pm$ 1.1  20 $\pm$ 4  22 $\pm$ 4  -6.3 $\pm$ 5.5  92 $\pm$ 6	9 to 11 am 3 to 5 pm 7.29 $\pm$ 0.11 7.29 $\pm$ 0.11 (7.38 $\pm$ 0.12) (7.38 $\pm$ 0.12) 42 $\pm$ 9 (32 $\pm$ 7) 46 $\pm$ 10 (35 $\pm$ 8 81 $\pm$ 19 $\dagger$ 94 $\pm$ 21 $\dagger$ (54 $\pm$ 15) (64 $\pm$ 16) 2.7 $\pm$ 1.1 4.2 $\pm$ 3.4 20 $\pm$ 4 22 $\pm$ 4 22 $\pm$ 4 24 $\pm$ 5 -6.3 $\pm$ 5.5 -4.3 $\pm$ 5.3 92 $\pm$ 6 95 $\pm$ 3	9 to 11 am 3 to 5 pm $P$ value <sup>d</sup> 7.29 ± 0.11 7.29 ± 0.11 0.499  (7.38 ± 0.12) (7.38 ± 0.12) (0.500)  42 ± 9 (32 ± 7) 46 ± 10 (35 ± 8) 0.186 (0.187)  81 ± 19† 94 ± 21† 0.043  (54 ± 15) (64 ± 16) (0.044)  2.7 ± 1.1 4.2 ± 3.4 0.192  20 ± 4 22 ± 4 0.111  22 ± 4 24 ± 5 0.134  -6.3 ± 5.5 -4.3 ± 5.3 0.199  92 ± 6 95 ± 3 0.027	9 to 11 am       3 to 5 pm       P value <sup>d</sup> 9 to 11 am $7.29 \pm 0.11$ $7.29 \pm 0.11$ $0.499$ $7.31^e$ $(7.38 \pm 0.12)$ $(7.38 \pm 0.12)$ $(0.500)$ $(7.36 \pm 0.13)$ $42 \pm 9$ ( $32 \pm 7$ ) $46 \pm 10$ ( $35 \pm 8$ ) $0.186$ ( $0.187$ ) $49^d$ ( $36 \pm 7$ ) $81 \pm 19^{\dagger}$ $94 \pm 21^{\dagger}$ $0.043$ $46 \pm 23$ $(54 \pm 15)$ $(64 \pm 16)$ $(0.044)$ $(30 \pm 15)$ $2.7 \pm 1.1$ $4.2 \pm 3.4$ $0.192$ $2.3^e$ $20 \pm 4$ $22 \pm 4$ $0.111$ $22 \pm 5$ $22 \pm 4$ $24 \pm 5$ $0.134$ $24 \pm 5$ $-6.3 \pm 5.5$ $-4.3 \pm 5.3$ $0.199$ $-5 \pm 6$ $92 \pm 6$ $95 \pm 3$ $0.027$ $84^e$	9 to 11 am       3 to 5 pm       P value <sup>d</sup> 9 to 11 am       3 to 5 pm         7.29 ± 0.11       7.29 ± 0.11       0.499       7.31°       7.25°         (7.38 ± 0.12)       (7.38 ± 0.12)       (0.500)       (7.36 ± 0.13)       (7.32 ± 0.15)         42 ± 9 (32 ± 7)       46 ± 10 (35 ± 8)       0.186 (0.187)       49 d (36 ± 7)       56 d (42 ± 11)         81 ± 19†       94 ± 21†       0.043       46 ± 23       37 ± 15         (54 ± 15)       (64 ± 16)       (0.044)       (30 ± 15)       (24 ± 9)         2.7 ± 1.1       4.2 ± 3.4       0.192       2.3°       3.5°         20 ± 4       22 ± 4       0.111       22 ± 5       24 ± 4         22 ± 4       24 ± 5       0.134       24 ± 5       25 ± 4         -6.3 ± 5.5       -4.3 ± 5.3       0.199       -5 ± 6       -4 ± 6         92 ± 6       95 ± 3       0.027       84°       49°

<sup>&</sup>lt;sup>a</sup>Arterial and venous blood samples were collected in the morning and afternoon to determine the effect of circadian rhythm on blood gas parameters. <sup>b</sup>Values in parentheses are corrected for a body temperature of 30°C. <sup>c</sup>Data are reported as mean  $\pm$  SD unless indicated otherwise. <sup>d</sup>A value of P < 0.05 was considered significant. <sup>c</sup>Values are reported as medians. <sup>f</sup>Oxygen saturation as measured by pulse oximetry.



**Figure 1.** Surgical placement of a telemetry blood pressure monitoring device in a green iguana. Left – placing a stay suture around the distal carotid; Middle – placing a vasculature pic through a nick in the arterial wall to facilitate the insertion of the arterial catheter; Right – Subcutaneous placement of the telemetry end of the catheter to permit hand-free blood pressure monitoring.



**Figure 2.** Continuous direct blood pressure reading from a conscious green iguana using an implanted telemetry arterial catheter. In this case, the animal has a heart rate of 60 beats/min, with a SAP of 81 mmHg and DAP of 70 mmHg.

# INVERTEBRATE ANTINOCICEPTION: ARE OPIOIDS EFFECTIVE IN TARANTULAS?

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### Abstract

The concept of nociception in invertebrates is complex. While it is recognized that many different invertebrates, including mollusks, nematodes, insects and crustaceans, exhibit nociception (response to a noxious stimulus), the idea that invertebrates might "feel" pain is still open to interpretation. At the heart of this issue is the fact that it is difficult to determine whether stimulus avoidance behavior in invertebrates is more than a reflex, and whether discomfort registers in the emotional sense that we associate pain with in higher vertebrates.

The presence of opioid receptors in the nervous system of invertebrates has been confirmed in nematodes, mollusks and some insects.<sup>3</sup> Administration of opioids, such as morphine, increases the latency of response to a stimulus, such as an electric shock or heat, in many species of invertebrate, whereas opioid antagonists, such as naloxone, seem to abolish this effect.<sup>2</sup> However, the data available are not consistent across invertebrate taxa. Information on arachnid nociception is particularly sparse. Our preliminary experiments on Chilean rose tarantulas (*Grammostola rosea*), using a noxious thermal stimulus, suggest that tarantulas consistently remove the affected limb from the stimulus, and that opioids such as morphine and butorphanol alter this behavior. These findings will be discussed as well as invertebrate nociception and implications for captive management and research.

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# CLINICAL FISH ANALGESIA: SWIMMING THROUGH THE MURKY WATERS OF FISH PAIN

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## **Abstract**

The objective of this presentation is to describe and highlight what is currently known about pain (nociception) and analgesia (antinociception) in fish. The primary question regarding fish analgesia is whether fish "experience" pain or are fish species merely capable of demonstrating a "reflexive" response to a noxious stimulus (nociception)? Of critical importance is the concept of whether we can recognize pain in fish, and is the perception of pain by a fish equivalent to that of a mammal? We will never be able to fully and objectively answer these questions, because fish simply cannot tell us. Many would argue that fish do not have the same anatomic and/or physiologic capabilities to "process" pain. 13 In other words, fish are merely responding and passively reacting to stimuli to which they are exposed, with little or no ability for cognition or self-awareness.<sup>13</sup> However, recent research has demonstrated that the transmission of peripheral sensory signals, via the spinal cord, to midbrain and forebrain regions that are homologous to mammalian cortical and limbic structures. 2,5,7,15-17 Additionally, the endogenous opioid system, which is activated in response to nociception and contributes to analgesia, is also well conserved throughout vertebrate phylogeny. 4,6,15 Thus, the physiologic and anatomic requirements for pain and analgesia appear to be remarkably similar among all vertebrate species, and therefore, there is substantive and compelling evidence from the neuroanatomic, neurophysiologic and behavioral literature to suggest that, at some level, a variety of fish species experience pain under certain contexts. In my clinical experience, both kappa- and mu-opioid agonists appear to be affective in providing pain relief, particularly post-surgically; however, mu-opioid agonists appear to provide fewer deleterious side effects. 1,8-10,12

Many veterinary clinicians argue that the administration of analgesics is risky to the patient and may mask behavioral signs of pain, which are considered evolutionarily adaptive for survival. However, veterinarians have an ethical obligation to treat painful conditions in all animals, including fish, as effective pain management reduces stress-induced disruption to homeostatic mechanisms, and also decreases morbidity and mortality associated with trauma or surgery. Several obstacles limit successful analgesic use in fish, including subjectivity in pain assessment, inadequate knowledge of analgesic efficacy across species, pharmacokinetics of analgesic drugs, and the unknown relationship between risks and benefits for specific drugs. It is my hope that future research will help us to determine if fish feel pain. Until then, we must use all available evidence, especially in those species most closely related to the species being studied, to err on the side of animal in subjectively assessing that a procedure considered painful in a mammal, should also be considered potentially painful in a fish species.

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Table 1. Published analgesic protocols commonly used in fish species

	Dose	Route	Frequency	Comments	Ref.
	[mg/kg]/				
<u>Opioids</u>					
Butorphanol	0.4; 10.0	i.m.	Once	Analgesic efficacy at higher dosages in koi; buoyancy anomalies and respiratory depression at high dosages	1, 7
Morphine	5.0	i.m.	q 24 hr	Post-surgical analgesic efficacy in koi; hyperactivity at higher	1
	40.0, 50.0	i.m.	Once	dosages.  No measureable analgesic efficacy	11
	7.0 (approx) - 30.0	i.ce	Once	in goldfish exposed to noxious heat ED50 = $6.7 \pm 0.8$ mg/kg in trout for antinociception	8
<u>NSAIDS</u>					
Ketoprofen	2.0	i.m.	Once	No evidence of analgesic efficacy	7
	1.0, 1.5, 2.0, 4.0	i.m.	Once	Plasma concentrations equivalent to those with efficacy in mammals with > 24h duration in plasma	3

# PHARMACOKINETICS OF TRAMADOL AND O-DESMETHYLTRAMADOL IN LOGGERHEAD SEA TURTLES (Caretta caretta)

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### Abstract

Trauma is the most common reason for sea turtles to be presented to the Georgia Sea Turtle Center for rehabilitation. Boat strike injuries account for over 20 percent of our caseload. These injuries are likely to be extremely painful. Although pain management in reptiles has made some recent advances, data are lacking for sea turtles. The objective of this study was to determine the pharmacokinetics of two orally administered doses of tramadol (5 and 10 mg/kg) and its major metabolite (O-desmethyltramadol, M1) in loggerhead sea turtles (Caretta caretta). After oral administration, the half-life of tramadol administered at 5 mg/kg and 10 mg/kg was 20.35 and 22.67 hr, respectively, whereas the half-life of M1 was 10.23 and 11.26 hr, respectively. The maximum concentration (Cmax) for tramadol after oral administration at 5 mg/kg and 10 mg/kg was 373 and 719 ng/ml, respectively, whereas that of M1 was 655 and 1376 ng/ml, respectively. We were able to determine that tramadol administered orally to loggerhead sea turtles at both dosages provided measurable plasma concentrations of tramadol and O-desmethyltramadol for Plasma concentrations of tramadol and Oseveral days with no adverse effects. desmethyltramadol remained ≥ 100 ng/ml for at least 48 hr and perhaps as long as 96 hr when tramadol was administered at 10 mg/kg. Based on therapeutic levels that are achieved in humans, <sup>2</sup> a dosage of 10 mg/kg every 48 hr should produce similar levels, but further studies are needed to confirm this information including multi-dose and pharmacodynamic studies.

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## AVIAN ANALGESIA: CURRENT RESEARCH AND CLINICAL APPLICATIONS

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### Abstract

It is difficult to define and recognize when birds feel pain, and it can be even more challenging to objectively determine whether a pain medication is effective in the avian patient. To determine the efficacy of an analgesic in any species, it is important to determine the pharmacokinetic (PK) and pharmacodynamic (PD) properties of the drug in that species. Integrating PK and PD data can also provide a basis for selecting clinically relevant dosing schedules for subsequent evaluation in disease models and clinical trials. PK studies of analgesic drugs are often insufficient to determine appropriate doses and dosing frequencies as plasma concentrations do not always correlate with delivery of analgesia. <sup>6,7</sup>

Anesthetic sparing studies, inflammatory models to evaluate repeatable behaviors or quantifiable weight bearing, and evaluating specific responses to a noxious stimulus can provide PD techniques for objective evaluation.<sup>5,8-12</sup> Experimental PD models have been developed in chickens and parrots, but these models may not extrapolate to pain behaviors relevant to clinical pain. Therefore, the doses and dosing frequencies recommended in the published reports should always be critically evaluated case-by-case when clinically applied. The goals of this presentation are to discuss the current literature pertaining to studies in avian analgesia, with an emphasis on clinical applications.

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Table 1. Selected NSAID, opioid and opioid-like analgesics evaluated in avian species by either pharmacokinetic

(PK)<sup>a</sup> or pharmacodynamic (PD)<sup>b</sup> studies.

Drug	Dosage mg/kg	Route	Frequency qhr	Species	Comments <sup>c,d</sup>	Type of Study	Reference
Butorphanol	5	p.o.	Single dose	Hispaniolan Amazon parrots	Oral bioavailability< 10%; do not recommend this route of administration	PK	4
	2-5	i.m., i.v.	Single injection	Hispaniolan Amazon parrots	PK: low mean plasma concentrations at 2 hr after injection PD:withdrawal thresholds to electrical stimuli no different from	PK/PD	4,13
Fentanyl	0.15-0.5	i.v.	Constant rate	Red-tailed hawks	controls after 2 mg/kg i.m. Reduced isoflurane MAC 31-55% in a dose-related manner, without	PD	11
2 0210111, 2	μg/kg/min		infusion		significant effects on heart rate, blood pressure, paCO2, or paO2		
Meloxicam	1	i.m.	12	Hispaniolan Amazon parrots	Improved weight bearing on arthritic limb	PD	3
				Chickens, ostrich, ducks, turkeys,		PK	
	0.5	i.v.	Single injection	pigeons	Variable distribution, slow clearance except ostrich		1
				Cape		PK	
	2	i.m., p.o.	Single treatment	Griffon vultures	Short $t_{1/2}$ less than 45 min	T K	7
	12.5			Hispaniolan Amazon	PK: t1 / 2 i.m. and i.v. less than 0.35 hr	PK/PD	
Nalbuphine HCl	25	i.m.	Single injection	parrots	Excellent i.m. bioavailability PD: 12.5 mg/kg produced 3 hr		6 12
	50				analgesia; higher doses did not increase analgesic time		
Tramadol	7.5	p.o.	Single dose	Peafowl	PK: maintained plasma human therapeutic concentrations for 12-24 hr	PK	2
	11	p.o.	Single dose	Red-tailed hawks	PK: maintained human plasma therapeutic concentrations for appx. 4 hr (but only three birds in study)	PK	16
	11	p.o.	Single dose	American bald eagles	PK: p.o. bioavailability high, 5 mg/kg p.o. q 12 hr suggested based on study; sedation with multiple dosing	PK	14
	30	p.o.	Single dose	Hispaniolan Amazon parrots	PK: maintained human plasma therapeutic concentrations for appx. 6 hr PD: Reduced thermal withdrawal response for appx. 6 hr	PK/PD	(Guzman, DS-M: pers comi

<sup>&</sup>lt;sup>a</sup>pharmacokinetic <sup>b</sup>pharmacodynamics

ctox=oxicity d  $t_{1/2}$  = half-life

# ANALGESIA FOR THE "BIG-UNS," ELEPHANTS, RHINOS, GIRAFFES AND HIPPOS

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## **Abstract**

Inherent in the Veterinarian's Oath is the prevention and relief of animal suffering; and, for zoological veterinarians, this includes a diverse array of animal groups, including the 'big-uns' – the megavertebrates. Provision of analgesia is fundamental to relief of suffering. Analgesia can be defined as the relief of pain without loss of consciousness.<sup>4</sup> Effective pain relief in megavertebrate mammals has been limited by lack of science-based information addressing pharmacodynamics and effectiveness of analgesics; the role of the rumen and its contents on pharmacokinetics of analgesics in large ruminants; the challenges of pain recognition; the difficulty in assessing pain location, intensity and response to analgesic therapy; and the obstacles associated with analgesic administration and patient acceptance/compliance. Only four pharmacokinetic studies for analgesic use in elephants have been published; three for the nonsteroidal anti-inflammatory agents ibuprofen<sup>1</sup>, phenylbutazone<sup>2</sup>, and ketoprofen<sup>3</sup> and one for the opioid butorphanol. No known pharmacokinetic publications are available in the literature for rhinos, giraffes, and hippos. Most of the information regarding use of analgesics in megavertebrates is anecdotal and is dispersed in case reports, abstracts, and book chapters throughout the literature or passed on by work of mouth from one veterinarian to another. The recommended dosages for analgesics from these sources are generally extrapolated from domestic animal studies and then modified as indicated by experience. Metabolic/Allometric scaling has been used to scale antibiotic dosages from domestic species to the elephant; however, the scaled dosages were considerably different from dosages recommended by pharmacokinetic studies. Consequently, it is unlikely that metabolic scaling will work for estimating dosages of analgesics in megavertebrates, especially if the drugs are protein bound or have unusual metabolic pathways. It is clear that there is a need for more pharmacokinetic studies involving analgesic drugs in all of the megavertebrate species, if we are to care for these species properly.

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# MARINE MAMMAL ANALGESIA: WHERE HAVE WE BEEN AND WHERE DO WE STILL NEED TO GO?

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### Abstract

There is an increasing effort to understand, evaluate, and minimize pain in marine mammals. Pinnipeds, otters, and cetaceans admitted to rehabilitation centers often present with traumatic injuries or other presumably painful conditions. Recent advances in anesthetic drugs, patient monitoring, and our understanding of marine mammal physiology have resulted in increased numbers of surgical procedures on marine mammals maintained in zoos and oceanaria. Nutrition and husbandry continue to improve and many marine mammal species are living long enough to develop typically painful geriatric diseases such as non-infectious arthritis, neoplasia and periodontal disease. Lastly, wildlife biologists whose research may involve potentially painful procedures be performed on free-ranging animals are increasingly working with veterinarians to minimize animal discomfort and provide appropriate analgesia. As professional advocates for and effectors of animal health and welfare, marine mammal clinicians are continually challenged to address analgesia in animals under our care.

However, our understanding of how best to assess pain and provide analgesia remains very limited, particularly in marine mammals. Analgesics are often chosen based on empirical data from other species and individual clinical experience. There are few pharmacokinetic trials and even fewer studies that actually evaluate efficacy of various analgesics in different marine mammal species. There are potentially very serious side-effects of many analgesic agents and more research on effective dosages, dosing schedules, and routes of administration is desperately needed. Below is a list of some analgesic agents that have been used in marine mammals.

### ACKNOWLEDGMENTS

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**Table 1.** Some analgesic agents used in various marine mammals. Please note that these agents have not been fully evaluated for safety and efficacy.

Drug	Dosage	Species	Note	
Acetaminophen	2.5 - 5 mg/kg p.o., b.i.d.	cetaceans	use with caution	
Acetylsalicylic acid	5 mg/kg p.o., b.i.d.	pinnipeds, sea otters	use with caution	
Buprenorphine	0.01 mg/kg s.c., i.m.,	pinnipeds		
	i.v., b.i.d.			
Butorphanol	0.05 - 0.3  mg/kg s.c.,	pinnipeds, sea otters,	0.05 - 0.15  mg/kg in	
	p.o., i.m., i.v., q.i.d.	cetaceans	cetaceans	
Carprofen	2-4 mg/kg p.o., s.i.d.	pinnipeds, sea otters		
Fentanyl	Patch	Zalophus Californianus	Injectable form	
			associated with seizures	
Flunixin megulmine	1 mg/kg i.m., p.o., s.i.d.	pinnipeds, cetaceans	up to 3 days	
Gabapentin	1 mg/kg p.o., s.i.d.	pinnipeds		
Ketoprofen	1 mg/kg i.m., s.i.d.	pinnipeds	up to 3 days	
Local blocking agents:	As required			
Lidocaine				
Bupivicaine				
Medetomidine	0.01 - 0.05  mg/kg i.m.	pinnipeds		
Meloxicam	0.1 - 0.2  mg/kg p.o.,	pinnipeds, sea otters,	0.1 mg/kg s.i.d. up to 3	
	i.v., i.m., s.c.	cetaceans	days in cetaceans	
Meperidine	0.5 - 2.0  mg/kg i.m.	cetaceans		
Ophthalmic ketorolac		pinnipeds, sea otters,		
		cetaceans		
Ophthalmic morphine		pinnipeds, sea otters,		
		cetaceans		
Ophthalmic nepafenac		pinnipeds, sea otters,		
		cetaceans		
Piroxicam	0.2 - 0.3  mg/kg p.o.,	pinnipeds	Adjunctive	
	s.i.d.		chemotherapeutic agent	
			for squamous cell	
			carcinoma	
Prednisone	0.05 - 0.3  mg/kg p.o.	pinnipeds, cetaceans	analgesic?	
Tramadol	0.5 - 2.0  mg/kg p.o.,	pinnipeds, cetaceans	variable efficacy in	
	b.i.d.		cetaceans	

# UPDATE ON PRIMATE ANALGESIA

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### Abstract

Compared to the human primate, our understanding of pain in nonhuman primates is relatively nascent. There are no vigorously validated pain scales available, and current recommendations regarding pain management are mostly empirical or, at least in great apes, extrapolated from human medicine. Signs of pain may be quite obvious or completely absent, depending on factors such as the site and severity of pain or rank within the social hierarchy. Some reported signs of pain include depression, lethargy, inappetance, weight loss, crouched or other abnormal postures, grimacing or facial contortions, teeth clenching, grunting/ moaning/ other vocalizations, head pressing or leaning the head against a wall, reduced or absent grooming, withdrawal from interaction with conspecifics, and guarding, holding, touching, or picking at the painful site. Some submissive behaviors (e.g., lying down) may look like signs of pain but can be differentiated from pain by segregating the patient from higher-ranking conspecifics. Providing areas for primates recovering from painful procedures to hide from aggressors after reintroduction to their group may help reduce stress and permit analgesic drugs to work better. Cameras are useful for detecting signs of pain which may not be manifested if the primate knows it is being watched. Analgesic drugs should be given not just after but before surgery (preemptive analgesia), as well as in combination (multimodal analgesia), for maximal efficacy. Not all analgesic regimens will be effective in all cases, and different drugs may need to be tried before an effective one is found for a given patient. Furthermore, some patients will develop side effects, whereas others will not. Acute pain (e.g., from traumatic injuries, biopsies, tooth extractions, etc.) can usually be managed effectively using a combination of opioids, nonsteroidal anti-inflammatory drugs (NSAIDs), and when possible, locoregional anesthesia. At least 72 hr of some form of analgesic therapy is recommended following injury or surgery. Opioids can cause sedation, inappetance, pruritus, nausea, ileus/ constipation, and respiratory depression; however, these potential side effects should not preclude the use of these drugs as long as judicious doses are administered and the patient is closely monitored. Buprenorphine, a partial µ agonist, appears to be the most commonly utilized opioid in primates today and seems to provide acceptable analgesia for most mildly to moderately painful conditions encountered in zoos, especially when used as part of multimodal analgesia. In the author's experience, however, it does not reduce inhalant requirements or the sympathetic outflow caused by surgical stimulation as profoundly as full  $\mu$  opioid agonists (e.g., morphine, fentanyl). In the uncommon event a primate undergoes a major surgery, a full µ agonist administered pre- and/or intraoperatively as a bolus(es) or constant rate infusion, as long as the patient's ventilation can be controlled, can provide excellent cardiovascular stability and preemptive analgesia. Depending on severity of pain, postoperative analgesia can be provided with buprenorphine or a full  $\mu$ agonist. A topical preparation of fentanyl designed to provide 72 hr of analgesia is being developed and may be an option for post-operative analgesia in the future. Fentanyl lozenges

2012 PROCEEDINGS AAZV CONFERENCE 109

have been used for sedation of great apes, and lower doses in this form for analgesia with less sedation may also prove useful. This author does not recommend the use of opioid patches (e.g., fentanyl, buprenorphine) unless they can be concealed under a jacket of some kind and the patient monitored closely because of the possibility that a primate will remove a patch, ingest it, and overdose. When alimentation is possible, oral opioids are an option. Morphine (available in immediate and sustained-release preparations), methadone, oxycodone, or hydrocodone are commonly sent home with people and might be useful in at least the great apes; however, this author has no experience using these drugs in lower primates. Stool softeners and antiemetic drugs can alleviate constipation and nausea/ vomiting, respectively, caused by opioids if these occur. Tramadol is an analgesic drug with weak μ agonist properties that also inhibits serotonin and norepinephrine reuptake; it causes less sedation and gastrointestinal side effects and provides analgesia comparable to oral NSAIDs in humans. NSAIDs work well in combination with opioids for acute pain; carprofen and meloxicam appear to be the most commonly used. As with opioids, these drugs should be used at judicious doses as part of multimodal analgesia with close patient monitoring for side effects such as acute renal failure or gastrointestinal ulceration. Long-acting local anesthetics such as bupivacaine or ropivacaine can be infiltrated locally or around specific peripheral nerves (e.g., digital nerves for a finger bite wound); if a severely painful, major surgical procedure is to be performed, an epidural or spinal injection or a major peripheral nerve block might be feasible. Icing is a simple, inexpensive, effective way to decrease inflammation around a surgical site and provide analgesia. Chronic pain (e.g., osteoarthritis or degenerative disk disease in a geriatric primate) is usually more challenging to treat as it may not respond as well to traditional analgesic therapy. Nutraceuticals (e.g., glucosamine and chondroitin) can be highly effective in some humans with osteoarthritis. NSAIDs often form the foundation of chronic pain management; chronic NSAID use can often be cut back with time (e.g., a half dose every other day or twice weekly) to reduce side effects while maintaining efficacy, especially if other drugs and non-pharmacologic modalities are used simultaneously. These might include low doses of opioids, tramadol, anticonvulsants (e.g., gabapentin, pregabalin), antidepressants, and acupuncture or laser therapy performed during immobilization, among others (Bourgeois). Intraarticular (e.g., for osteoarthritis) or epidural (e.g., for radiculitis) injection of a local anesthetic plus corticosteroid (e.g., triamcinolone) are performed to provide longer-lasting analysesia in humans and can be highly effective. Finally, some drugs used for immobilization are analgesic drugs as well, notably ketamine and  $\alpha_2$ agonists, and may contribute to analgesia for at least some time after recovery.

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**Table 1.** Analgesic drugs for use in primates.

Drug	Dose	Route	Dosing interval (hr)	Comments		
Buprenorphine	0.005-0.03 mg/kg	s.c., i.m., i.v.	4-12	1-2 doses recommended after major surgery as part of multimodal analgesic protocol in monkeys		
Buprenorphine SR (ZooPharm)		s.c.		Data in primates pending but not published at time of writing		
Morphine	0.025-0.1 mg/kg <sup>a</sup> 0.01-0.03 mg/kg <sup>a</sup>	i.v. i.v.		Can be used to reduce inhalant requirements and provide preemptive analgesia during surgery		
	0.1-2 mg/kg	s.c., i.m.	3-6			
Morphine solution Morphine controlled-release	10 mg <sup>b</sup>	p.o.	3-4	anargesia during surgery		
	15 mg <sup>b</sup>	p.o	8-12			
Hydromorphone	0.015-0.02 mg/kg <sup>a</sup> 2-4 mg <sup>b</sup>	i.v. p.o.	4	Can be used to reduce inhalant requirements and provide preemptive analgesia during surgery		
Methadone	5-10 mg <sup>b</sup>	p.o.	12 p.r.n.			
Fentanyl	1-2 mcg/kg <sup>a</sup>	i.v.	during surgery			
	5-10 mcg/kg (bolus) 10-25 mcg/kg/hr (infusion)	i.v.	p.r.n. during surgery			
Codeine	30-60 mg <sup>b</sup> (Morgan)	p.o.	4			
Hydromorphone	2-4 mg <sup>b</sup> (Morgan)	p.o.	4			
Oxycodone	5-20 mg <sup>b</sup>	p.o.	4-6			
Hydrocodone	5-20 mg <sup>b</sup>	p.o.	4-6			
Oxycodone + acetaminophen	10 mg oxycodone + 325 mg acetaminophen <sup>b</sup>	p.o.	4-6			
Hydrocodone + acetaminophen	10 mg hydrocodone + 325 mg acetaminophen <sup>b</sup>	p.o.	4-6			
Tramadol	50 mg <sup>b</sup>	p.o.	6			
Gabapentin	600-800 mg <sup>b</sup>	p.o.	8	Antiepileptic drug used to treat neuropathic pain		
Pregabalin	75 mg up to 600 mg/day <sup>b</sup>	p.o.	8	Antiepileptic drug used to treat neuropathic pain		
Aspirin	500-1000 mg <sup>b</sup>	p.o.	4			
Acetaminophen	500-1000 mg <sup>b</sup>	p.o.	4			
TI C	40 mg/kg	p.o.	4			
Ibuprofen	400 mg <sup>b</sup>	p.o.	4-6	Desire frances and being a figure and for a		
Carprofen	2-4 mg/kg	s.c., i.m.	24	Dosing frequency can be increased for 2-3 doses if required.		
Meloxicam	0.1 mg/kg (multiple species)	p.o., s.c.	24	Can be administered for at least up to 4-5 days if needed; dosing frequency can be increased for 2-3 doses if required		
	0.2 mg/kg (macaque)	p.o., s.c.	24			
	7.5 mg <sup>b</sup>	p.o.	24			

<sup>&</sup>lt;sup>a</sup>Dose used for human pediatric patients <sup>b</sup>Dose used for average adult human.

# NEMATOPHAGOUS FUNGUS (Duddingtonia flagrans) PILOT TRIALS FOR TRICHOSTRONGYLE PARASITE CONTROL IN EXOTIC ARTIODACTYLID SPECIES

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# **Abstract**

Internal nematode parasites, specifically the abomascal trichostrongyle *Haemonchus* spp., are a significant health concern in domestic and exotic ruminants in the southeastern US and abroad. 1-3 Non-chemical alternatives should be investigated in exotic species, similar to domestic ruminant studies, to reduce traditional drug selection pressure and address resistance issues. nematophagous fungus, Duddingtonia flagrans, has shown promise in domestic small ruminants for environmental control of the infective L3 larvae in the feces in the environment.<sup>3</sup> Two pilot trials were conducted to evaluate a non-chemical method for controlling gastrointestinal nematode parasites in captive ruminant hoof stock at Disney's Animal Kingdom® and Disney's Animal Kingdom Lodge<sup>®</sup>. The specific trials involved feeding spores of the nematode-trapping fungus Duddingtonia flagrans to selected captive exotic hoof stock in order to reduce infective larvae survival/development in feces and, thus, reduce exhibit forage contamination. The fungal spores were fed daily at 250,000 spores/kg BW for five days (2010 pilot trial) and 30,000 spores/kg BW for five days (2011 pilot trial). Fecal samples were collected from control and treatment animals before, during, and after the fungus treatment course to look at fecal egg counts and larval culture rates. Both doses showed successful reduction in survival of L3 larvae in vitro implying that doses of 30,000 spores/kg BW may be an effective tool for environmental control of *Haemonchus* spp. in exotic artiodactylids.

#### **ACKNOWLEDGMENTS**

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nematode-trapping fungus Duddingtonia flagrans to reduce infective larvae of gastrointestinal nematodes in goat feces in the southeastern United States: dose titration and dose time interval studies. Vet. Parasitol. 120 (2004) 285–296.

# CHRONIC RENAL FAILURE IN MULTIPLE SOUTHERN WHITE RHINOCEROS (Ceratotherium simum)

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# Abstract

Chronic renal failure (CRF) was diagnosed in three aged (36-42 yr) and one young (9 yr) southern white rhinoceros (Ceratotherium simum simum) at Fossil Rim Wildlife Center. The aged rhinoceros had loss of body condition, lethargy, and decreased appetite. Clinicopathologic findings in these geriatric rhinoceros included azotemia, hypoalbuminemia, hyponatremia, hypochloremia, and hypophosphatemia; additionally, two of three older rhinoceros had hypercalcemia. Isosthenuria and proteinuria were present on the urinalysis. Fractional excretion of sodium was elevated compared to horse parameters, suggesting inadequate tubular function. While primary renal tubular disease was the most likely cause for the observed findings, eventual histopathologic findings from necropsy only confirmed end-stage kidney disease with no clear etiology in all three geriatric rhinoceros. The young rhinoceros was smaller than normal and initially presented with lethargy, decreased appetite, swollen limbs, and ulcerated lesions on the foot pads. Clinicopathologic findings suggesting renal disease were limited to severe proteinuria and hypoalbuminemia, consistent with glomerular disease. After clinical recovery from this initial episode, proteinuria continued. Thirty months after initial presentation, the rhinoceros again presented with lethargy and decreased appetite. Clinicopathologic changes indicative of CRF were present: azotemia, hypophosphatemia, hypercalcemia, hyponatremia hypochloremia, in addition to continued hypoalbuminemia and proteinuria. Fractional excretion of sodium was elevated. Currently, this animal is maintaining body condition. These rhinoceros exhibited similar physical and biochemical changes to horses with CRF. Dietary reduction of calcium, oral supplementation of phosphorus, and provision of a higher calorie, more palatable diet resulted in temporary improvement in clinical signs and several clinicopathologic abnormalities.

# REPRODUCTIVE ANATOMY OF THE MALE GIRAFFE (Giraffa camelopardalis)

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#### **Abstract**

The giraffe (*Giraffa camelopardalis*) is a commonly kept species in zoological gardens throughout the world. Although still classified as a "Least Concern" species, the wild giraffe population is decreasing, with some subspecies even listed as "Endangered". In the future, breeding giraffes in zoological gardens might be a significant step in conservation and may include artificial insemination. This study of the immature male giraffe reproductive system compared transrectal ultrasound findings with gross anatomy at post mortem. Transrectal ultrasounds were performed on immature male giraffes (n=7) to assess and measure accessory sex glands. Measurements from ultrasound were compared to gross anatomy post mortem in the same individuals. In addition, histology was performed on the whole male giraffe reproductive system (n=14).

This study provides information for the use of ultrasound as a diagnostic tool to assess the reproductive status of male giraffes,<sup>4</sup> and is the first anatomic report of the male giraffe genital system.

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# THIAFENTANIL (A3080): WILL IT REPLACE ETORPHINE AND CARFENTANIL? WHAT SPECIES DOES IT WORK OR NOT WORK IN?

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#### Abstract

Thiafentanil has been in the pharmaceutical development and registration pipeline for use in hoofstock for almost 20 yr. During that time it has been used by hundreds of veterinarians in zoos and free ranging situations in which useful data and experience has been collected. As it is now fully approved in South Africa and exported to numerous countries from there and nearing a FDA MUMS Index approval here in the US, this may be an appropriate time to give a broad overview of its comparative applications, advantages and disadvantages to the currently available potent opiates etorphine and carfentanil.

Etorphine has been used in wildlife and zoological medicine for over 60 yr. Its advantage is that it has been used in almost every species possible. The knowledge base as to what combinations of alpha-two agonists, buterophrenone, or dissociative with etorphine is effective in what species is vast. Concurrently, we have discovered that with etorphine in many species, we experience prolonged excitement phases during induction, hypertension in many cases, respiratory depression and muscle rigidity. In many species there is more regurgitation with etorphine compared to carfentanil and thiafentanil. Many concurrent uses of the alpha-two's, azaperone, and the sedatives have been used in an attempt to manage these side effects. Etorphine anesthesia protocols were eventually worked out for most major species, even with its side effects, and became widely used in larger hoof stock and equids.

Carfentanil was first available for use in the early 1980's. Its first applications were explored in South Africa and later in North America. Its most apparent immediate advantages over etorphine were the low dose volumes possible and slightly shortened induction times in many key species. It still had most of the classical opiate issues such as muscle rigidity, respiratory depression and hypertension that had to be managed. It was rapidly learned that wild members of the Perissodactyla were refractory to its effect or showed more adverse side effects-much to the dismay of the wildlife medicine community.

Thiafentanil oxalate (A3080, Thianil) was first available for field use in the early 1990's. The first field reports and publications immediately demonstrated the dramatically shortened induction times in most species. Its expanded field use demonstrated improved efficacy in many species and it now is considered the drug of choice for hoofstock such as gemsbok (*Oryx gazella*), Liechtenstein's hartebeest (*Sigmoceros lichtensteini*), impala (*Aepyceros melampus*), kudu (*Tragelaphus strepsiceros*), nyala (*Tragelaphus angasii*), reedbuck (*Redunca* sp), rhebok (*Pelea caoreolus*), roan (*Hippotragus equinus*), sable (*Hippotragus niger*), waterbuck (*Kobus ellipsiprymnus*), and klipspringer (*Oreotragus oreotragus*). Published reports indicate that it is also the opiate of choice for use in giraffe (*Giraffa camelopardalis*). Field work in Thailand

also indicate that it is the drug of choice for gaur (*Bos gaurus*) and banteng (*Bos javanicus*) (M. Bush, pers. comm., 2012). With all this success in these species, the Perissodactyla remain refractory to thiafentanil, and etorphine remains the drug of choice for these species as well as the rhino and elephant, although field use of thiafentanil in elephant and rhino is becoming more common in Africa (J. Raath, pers. comm., 2012). The rapid metabolism of thiafentanil may be a drawback in some situations as supplementation may be necessary before all procedures are completed in contrast to the longer metabolic half-life of etorphine and carfentanil.

In North America due to the improved shortened induction period of thiafentanil in species such as elk, it has become the drug of choice for aerial capture for this species.<sup>3</sup> The reduced induction time enables immobilization of more animals with less helicopter time. Its efficacy in pronghorn makes it the only drug that will reliably immobilize this species.<sup>4</sup>

Based on the published literature and the field reports available today, it is most probable that thiafentanil will replace both carfentanil and etorphine in most free ranging hoof stock, other than the Perrisodactylids, due to its shortened induction time, low dose volume, and fewer side effects in some species. Etorphine will remain in use as the drug of choice in the Perrisodactylids for the foreseeable future.

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# A PILOT STUDY ON THE EFFECTS OF A LOW-STARCH DIET ON INSULIN RESISTANCE IN TWO CAPTIVE BLACK RHINOCEROS (Diceros bicornis) AT THE CLEVELAND METROPARKS ZOO

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#### **Abstract**

Black rhinoceros captive breeding programs are not self-sustaining due to metabolic disorders (including hemolytic anemia, necrolytic dermatopathy, iron storage disorder, and rhabdomyolysis) not usually seen in free-ranging populations. Researchers hypothesize that these diseases are due to obesity-mediated chronic inflammation that contributes to iron overload, insulin resistance, and hypophosphatemia. Preliminary data from ongoing projects at Cleveland Metroparks Zoo (CMZ) indicate measurable differences between potential markers of insulin resistance and inflammation in captive versus free-ranging black rhinos. Original rhino diets were formulated at CMZ based on the Rhino SSP Husbandry Manual and National Research Council domestic horse diet recommendations.<sup>2</sup> Similar to domestic horses, rhinos are hind-gut fermenters, and low-starch diets help manage insulin resistance in horses.<sup>4</sup> Working within the parameters of the previously established diets, we replaced high-starch grain pellets (Mazuri® ADF#16) with low-starch (25% less starch) grain pellets (Mazuri® 5V05). Total quantities (with similar caloric content) of the diet remained unaltered. All animals were weighed regularly. We collected baseline blood samples prior to the diet change and continued collecting samples bi-weekly. Serum samples were analyzed for potential markers of insulin resistance (serum insulin and glucose) using enzyme-linked immunosorbent assays (ELISAs) previously validated at the CMZ endocrinology lab for use with black rhino serum. Preliminary data indicate declining averages in both insulin and glucose serum concentrations in both rhinos as compared to past averages. These declines may indicate an increase in insulin sensitivity and one step towards decreasing the incidence of metabolic disorders in black rhinos.

#### **ACKNOWLEDGMENTS**

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# E. coli SIDEROPHORE VACCINATION TO AUGMENT HEALTH MANAGEMENT OF DOMESTIC GOATS (Capra hircus) IN GUEST CONTACT ROLES

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# Abstract

An *E. coli* siderophore vaccine (E. coli bacterial extract vaccine with SRP®, Pfizer Animal Health/Epitopix, Willmar, MN 56201, USA) with USDA approval for beef cattle reduces potential disease risk by immunologic interference with gastrointestinal coliform's iron-harvesting receptors.<sup>4</sup> Complete vaccination eliminated *E. coli* O-157 presence for 85% of treated individuals and reduced shedding by 98% for those without complete elimination.<sup>5</sup> The vaccine is applied optimally as cattle depart pasture for the feedlot so as the animals actually enter the end production chain, they present less contamination risk to the consumer.<sup>4,5</sup> In production applications, the siderophore vaccine has been applied experimentally to younger and smaller species of production ruminants with similar benefit with lower vaccine doses at reduced frequency (V. Cortese, personal communication).

It is well established that human-animal interfaces present opportunities for zoonotic disease transmission.<sup>1-3</sup> Implicated in the zoo community as a source of this concern are "petting yards" with domestic livestock which often include young animals.<sup>1</sup> In the zoo guest contact yard, it should be considered that the animals may experience some of the same social issues experienced by production purposed animals. Further consideration suggested that zoo guests, especially seasonally, would be considered within high at-risk age categories. Although not a replacement for good veterinary care and encouragement of appropriate guest hygiene, <sup>1-3</sup> a product which could reduce actual contamination potential from contact program ruminants suggested a novel application for the siderophore product.

# ACKNOWLEDGMENTS

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# UPDATES ON ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV) EEHV-5 INFECTIONS IN ASIAN ELEPHANTS (*Elephas maximus*)

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# **Abstract**

Elephant endotheliotropic herpesviruses (EEHVs) can cause acute hemorrhagic disease with high mortality rates in Asian elephants (*Elephas maximus*). Recently, a new EEHV type known as EEHV-5 has been described, but its prevalence and clinical significance remains unknown. To address this issue, we looked for EEHV-5 in 2 captive herds in North America and in over 50 elephants from India. In the first captive herd, a 42-yr-old wild-born female Asian elephant demonstrated signs of illness (swollen temporal glands, oral hyperemia, and generalized depression) over a three week period during the spring of 2011 that coincided with EEHV-5 viremia as detected via real time PCR on whole blood samples. Retrospective analysis of stored blood samples and trunk washes during the spring of 2011 from the other six elephants in the herd demonstrated shedding of EEHV-5 in trunk secretions in all six elephants and EEHV-5 viremia in five elephants. EEHV-5 trunk shedding and viremia without associated clinical signs was also detected in an elephant that was recently transferred between herds within North America. Finally, EEHV-5 was detected in 20% of trunk washes obtained from over 50 Asian elephants living in India. The results suggest that EEHV-5 infection might be common within captive and range country Asian elephants and in some cases it can cause illness.

# NEW THOUGHTS ON PERACUTE MORTALITY IN GIRAFFE

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# Abstract

Peracute mortality syndrome, or giraffe wasting disease, has been a leading cause of mortality in giraffe since the 1970s. It generally presents as acute death without premonitory signs. It is characterized by serous atrophy of adipose tissue and weight loss. Other lesions are non specific. Several articles have been published suggesting a potential nutritional component.<sup>2,4-6,8</sup>

Over the past 15 yr, evaluation of the serum, omental, and pericardial adipose tissue relative % fatty acids (R%FA) in captive North American giraffe deaths has demonstrated an inverted linolenic:linoleic acid ratio when compared to wild giraffe values in virtually all animals.

Linolenic acid is metabolized at a lower temperature than linoleic acid. When linolenic to linoleic acid ratios are inverted for a prolonged period, giraffe enter a negative energy balance and subsequently lose weight. Cold stress is a well known inducer of lipolysis. Due to the large surface area of giraffe and minimally protective pelage, conservation of body heat is not possible during suboptimal environmental temperatures. Since linolenic acid burns at a lower body temperature than linoleic acid, we postulate that prolonged suboptimal environmental temperatures cause serous atrophy of fat reserves and predisposes to acute mortality.

Very few species of native plants in the United States provide a linolenic to linoleic acid ratio >1:1. *Perilla futrescens*, *Linum usitatissimum*, and *Salvia columbariae* plants provide a linolenic:linoleic acid ratio > 2:1. <sup>1,7</sup> Alfalfa hay, the most common component of giraffe diets, provides a significant inverse ratio of linolenic to linoleic acid. <sup>1</sup> To find a plant that provides a linolenic to linoleic acid ratio >2:1, we looked for a North American equivalent to African *Acacia* species, the predominant forage of wild giraffe. <sup>3</sup>

Prairie bundle flower (*Desmanthus illinoisensis*), a common native plant, was analyzed for relative % fatty acid (R%FA) profiles and found to have a greater linolenic:linoleic acid ratio than any other plant studied. (We theorize that giraffe, as well as other ruminants have an as yet unknown mechanism to detect and seek out plants based on their linolenic:linoleic ratio, as they actively seek this plant out over other browse items offered). Plants were grown on zoo grounds, harvested and analyzed at various stages of seasonality. In addition, plants were dried for 30, 60 and 90 days, and then analyzed for R%FA analyses. No adverse effects were noted when supplementing prairie bundle flower to giraffe. Results are encouraging and may provide a basis for year round supplementation to captive giraffe diets, minimizing the need for high energy, alfalfa based diets that may predispose to rumen acidosis, urinary calculi and urethral blockage, the latter of which is commonly diagnosed as a cause of death in male giraffe. Additional research into fatty acid content of wild *Acacia* sp. and supplementation is ongoing.

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# MORTALITY REVIEWS: HOW TO MINIMIZE BIAS AND DRAW APPROPRIATE CONCLUSIONS

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#### Abstract

Bias is unavoidable in research, but efforts to minimize it are essential if we want to draw valid conclusions. Mortality reviews can be useful tools for managing population health but are fraught with difficulties that are often overlooked.<sup>1-4</sup> Bias occurs in the creation of pathology reports when different pathologists use different diagnostic criteria, have differing levels of experience and confidence, and conduct investigations of varying depth. The only way to minimize these types of bias is to have pathologist(s) review and standardize each report. Bias is introduced in the extraction of data from reports when the investigator subjectively interprets a finding in a way that gives the desired diagnosis or outcome, when interpretation of one finding influences the interpretation of another, and when the investigator only relies on the most readily available data rather than the most reliable. These types of bias can be minimized by developing detailed case definitions, protocols for record review, and procedures for data interpretation in advance. Bias in the final interpretation can occur if missing data are not handled properly, when causal inferences are drawn without appropriate controls, when a single disease process is misinterpreted as multiple processes and vice versa (often due to confusion over diagnostic terminology), and when the population to which the results apply (the population at risk) is misidentified. Avoiding these types of bias requires careful study design, addressing the other types of bias described above, and if necessary, recruiting additional expertise for the study.

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MANAGING A TUBERCULOSIS OUTBREAK: DEVELOPMENT AND IMPLEMENTATION OF SCREENING PROTOCOLS FOR Mycobacterium tuberculosis IN ZOO MAMMALS

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#### Abstract

Tuberculosis (TB) due to *Mycobacterium tuberculosis* was diagnosed in an Asian elephant (*Elephas maximus*) and a chimpanzee (*Pan troglodytes*) at Taronga Zoo, Australia. Detailed investigation into the disease outbreak included development of screening protocols for TB in all collection mammals. Early, rapid detection of disease is essential to prevent the silent spread of TB through a collection and to protect the health of staff and visitors. Ante-mortem diagnosis of TB in zoo animals is challenging due to lack of validated or standardized diagnostic techniques in most species and the limited sensitivity and specificity of most tests.

Over 600 animals of 78 species were prioritized for TB screening based on reported susceptibility to *M. tuberculosis*, exposure risk, availability of a recognized testing protocol and logistics of animal restraint. Where possible, a combination of diagnostic test modalities was used for each species, including non-specific tests (clinical examination, CBC and serum biochemistry, radiography, gross necropsy); direct sampling for organisms (Ziehl-Neelsen staining, PCR and culture of tracheo-bronchial lavage, trunk wash or nasal wash material, fine needle aspirates of lymph nodes or tissue collected at necropsy); and immunologic tests based on cell mediated immune response (comparative tuberculin skin test and Interferon-γ release assay) or humoral response (Elephant TB Stat-Pak<sup>®</sup> (Chembio) or Dual Path Platform Vet<sup>®</sup>TB test<sup>TM</sup> (Chembio)). Suspect or positive results were interpreted in light of the potential limitations of the tests and prompted further investigation. The zoo's long term TB surveillance program will be modified according to the results of ongoing screening and exposure risk.

### ACKNOWLEDGMENTS

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USE OF PCR-DGGE TO CHARACTERIZE THE DISTRIBUTION OF BACTERIAL POPULATIONS IN FECES OF RETICUALTE GIRAFFES (Giraffa camelopardalis reticulata), AFRICAN ELEPHANTS (Loxodonta africana) AND WHITE RHINOCEROS (Ceratotherium simum)

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# **Abstract**

The gastrointestinal microbiota play a vital role in overall health of people and animals by helping break down and digest food, producing vitamins and hormones, training the immune system and preventing pathogenic bacterial overgrowth. Little is known, however, about the ecology of microbiota of large exotic herbivores and basic questions need to be answered. The objectives of this study were to evaluate whether bacterial populations are evenly distributed throughout fecal excretions in three exotic herbivore species and to compare the inter- and intraspecies variability of bacterial populations. Fecal samples were collected from reticulated giraffes (Giraffa camelopardalis reticulata) (n=6), African elephants (Loxodonta africana) (n=7) and white rhinoceros (Ceratotherium simum) (n=3) at the Indianapolis Zoo. PCR targeting the 16S rRNA gene was performed followed by denaturing gradient gel electrophoresis (DGGE) to create bacterial community fingerprints for each individual sample. A homogenized sample of an entire bolus (elephants and rhinos) or multiple pellets (giraffes) was compared against five individual samples randomly collected throughout the excretion to evaluate differences in bacterial populations. Pairwise comparisons were made and a cluster analysis performed to evaluate inter- and intraspecies relatedness. The study found that dominant bacterial populations were evenly distributed throughout the fecal excretion in each species, suggesting that a small sample is indeed representative of the entire excretion. This is important to know when collecting samples for microbiologic culture. Differences in microbial communities were observed, with the greatest contributing factor in variability being species, followed by age. One giraffe being treated with antibiotics for a chronic leg infection demonstrated decreased species richness and differed from the other giraffes in the bacterial populations present.

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# PLASMA PROTEIN ELECTROPHORESIS IN GRAND CAYMAN BLUE IGUANAS (Cyclura lewisi)

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#### Abstract

A retrospective study was conducted on banked heparinized plasma samples (n=139) collected from healthy Grand Cayman iguanas (Cyclura lewisi) on Grand Cayman to measure protein fractions via protein electrophoresis (EPH). Data were analyzed by year (2004-2011), season (summer (n=67) vs. fall (n=72)), age class (juveniles (n=80) vs. adults (n=59)), origin (wild (n=17) vs. captive (n=122)), and gender (unknown=2, males=69, females=68). When juveniles were excluded from analysis, gender significantly (p < 0.05) influenced albumin and  $\alpha$ 1 globulins with higher values in females and males, respectively. Albumin was not significantly influenced by year, season, age class, or origin. All globulins fractions were significantly related to age class with higher values in adults, and all except  $\gamma$  globulins were significantly influenced by season with higher values in the fall. A significant relationship was also present between  $\alpha 2$ and  $\beta$  globulins and year with an increase in these fractions over time. In addition,  $\beta$  globulins were significantly related to origin, being higher in wild iguanas. By using the combined data, the protein EPH fractions (mean  $\pm$  standard deviation) are as follows: total protein 7.8  $\pm$  1.6 g/dL, albumin 3.31  $\pm$  0.5 g/dL,  $\alpha$ 1 globulins 0.79  $\pm$  0.18,  $\alpha$ 2 globulins 0.20  $\pm$  0.05 g/dL,  $\beta$ globulins  $2.60 \pm 0.90$  g/dL,  $\gamma$  globulins  $0.87 \pm 0.41$  g/dL, and albumin/globulin ratio  $0.80 \pm 0.21$ . Knowledge of reference intervals for protein EPH fractions in this critically endangered species will aid in the care and management of both captive and wild Cyclura spp. populations.

#### **ACKNOWLEDGMENTS**

The authors thank the WCS and Blue Iguana Recovery Programme staff and volunteers who participate in this endangered species recovery effort and are grateful for their assistance with data and sample collection. The authors also thank the St. Matthews Veterinary School for provision of laboratory space on Grand Cayman, David Powell for discussing statistical analysis of the data, and Joan Maurer and the Milwaukee County Zoo and Society for project support.

# THE ROLE OF HEPCIDIN IN REGULATION OF IRON BALANCE IN BATS

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### Abstract

Hemochromatosis has been associated with liver disease and mortality in captive Egyptian fruit bats (Rousettus aegyptiacus). Although evolutionary adaptation to low levels of iron in the natural diet has been implied, the physiologic basis for susceptibility has not been established. In humans, the iron regulatory protein hepcidin appears to play a crucial role in iron balance and the development of hereditary hemochromatosis. A deficiency or resistance to hepcidin has been implicated in human hereditary hemochromatosis and may play a role in Egyptian fruit bat hemochromatosis. A preliminary investigation was carried into the role of hepcidin in iron metabolism in bats. The coding gene sequence of the hepcidin gene was determined for three species with variable susceptibility to hemochromatosis; Egyptian fruit bat, straw-coloured fruit bat (Eidolon helvum), and common vampire bat (Desmodus rotundus). Baseline blood parameters were compared to those obtained 14 days after intramuscular administration of 100 mg/kg iron dextran (Dextafer®) in the Egyptian fruit bat and straw-colored fruit bat. Hematologic parameters assessed included plasma ferritin, transferrin saturation, plasma iron, and a complete blood cell count (CBC). Liver biopsy samples were obtained at baseline and 14 days after iron administration from all three species and assessed for morphology (histopathology), liver iron content (atomic absorption spectrophotometry), and relative gene expression of hepcidin (RTqPCR). Results were compared between all three species, including two distinct populations of Egyptian fruit bat, with and without underlying hemochromatosis.

130 2012 PROCEEDINGS AAZV CONFERENCE

# POLYARTHRITIS ASSOCIATED WITH A NOVEL POXVIRUS IN BIG BROWN BATS (Eptesicus fuscus)

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#### **Abstract**

To the authors' knowledge, there are no previous reports of any kind of poxvirus infection in bats. From 2009-2011, 6 big brown bats (Eptesicus fuscus) were submitted to Northwest ZooPath for histologic evaluation. All bats were adults found down and unable to fly in the late Spring or Summer. Five were males and sex was unknown for one. All but one of the bats had one or more visibly swollen and sometimes contused joints involving the long bones of the legs and wings, and one had contusions of the oral commissures. All bats received care that included antibiotics, nutritional and fluid support with minimal or no clinical improvement, progressive joint swelling and increased lethargy. All bats were eventually euthanatized. Gross lesions were limited to the joints in all bats. Histologically, all bats had severe fibrino-suppurative and necrotizing tenosynovitis and osteoarthritis with occasional localized vasculitis. No infectious agents were seen by light microscopy with hematoxylin and eosin, giemsa, Warthin-Starry, Brown and Brenn or Gomori methenamine-silver stains or in a Wright-Giemsa stained cytologic preparation of a joint aspirate. Aerobic, anaerobic and mycoplasma cultures of the joint from one bat were negative. Transmission electron microscopic examination of the affected joint capsule from one bat identified poxvirus particles in the cytoplasm of apparent synovial cells. Poxvirus DNA was isolated from the wing web and joint of one bat and preliminary phylogenetic studies indicate that the virus is distinct from any currently known poxvirus, but is distantly related to sheeppox, goatpox and lumpy skin disease pox. Poxvirus-induced bone lesions are apparently rare, and the histologic findings in these bats resemble those associated with the bone lesions induced by smallpox in children.<sup>1</sup>

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DEVELOPMENT OF A QUANTITATIVE PCR FOR RAPID AND SENSITIVE DETECTION OF AN INTRANUCLEAR COCCIDIAN PARASITE OF TORTOISES (TINC) AND IDENTIFICATION OF TINC IN THE CRITICALLY ENDANGERED ARAKAN FOREST TURTLE (Heosemys depressa)

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#### **Abstract**

The tortoise intranuclear coccidian parasite (TINC) was first reported in radiated tortoises, *Geochelone* (*Astrochelys*) radiata, presenting with severe anorexia and lethargy.<sup>2</sup> It has since proven to be a significant cause of disease of tortoises causing high mortality and affecting several threatened chelonian species.<sup>1</sup> Diagnostic testing has been limited to relatively labor intensive and expensive pan-coccidial PCR and sequencing techniques with a long turnaround time. This report describes the development a quantitative PCR (real-time or qPCR) that provides a rapid, analytically specific, and economical detection of TINC. A qPCR probe targeting a specific and conserved region of TINC 18S rRNA was designed. The qPCR reaction was run on samples known to be TINC positive and the results were consistent and analytically specific. The assay was able to detect as little as 10 copies of target DNA in a sample. The development of this assay enables studies optimizing diagnostic sampling, describing geographic disease prevalence, and investigating life cycles. Testing of soil and invertebrates from enclosures of positive animals was negative and did not provide any further insights into the life cycle of the parasite. This assay was used to identify TINC in a novel host species, the critically endangered Arakan forest turtle (*Heosemys depressa*).

# ACKNOWLEDGMENTS

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# TICK TALK: TRYING TO UNDERSTAND (AND KILL) THE SPINOSE EAR TICK (Otobius megnini)

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### Abstract

Spinose ear tick (Otobius megnini) infections of ungulates at Fossil Rim Wildlife Center (FRWC) are commonly seen. This one-host soft tick (Acari: Argasidae) is native to southwestern United States and Mexico. Larval and nymphal ticks are parasitic, often feeding deep in the ear canal. The adults, however, do not feed. Final molt and reproduction occur off the host. This tick has not been reported to transmit pathogens,<sup>2</sup> and in domestic animals morbidity may be limited to irritation, secondary infections and potential impacts on production. 1,3,4 From October 2009 through March 2012, ear ticks were found in 13 of 21 ungulate species and 84 of 148 individuals examined (Table 1). Infected ears were treated with ivermectin paste (Vetrimec Paste 1.87%, Vet One, Meridian, ID 83680 USA) applied in the ear canal after manual removal of ticks. Adult and larval ticks have been collected at FRWC from animal sheds and in the natural environment near sheds. Adult ticks were most often found in the crevices behind beams, the base of posts, and debris under ledges, and were collected most successfully with a debris-filtering technique. Larvae were successfully collected using carbon dioxide traps. Current research is underway to further document spatial and temporal distribution; this data will be used to better manage tick populations off the host by altering the environment at the most advantageous times and locations. Off host treatment will likely prove essential in controlling tick populations due to limited handling of FRWC ungulates.

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Table 1. Prevalence of Otobius megnini by species.<sup>a</sup>

Common name	Species	No.	Ticks	No ticks	Prevalence (%)
Addax antelope	Addax nasomaculatus	46	42	4	91.3
Ankole-Watusi cross	Bos taurus	1	1	0	100
Arabian oryx	Oryx leukoryx	1	1	0	100
Fallow deer	Dama dama	2	1	1	50
Gemsbok	Oryx gazella	8	5	3	60
Giraffe	Giraffa camelopardalis	1	1	0	100
Grevy's zebra	Equus grevyi	2	2	0	100
Grant's zebra	Equus burchelli	14	6	8	42.9
Hartmann's mountain	Equus zebra hartmannae	10	8	2	75
zebra					
Roan antelope	Hippotragus equinus	4	4	0	100
Sable antelope	Hippotragus niger	11	5	6	35.7
Scimitar-horned oryx	Oryx dammah	6	5	1	83.3
Wildebeest	Connochaetes taurinus	24	3	21	12.5

<sup>a</sup>Species, with number of individuals examined, found to be negative for the presence of *Otobius megnini*: aoudad, *Ammotragus lervia* (2), blackbuck, *Antilope cervicapra* (2), domestic goat, *Capra hircus* (4), domestic sheep, *Ovis aries* (1), greater kudu, *Tragelaphus strepsiceros* (1), Przewalski's horse, *Equus przewalskii* (2), red deer, *Cervus elaphus* (4), waterbuck, *Kobus ellipsiprymnus* (2).

NON-LETHAL ACQUISITION OF LARGE LIVER SAMPLES FROM FREE-RANGING STURGEON (Scaphirhynchus spp.) USING NOVEL OPTICAL BIOPSY FORCEPS

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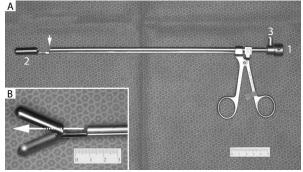
#### Abstract

The harvesting of liver samples for toxicologic and other laboratory analyses is frequently undertaken in free-ranging fish in order to evaluate accumulations of various pollutants and chemicals.<sup>1-13</sup> However, commonly used techniques of collecting liver are lethal and unacceptable when dealing with charismatic, threatened or endangered species. We report the use of a non-lethal, single-entry, endoscopic technique using saline infusion to examine and collect large liver samples using optical biopsy forceps (62046GS, Karl Storz Veterinary Endoscopy America Inc [KSVEA], Goleta, CA 93117 USA; Figure 1) from 15 free-ranging shovelnose sturgeon (Scaphirhynchus platorynchus), and one pallid sturgeon (S. albus). Under tricaine methanesulfonate general anesthesia, a 1 - 2 cm ventral midline skin incision permitted the introduction of the forceps, which incorporated a 5 mm telescope (62033APA, KSVEA). Liver examination and liver biopsies up to 1.4 grams in weight, and representing up to 14% of total liver tissue were successfully obtained. All fish made uneventful recoveries and those that were subjected to necropsy examinations the following day failed to indicate any significant hemorrhage or iatrogenic trauma. The use of large optical biopsy forceps are recommended as a practical, non-lethal alternative for the collection of large liver biopsies from sturgeon and other fish.

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**Figure 1.** Endoscopic optical biopsy forceps. (A) 5 mm x 29 cm rigid endoscope is inserted through the opening (1) such that the terminal lens is positioned as shown by the arrow. This provides a clear view of the large biopsy cups (2). The port (3) provides an ingress for sterile saline to create the necessary insufflation. (B) Close-up of the large biopsy forceps in the open position. Holes at the back of the biopsy cups permit direct observation of the tissue to be sampled (arrow).

# OBSERVATIONS ON PRAZIQUANTEL CONCENTRATIONS DURING EXPERIMENTAL AND CLINICAL USE IN MARINE AQUARIA

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#### Abstract

Praziquantel baths are a routine part of marine fish quarantine; however, data to support doses, dosing intervals, or stability of the drug in marine systems are lacking. Over the past 2 yr, New England Aquarium has conducted several modest experiments to assess praziquantel potency, stability, limit of detection, and concentrations during clinical use. In addition, the effects of ozone and activated carbon on praziquantel concentrations were investigated. Results suggest that the residence of time of praziquantel in marine systems is very variable, from less than 24 hr in some systems, to over a week in other systems, even during active attempts to remove it faster.

#### Introduction

Several reports have documented effective clinical use of praziquantel baths for treatment of external trematode infections of marine fish, and many institutions use praziquantel baths as a routine part of marine fish quarantine. In a recent survey of fish quarantine practices at zoos and aquaria, 75% of institutions reported routine use of praziquantel baths, while only 3% tested praziquantel concentrations in treated water. Clinically used protocols vary widely (e.g. 20 ppm for 90 min, 2 ppm for several weeks); however, data to support specific doses, dosing intervals, or stability of the drug in marine systems are lacking. At this time, testing for praziquantel concentrations in sea water is conducted by only three laboratories in the United States, and is moderately expensive (\$30-\$150 per test). As such, monitoring therapeutic concentrations of praziquantel is not routine.

# **Methods**

Over the past 2 yr, New England Aquarium has conducted several modest experiments to assess praziquantel potency, stability, limit of detection, and concentrations during clinical use. Praziquantel assays were conducted by high performance liquid chromatography at Analytical Research Laboratories, Oklahoma City, OK 73104 USA. Praziquantel was purchased in bulk powdered form from Fishman Chemical, Hobe Sound, FL 33455 USA. Pure samples from three separate lots of praziquantel stored in three separate locations were submitted for potency testing, which was >99% for each sample. For addition of praziquantel to sea water, powder was weighed based on desired concentration and volume of water, and was distributed into the water by squeezing it through a nylon stocking until fully dissipated. Components of life support systems that may remove or denature praziquantel were not used during clinical treatments (i.e. ozone, ultraviolet sterilizers, or activated carbon), but were used experimentally and clinically to reduce concentrations when desired. All praziquantel baths were prepared using natural sea water.

#### Results

Assays of a serially diluted 6 ppm praziquantel solution in natural sea water (water temp 24°C) demonstrated an approximate limit of detection between 0.015 and 0.04ppm. Detailed accuracy tests were not conducted; however, for four systems in which concentrations were measured 60 min after dosing, concentrations were 73%, 92%, 95%, and 126% of expected values (water temperature 24-26°C). It is very possible that this represented incomplete mixing of the drug rather than test inaccuracy, but specific accuracy testing would be required to assess this possibility.

To assess stability under the influence of ozone and activated carbon, concentrations were monitored in an experimental 3800 gallon system with no animals (water temperature 24-25°C), with a starting concentration of 3.5ppm. Concentrations were reduced to 65% (24h), 27% (72h), 10% (120h), 4.5% (7d), and 2.5% (8d) after starting ozone and carbon.

During routine quarantine treatments, with initial concentrations of 5 ppm or 10 ppm, four systems were surprisingly found to have undetectable praziquantel concentrations within 24 to 96 hr after dosing (water temperatures 24-26°C).

Praziquantel was used clinically for treatment of *Benedeniella posterocolpa* on a group of newly acquired cownose rays (*Rhinoptera bonasus*). Rays were housed in a 30,000 gallon closed system (water temperatures 23-24°C) which was treated on Day 1 at a theoretical praziquantel concentration of 3.5 ppm, with desire to maintain this concentration for 1 mo. Supplemental redoses of 50% were applied on Day 5 and Day 16, and a 100% re-dose was applied on day 22 based on measured concentrations. Measured concentrations were 2.75 ppm (Day 2), 2.55 ppm (Day 4), 4.0 ppm (Day 7), 3.38 ppm (Day 11), 2.44 ppm (Day 14), 1.72 ppm (Day 18), 0.94 ppm (Day 21), and 1.03 ppm (Day 25). Treatment was effective in eradicating the parasites based on examinations over the subsequent 20 mo. For compliance with regulations for the discharge of the treated water, ozone and carbon was applied to the system on Day 29. Concentration on Day 32 was 0.045 ppm, and on Day 40 was undetectable.

# Discussion

Collectively, these preliminary observations suggest that the residence of time of praziquantel in marine systems is highly variable, from less than 24 hr in some systems, to over a week in other systems, even during active attempts to remove it faster. Based on these findings, clinicians should consider monitoring therapeutic praziquantel concentrations during treatment. Increasingly strict regulations for discharge water may also increase the need for such testing. Test accuracy, stability during shipping, and variables that influence the persistence or elimination of praziquantel in marine systems are worthy of further study.

#### **ACKNOWLEDGMENTS**

The author thanks the many members of the Fishes Department and Animal Health Department that contributed to the execution of these experiments and treatments.

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DOES ORALLY ADMINISTERED DOXYCYCLINE ACHIEVE ADEQUATE CONCENTRATION IN THE PLASMA AND TEARS OF ELEPHANT SEALS (Mirounga angustirostris)?

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#### **Abstract**

Keratitis, a common, painful, and potentially blinding disease of pinnipeds frequently involves bacterial infection as either a primary or secondary factor. Topical antimicrobial treatment is rarely an option due to animal lifestyle and temperament. This project assessed plasma and tear doxycycline concentrations following oral doxycycline (Doxycycline Hyclate, Medisca Pharmaceuticals, Las Vegas, NV 89119 USA) administration to elephant seals. The study involved eighteen juvenile elephant seals without ocular disease who were housed at The Marine Mammal Center. Doxycycline (10 or 20 mg/kg) was administered orally every 24 hr for 4 days. Tear and plasma samples were collected at fixed times, and doxycycline concentration assessed using liquid chromatography/mass spectrometry. Concentration-time data were Following administration of 10 mg/kg/day calculated using noncompartmental analysis. doxycycline, maximum plasma doxycycline concentration (C<sub>max</sub>) on Day 4 was 1.5 µg/mL at 4.0 hr. Administration of 20 mg/kg/day doxycycline produced C<sub>max</sub> on Day 4 of 1.9 µg/mL at 5.8 hr. Doxycycline elimination half-life on Day 4 in animals receiving 10 or 20 mg/kg/day doxycycline was 6.7 or 5.6 hr, respectively. Plasma:tear doxycycline concentrations averaged over all days were not significantly different between the low-dose (9.85) and high-dose (9.83) groups (P = 0.99). Doxycycline was detectable in tears for at least 6 days following cessation of oral dosing. Doxycycline administered orally to elephant seals at these doses achieved concentrations in tears and plasma likely to have some antimicrobial and anti-inflammatory effects at the ocular surface and systemically and should be considered for treatment of corneal disease in this and possibly other similar species.

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RESPONSE TO HUMAN RECOMBINANT GRANULOCYTE COLONY-STIMULATING FACTOR (FILGRASTIM; NEUPOGEN®) IN NEUTROPENIC CETACEANS

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# **Abstract**

Neutrophils are one of the initial lines of protection against pathogens. concentrations in the blood decrease markedly, animals become highly susceptible to infections.<sup>10</sup> Neutropenia is caused by increased demand, increased consumption, decreased production, or destruction of neutrophils or their precursors. In domestic animals, causes of neutropenia include infectious diseases such as viral and rickettsial infections, bacterial pneumonia or sepsis, drug reactions, primary bone marrow disease, and immune-mediated disease.<sup>1,9</sup> Neutropenia has been reported in cetaceans secondary to systemic sulfa<sup>2,6</sup> and ketoconazole use<sup>4</sup> and chronic, severe infection.<sup>8</sup> Filgrastim (Neupogen®, Amgen Manufacturing, Limited, Thousand Oaks, CA 91320 USA), is a human recombinant granulocyte colony-stimulating factor that is effective in increasing peripheral neutrophil counts in a number of species.<sup>3,5,7</sup> We report the use of filgrastim to treat neutropenia in three cetacean species [killer whale (Orcinus orca; n=6), bottlenose dolphin (Tursiops truncatus; n=4), and beluga whale (Delphinapterus leucas; n=1)] ranging in age from 1 week to greater than 24 yr. In most cases the cause of neutropenia was undetermined (n=8). Bacterial septicemia (n=1) and drug reaction to systemic sulfa (n=2) were identified causes. In all but two instances, neutrophil counts increased within 24-48 hr of one dose of filgrastim (1-7 µg/kg). In the majority of cases the response was characterized by an initial rise in band neutrophils followed by an increase in mature neutrophils. The number of doses of filgrastim administered, intensity of monitoring, and degree and duration of response varied among cases. No adverse reactions were seen.

# ACKNOWLEDGMENTS

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RETROSPECTIVE REVIEW OF MORBIDITY AND MORTALITY IN GIANT PACIFIC OCTOPUS (Enteroctopus dofleini) AT THE NATIONAL AQUARIUM FROM 2004 – 2012

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### Abstract

The giant Pacific octopus (*Enteroctopus dofleini*) is a popular exhibit species in many public display aquaria, though information on veterinary care is limited. A retrospective review of electronic records (Tracks©) was conducted looking specifically at time in collection, antemortem clinical signs, and post-mortem histopathology. Between March 1, 2004 and March 4, 2012 the National Aquarium housed 18 giant Pacific octopuses, 16 of which died during the review period.

There were 7 males, 8 females and 1 animal whose sex was not noted. Average time in captivity for all animals was  $350 \pm 174$  days (male:  $312\pm108$  days, females:  $399\pm220$  days). The giant Pacific octopus is semelparous – males and females die after gamete release. Nine (56%) of the animals in this review were sexually mature at the time of death, confirmed either by histopathology or observation of gamete release.

Common ante-mortem clinical signs included anorexia, behavior changes (i.e., decreased interaction with staff, lethargy, and color changes), skin lesions, ocular changes, and self-mutilation. Histopathologic diagnoses included infectious/inflammatory processes affecting multiple organ systems including gastrointestinal, cardiovascular, respiratory, ophthalmic, renal, integumentary, and reproductive. Integumentary lesions of the mantle and arms included focal ulcerations, cellulitis, lacerations and necrosis. A number of parasitic organisms were noted including *Ichythyobodo* in the gills, amoeba in multiple organs, dicyemids in renal tissues, ocular nematodes, protozoa in the digestive gland and helminth like bacteria in the renal tissues. This information will be useful for in refining captive management of the species.

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# OBJECTIVE AND BEHAVIORAL RESULTS FOLLOWING CATARACT REMOVAL IN 80 PINNIPEDS

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# Abstract

Lens diseases are common in pinnipeds, affecting half of all pinnipeds under human care. Postoperative results and behavioral changes were evaluated following cataract removal. Eightyone pinnipeds (n=148 eyes) underwent unilateral (n=12) or bilateral (n=69) lensectomy between 2003 and April 2012. Questionnaires evaluating behavioral changes were sent to 12 California sea lion trainers. All but one animal was under human care; there were 38 females, 43 males, average age was 20.3 yr (range 7 mo to 35 yr); 45 Zalophus californianus, 16 Phoca vitulina, 1 Arctocephalus townsendi, 1 Mirounga angustirostri, 2 Arctocephalus pusillus pusillus, and 7 Neophoca cinerea, 4 Arctocephalus australis, 3 Arctocephalus forsteri, 1 Halichoerus grypus. Eyes with pre-existing anterior lens luxations (n=41) had persistent corneal fibrosis (otariids) or corneal edema (phocids). Hyphema developed intraoperatively (n=4) or post-operatively (n=2). Post-operative complications include infected corneal ulcers or chronic corneal opacities OU (n=3 animals due to water quality imbalances), endophthalmitis and retinal detachments (n=1 animal), intermittent corneal stromal abscesses (n=3 eyes). Vision improved in all but six eyes; pain was resolved in all but two eyes of two animals. Behavioral changes from 12 California sea lions included increased self-confidence and motivation to train, and a stronger relationship with trainers. All animals went from using tactile, verbal and/or auditory cues to visual cues alone, indicating improved sight. Overall, lensectomies in pinnipeds have proven successful in terms of sight, improved behavior and motivation to train, and stronger human-animal-bond.

# UTERINE AND OVARIAN DISEASE IN SINGLE GENDER HOUSED SOUTHERN STINGRAYS (Dasyatis americana)

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#### Abstract

Female southern stingrays (Dasyatis americana) housed in single gender groups have presented with reproductive disorders in a number of aquaria. Two of nine adult, female Southern stingrays, housed in one large mixed species aquarium presented with decreased appetite and a prominent bulge over their caudal dorsal surfaces. Physical examination, blood collection, endoscopy and ultrasound were performed on the animals. Ultrasound showed a severe accumulation of hypoechoic uterine and markedly enlarged ovaries with mixed size hypo- and hyperechoic structures. Endoscopy and fluid analysis of the uterine fluid in affected animals confirmed an overabundance of histotroph. Serum analysis revealed estrogen concentrations that were markedly higher in affected females. Examination of the remaining females revealed three additional affected animals. Necropsy results in the initial females corroborated ultrasound findings and showed hemorrhagic and necrotic ovarian tissue with cystic follicles and multiple retained masses filled with yolk material that was both inspissated and fluid. Ultrasound criteria were developed to differentiate between normal and abnormal female stingrays and hormone levels were obtained in both healthy and affected female stingrays. Initial data describes a progressive reproductive disorder, possibly linked to chronically elevated estradiol as a function of being maintained in an all female group. The repeated production but retention of follicles appears to result in an abnormally large ovary, which together with an over exuberant production of histotroph results in a large fluid filled uterus and a domed back. Further investigation into this disease process is ongoing together with the development of treatment strategies.

#### **ACKNOWLEDGMENTS**

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# BOTTLENOSE DOLPHIN ADENOVIRUS 1 AND CALIFORNIA SEA LION ADENOVIRUS 1: GENOME CHARACTERIZATION AND DEVELOPMENT OF qPCR TESTING

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#### Abstract

Adenoviruses are non-enveloped, double stranded DNA viruses with a medium sized genome of 26-45kbp. Adenoviruses are generally host specific, with many studies showing host-pathogen codivergence. The family Adenoviridae is widely distributed among vertebrates, and there are five recognized genera of adenoviruses: *Mastadenovirus*, *Aviadenovirus*, *Atadenovirus*, *Siadenovirus* and *Ichtadenovirus*. Of these, *Mastadenovirus* and *Atadenovirus* are known to infect mammals. Members of the genus *Mastadenovirus* are only found in mammals and are likely to originate in that group. In cetaceans, although there is evidence of adenoviruses in beluga, bowhead, and sei whales, no characterization has been done. In California sea lions (*Zalophus californianus*), adenovirus was first associated with hepatitis in 1979 and initial sequence characterization of a partial mastadenoviral polymerase gene has recently been reported. Genomic characterization of California sea lion adenovirus 1 and Bottlenose dolphin adenovirus 1 reveal that they are mastadenoviruses that cluster with viruses from other laurasiatherian hosts. Further, we present development of quantitative PCR assays to be used for surveillance and epidemiologic studies of these viruses, enabling rapid diagnosis. Ecology and evolution of these viruses will be discussed.

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# TREATMENT OF CUTICULAR MYCOSIS IN WILD-CAUGHT COMMON SEA FANS (Gorgonia ventalina)

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#### Abstract

Gorgonia ventalina, the common sea fan, is a protected coral species found throughout the Caribbean. Mass mortalities associated with infection from Aspergillus spp. have been documented in wild G. ventalina. Approximately 2 mo after entering captivity from a multispecies coral rescue, 23 G. ventalina colonies began experiencing clinical signs consistent with fungal infection including: purpling of tissue, gall formation, and focal death of skeleton. Cytology, biopsy, and culture confirmed multifocal cuticular mycosis with isolation of Aspergillus sydowii. Previously reported treatment of this disease has centered on surgical excision of diseased colonies, which can result in significant loss of tissue. In an effort to retain coral shape and size we divided the coral colonies into four medical treatment groups: daily itraconazole bath, every other day itraconazole bath, topical daily clotrimazole, and topical clotrimazole every other day. Best results were observed with the daily itraconazole bath, although significant improvement was observed in all four groups. Treatment duration ranged from 14 to 138 days and rate of regression of lesions was variable from colony to colony. Based on this treatment study our recommendation for medical treatment of sea fans with A. sydowii is daily itraconazole baths.

#### **ACKNOWLEDGMENTS**

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# ANALGESIA IN ZOO AND WILDLIFE ANIMALS: TRANSLATING AND CREATING EVIDENCE

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#### Abstract

Analgesic techniques in small animal pets is challenging because of the inability to communicate with the patient. While much progress has been made regarding research techniques to investigate the safety and efficacy of various drugs, these studies still have deficiencies. Three common problems are 1) the subjective outcome measures that many studies depend upon, 2) the results of studies generally address the average patient, not the individual patient, and 3) studies rarely address the caregiver placebo effect. Another setback in veterinary medicine is that many drugs used for people are directly translated to use in animals without proper investigation of the pharmacokinetics and pharmacodynamics of the drug in the proper species and even if these data are available they are commonly ignored. These problems are magnified when our profession addresses analgesia in zoo and wildlife animals.

Since it is unlikely that detailed analgesic drug pharmacokinetic and pharmacodynamic (PK / PD) research and field investigations will be available for most zoo and wildlife animals, evidenced based medicine will be caregivers translating available knowledge from similar species and combining that with their shared experiences. While this is not ideal, this is a common theme in veterinary medicine. For example, in small animal surgery a new treatment may become available. No published data exists, there is no oversight of the implants used, there is only how it might work because of how it worked in people and shared opinion. When faced with this situation I take the conservative path. The potential benefits must heavily outweigh the potential risks and the investment from the owner must not be greater than an available treatment that has greater evidentiary value.

For species where an untested drug for analgesic purposes is proposed one can translate some evidence better than other. Sodium potassium channels work similarly in nearly all species. Bupivacaine blocks the influx of sodium into nerve cells that prevents depolarization. I think this is an example of a drug where the outcome of treatment can be accurately predicted. Oral tramadol has been shown to have wildly variable metabolism in different mammals. This is a drug where the outcome of treatment can be accurately predicted as wildly variable.

Developing and sharing a clinical opinion about the safety and efficacy of an analgesic must be done with caution. Some perspective on the accuracy of our opinions in this area can be gained from a review of the caregiver placebo effect. The caregiver placebo effect is a bit different to the widely accepted placebo effect. This is because when a placebo effect occurs the patient actually feels better. When a caregiver placebo effect occurs, the patient feels nothing (they may not even know they have received a treatment) but the caregiver feels the patient is better. This is an enormous source of bias when a caregiver measures outcomes, and it has been consistently

demonstrated in human medicine when parents assess the effect of medication on their children. More recently in a study of dogs that had lameness secondary to osteoarthritis the care giver placebo effect for pet owners and veterinarians was measured. The result, not surprisingly, were similar to findings of caregivers of human patients. Pet owners had a caregiver placebo effect of nearly 60% and veterinarians (veterinary surgeons) of 50%. From this study what this means is that the patient was treated with a placebo for its lameness and did not improve (limb function measured by computational gait analysis in a FDA-GCP study) but the caregiver thought it was better. The effect was also shown when the patient actually worsened but the caregiver thought the patient was better or unchanged. There are many reasons why a caregiver placebo effect occurs; we want our patients to improve and believe that our treatments should work. This data convinced me that when treating an individual patient I must accept that any change I think I see could be from just my belief or from chance. The caregiver placebo effect can be overcome even within an individual patient and even within zoo and wildlife medicine. Implementing a single case design study can do this. I think this would be especially helpful if treating a patient for a long-term condition. It works by choosing an outcome measure(s) and documenting that outcome for a period of time (e.g. 2 weeks). Then the intervention (e.g. medication) is given for the same period of time and the outcome is measured. This is then repeated for another cycle. After 8 weeks the data can be reviewed and one can scientifically evaluate how the intervention affected an individual patient.

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### DIAGNOSTIC IMAGING IN ACUTE GASTROINTESTINAL DISEASE

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#### Abstract

When presented with acute gastrointestinal disease, one of the main clinical questions is whether or not surgical intervention is warranted. Diagnosis of gastrointestinal disease in zoo and wildlife species has the added challenge of species-specific anatomic variations. However valuable clinical information can be obtained through the appropriate application of diagnostic imaging modalities used in a step-wise approach. Beyond radiography and ultrasound that are often available in house, advanced imaging modalities may be very useful in individual cases. An understanding of the basic physics behind each modality and the associated limitations, including size limitations, allows appropriate study selection. Developing an in-house imaging library is very valuable for comparison, but even when not available, the application of some basic rules can help determine whether or not surgical intervention is warranted. Identifying markedly different intestinal diameters suggesting complete obstruction or free gas suggestive of perforation on radiographs warrants surgical intervention although ultrasound may provide additional information via a non-invasive route prior to a surgical procedure, particularly in geriatric animals where neoplasia may be a concern. In the case of partial obstructions due to foreign material ingestion, these may be successfully monitored during transit using an appropriate imaging technique thus avoiding unnecessary invasive procedures in some cases. Gastrointestinal neoplasia, if treated, will often require serial studies and appropriate modality selection for comparison over time is needed. In addition to modality specific limitations and applications, repeatability, transportation, cost and time needed under anesthesia are all factors that need consideration on a case-by-case basis.

2012 PROCEEDINGS AAZV CONFERENCE 151

### CHOPSTICKS OR FORKS? HOW TO CHOOSE YOUR SURGICAL WEAPONS

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### Abstract

Ninety years after his death, Halsted's Principles remain one of the most important creeds for the current day surgeon. But his instructions are sometimes easier to remember than to follow, especially when dealing with very small or fragile patients. And what does it really mean to minimize tissue trauma? How, exactly, does a surgeon handle tissues "gently" when surgery is intrinsically traumatic? This presentation will outline some important principles of surgery that have a real impact on outcome, and provide practical hints as to how to better follow Halsted's principles in your exotic patients.

# Hasted's Principles:

- Strict asepsis during preparation and surgery.
- Good hemostasis to improve conditions for the procedure and limit infection and dead space.
- Minimize tissue trauma.
- Use good surgical judgement ensuring elimination of dead space and adequate removal of material
- Minimize surgery time through knowledge of anatomy and technique.
- Correct use of instruments and materials used.

# Care and Handling of Tissue

Primum non nocere (above all else, do no harm). Minimizing tissue trauma through gentle tissue handling should always be a primary goal. The tissues are best cared for when we do the following:

- avoid excessive blunt dissection
- avoid excessive traction
- handle tissues only when absolutely necessary
- separate only those tissue planes necessary for visualization or excision
- avoid repeated changes in retractor position
- do not allow retractors to tear or stretch tissue excessively
- keep the tissues moist with regular application of saline
- avoid exposure to irritant or inflammatory substances like talc, lint, urine, bile or intestinal contents
- use appropriate instruments

#### Hemostasis

Although the consequences of profuse hemorrhage are understandably feared by surgeons, and discussed with clients, effective hemostasis is taken for granted during most surgical procedures. In very small patients, even a trivial-appearing amount of hemorrhage can represent a relatively large volume of blood loss and it clearly better avoided than controlled! Many small blood vessels are cut during even routine surgical procedures and a minority require intervention. Vessel retraction, platelet plugging and coagulation usually occur promptly and therefore it is usually only the larger, visible vessels that require ligation. However, there are numerous potential sources of blood loss if coagulation is impaired for some reason. Ongoing bleeding in a patient with a coagulopathy may not just occur from obvious surgical sites, but in the form of a slow ooze from all damaged surfaces, including those that have simply been handled during the procedure. Hemorrhage is detrimental for a number of reasons:

It may lead to hypovolemia and obscures the surgical field, increasing the risk of damage to local structures. Repeated attempts at clamp placement, ligation, cautery or just swabbing the tissues leads to additional trauma. Ongoing hemorrhage slows the surgery and increases operating time, thereby increasing tissue trauma and bacterial contamination. To maximize effectiveness, reduce the risk of tissue damage, and facilitate natural clotting, hemostasis should be attempted in the following sequence:

- 1. Digital pressure. This stems the flow while enough platelets accumulate to form a plug, or a stable clot forms. Pressure should be applied for at least 60 seconds in cases of minor hemorrhage and up to 5 min for more serious hemorrhage. Avoid dislodging the developing clots when swabbing the area. Digital pressure may not be indicated in a patient with a very small circulating blood volume, in which case you should go straight to step 2.
- 2. If simple digital pressure is ineffective, carefully apply a hemostat, using the tip of the instrument. The hemostats are left in position for at least 5 min, at which stage they may be released, or cautery applied, or a the vessel ligated.
- 3. If the bleeding point is deep within the tissues, within a body cavity, or in close proximity to a structure that might be damaged by hemostats (such as the facial nerve during total ear canal ablation) or the ureter during ovariohysterectomy, further pressure may be applied by packing the cavity tightly with surgical sponges. Sponges are packed on top of one another and held in position until blood stops oozing through the fabric. The packing is left in place for at least 5 to 10 min. It is helpful to use a clock or stopwatch, as time passes slowly under these circumstances! Always perform a sponge count to ensure that sponges are not retained.
- 4. For a final check of a wound or body cavity, flood the area with sterile saline. Hemorrhage appears as a tendril of blood rising like chimney smoke from the bleeding point, allowing careful application of thumb forceps or a hemostat. Pressure and other physical effects of the saline such as cold temperature will also sometimes stop the bleeding.

## **Surgical Instruments**

The instruments available to the surgeon have been developed for very specific scenarios and the chances are that they were not designed with reptiles and birds in mind! Most surgeons use a small number of key instruments during the course of every day practice and there are some instruments that are appropriate regardless of which species you use. Gentle tissue handling is facilitated by choosing the appropriate instrument for each task, reducing the number of times the tissues are grasped, released and grasped again, and ensuring the instruments are sharp, the locking devices reliable, and the jaws close smoothly and effectively. Good retraction and good lighting is essential to minimize tissue trauma. Stay sutures should be used in situations where hand-held or self-retaining retractors are not appropriate. In some instances, the tissues are too fragile to use surgical instruments (e.g., mediastinum, bladder wall).

#### Sterile Saline

Saline-soaked swabs and sponges are used to keep tissues moist, protect them from retractor blades, absorb blood and body fluids, swab the wound to keep it clear of blood while the surgeon is working and for packing when hemostasis is required. The surgeon or their assistant should always keep count of the number of swabs opened and make sure the count matches before the surgical wound is closed as retained swabs have been reported in many locations, including the thoracic and abdominal cavities, lumen of the stomach, airway and soft tissues following fracture repair or major soft tissue reconstruction.

Wound lavage should be used after lengthy procedures or those in which contamination is known to be present. Vigorous lavage using warm, sterile saline dislodges bacteria, lint from surgical sponges, tale from surgical gloves, blood clots, intestinal contents, urine and other foreign or irritant material. Repeated flooding of the site with saline, followed by suction, can be used to confirm whether hemorrhage is still occurring or there is ongoing air leakage following a lung lobectomy or biopsy, or biliary tract surgery. Lavage is most beneficial when appropriate volumes of saline are used (dilution effect), with physical dislodgment of debris by pulsatile application of hydrostatic pressure.

### **Surgical Suction**

Some form of surgical suction is essential for many of the procedures we perform, in order to remove contamination and tumor cells, clear the surgical site of blood to improve visualization, allows retrieval of saline used to flush away blood and debris, moisten tissues and identify bleeding points, and permits suctioning of aerosolized gases liberated during electrocautery. Gentle application of a fine suction tip is a very effective way to separate organs, break down adhesions and establish tissue planes.

### Retraction

There are many different types of retractor available, but few are delicate enough for very small patients. Judicious use of stay sutures can be a lot less traumatic than having your assistant tugging on a Senn retractor, and indeed can take the place of an assistant. The Lone Star retractor

(Lone Star retractor, Lone Star Medical Products, Stafford, TX) is very useful, eliminates the need for an assistant to hold retractors in many instances, and can exert constant force on tissues. Moistened sterile q-tips, used in the fashion of chopsticks, or in place of grasping instruments, are a great way to move tissues around without damaging them and have the added advantage of absorbing blood and fluid, thereby improving visualization

# The Surgical Assistant

Effective utilization of a surgical assistant does, however, contribute greatly to a successful outcome. The role of the assistant includes managing the surgical table, assisting with surgical retraction, ensuring diagnostic samples are not lost and keeping count of surgical sponges. Effective engagement of the surgical assistant ensures the surgery proceeds efficiently and with minimal interruptions.

# Surgical Lighting and Magnification

You cannot perform surgery safely if you cannot see! Ideally, an operating room should be equipped with at least two, ceiling-mounted lights capable of being focussed on the surgical site. The lights should not emit too much heat, should not cast shadows and should allow you to accurately interpret colors. You should be able to either apply sterilized light handle covers, or sterilize the handles themselves so as to control the light at your convenience. The two lights should have articulated attachments that allow them to be directed towards the patient at virtually any angle. They should be capable of moving independently. One light is used as a "primary" light, usually centered above the surgeon, and the other is a "secondary" light that is directed in at an angle and often moved during the course of the surgery. The surgeon or their assistant should take note of changes in lighting during the procedure and ensure the lights are positioned so as to avoid them being obscured by the surgeon or assistant. This is especially important when working in body cavities. Some time should be taken at each stage of the surgical procedure to ensure that the lighting is optimal. Poor visualization during surgery is often the result of poor light position.

Intraoperative illumination may also be achieved with surgical headlights. These are mandatory for microsurgery and delicate procedures in very restricted surgical fields. Likewise, fine detail work in tiny patients will be greatly facilitated by some form of magnification, even if you have great vision. Operating loupes, and minimally invasive cameras can provide this magnification. Loupes are great as long as you have them set up specifically for your eyes and use them regularly. If you only dust them off once every 12 mo then they will be uncomfortable, hard to focus through and feel like they are in the way. They need to become second nature in order to help rather than hinder you, but you and your patients will appreciate the results.

# THE ASSOCIATION OF ZOOS AND AQUARIUMS AND PFIZER ANIMAL HEALTH GROUP DONATION PROGRAM

Thomas W. deMaar, DVM

### Gladys Porter Zoo, Brownsville, TX 78520 USA

The Association of Zoos and Aquarium's (AZA) pharmaceutical donation program with Pfizer Animal Health continues to bring beneficial support to member's animal health program needs. Now in its third year, the Pfizer program has contributed over \$400,000 of free pharmaceuticals to AZA facilities. The donations are sourced by Pfizer from returned product, damaged packaging and short expiration date (but not less than 6 mo).

All U.S. based, AZA accredited, nonprofit zoos, aquariums and related facilities that retain a licensed veterinarian are eligible to participate. Initial registration is done through the AZA website (using the institution's log in); registrants provide a veterinarian contact with address, email and phone; and a Fed Ex or UPS shipping account number.

Pfizer Animal Health provides a new inventory of pharmaceutical products, approximately every quarter, available at no charge to participants. The list is edited to remove products not commonly used in zoo medicine, large-volume items, counter displays, products with shipping restrictions and is subsequently announced to registered participants. Participants have two weeks to request product via an AZA web portal that automatically monitors inventory. The assembled request is forwarded to Pfizer, which, in turn, ships the available product to the Gladys Porter Zoo (GPZ). Once product is inventoried at GPZ, each recipient makes a \$40.00 payment at AZA's website (\$25.00 to AZA for maintenance of the computer portal and \$15 to GPZ for handling time and supplies). GPZ ships the boxes to the grateful recipient after payment is received.

Product availability varies due to changes in the warehouse between the time the initial list was compiled and when shipment takes place. In addition, Pfizer may use the same pool of product for contributions to other organization and disaster relief response. During redistribution the staff at Gladys Porter Zoo utilizes two guiding principles: a) "first come, first serve" but also b) "spread the wealth". In this way, if an early requestor has asked for a large share of what is available then some product may be redistributed to a later requester, based on need.

In 2012 so far AZA participants shared product from an inventory valued at \$120,000. Pfizer Animal Health is committed to the veterinary profession, education, innovation, and philanthropy. These product donations allow us to give our zoo residents an enhanced level of care and put our resources to their most efficient use. Is your zoo or aquarium getting its share? To register, go to www.aza.org/pfizer

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# AZA AND AVMA: PARTNERSHIPS WITH THE BIG ORGANIZATIONS IN THE ZOO AND VET FIELDS

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#### Abstract

During its 52-yr history the American Association of Zoo Veterinarians (AAZV) has developed collaborations with a number of different veterinary organizations including the Association of Reptile and Amphibian Veterinarians (ARAV), the Association of Exotic Mammal Veterinarians (AEMV) and the European Association of Zoo and Wildlife Veterinarians (EAZWV). Common interests with the American Association of Wildlife Veterinarians (AAWV) led to the development of a formal Memorandum of Agreement between the two organizations to promote shared goals of the organizations. The majority of the over 900 members of the AAZV are employed as zoo veterinarians, and as such also have an interest in the two largest organizations representing the zoo and veterinary communities. The Association of Zoos and Aquariums (AZA) represents over 6,000 members from 224 accredited zoos and aquariums in North America. The American Veterinary Medical Association (AVMA) represents over 82,000 member veterinarians primarily in the United States and Canada. These two organizations present a number of opportunities for the AAZV to advance its strategic vision as a "leading resource offering expertise in health and welfare of wildlife" and "an influential contributor to the development of policies that affect the health, welfare and conservation of wildlife"

The AZA's Animal Welfare Committee has veterinary membership as a permanent part of its structure. The Animal Health Committee is the official liaison with the AAZV. The Accreditation Commission ensures that member zoos and aquariums meet AZA standards though a process of inspection and approval of AZA member institutions. The Accreditation Standards state that members "should adopt ....the Guidelines for Zoo and Aquarium Veterinary Medical Programs and Veterinary Hospitals developed by the American Association of Zoo Veterinarians (AAZV)"<sup>2</sup> The development of these standards by AAZV provides a powerful tool for influencing the practice of zoological medicine. The AVMA has five committees that include representatives from the fields of zoo, wildlife or aquatic medicine including the Animal Welfare Committee, the Clinical Practitioners Advisory Committee and the Committee on Environmental Issues. These committees provide the expert evaluation of issues for the policy makers in the AVMA. One of these policy makers is the House of Delegates and the AAZV has had a representative on the Advisory Panel to the House of Delegates since 2009. There are some challenges in coordinating among organizations with somewhat different visions and constituencies. However, given the high profile and much-regulated nature of the practice of zoological medicine it is important that we are represented in the process of developing policies and standards

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# REPORT ON A MULTI-STAKEHOLDER EXERCISE FOR AVIAN INFLUENZA PREPAREDNESS AND RESPONSE

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#### Abstract

Zoo veterinarians and the United States Department of Agriculture (USDA) have spent considerable time and resources in preparing the zoological community for Highly Pathogenic Avian Influenza (HPAI), but the opportunities for the community to evaluate that preparedness have been limited. This presentation will report on the outcomes of a preparedness exercise for sixteen Midwestern zoos called "Flu at the Zoo". Funded by USDA Animal Care Emergency Programs and facilitated through the University Of Illinois College Of Veterinary Medicine, the goals of the exercise were to enhance preparedness and communication among zoological personnel in Illinois, Indiana and Missouri in response to a simulated outbreak of HPAI in their facilities. This exercise also allowed evaluation and updating of the USDA/Association of Zoos and Aquariums (AZA) HPAI Outbreak Management Plan. This Plan was designed to be used as a guidance document for regulatory agencies when dealing with HPAI in a zoological facility.

Developed using Homeland Security Exercise and Evaluation Program (HSEEP) guidelines, the exercise brought together zoological personnel with USDA (Animal Care, Veterinary Services, Wildlife Services), State Animal Health officials, Public Health, academics and other stakeholders. HSEEP exercise structure was chosen as it promotes a standardized set of measures for exercise evaluation.

This presentation will discuss and evaluate the exercise structure and highlight lessons learned. While the scenario was developed to examine HPAI preparedness and response for the managed wildlife community, this exercise fulfilled the all-hazards approach to response to any infectious disease outbreak involving animals and/or humans associated with a zoological facility.

#### **ACKNOWLEDGMENTS**

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#### USDA APHIS ANIMAL CARE: ACTIVITIES AND OPPORTUNITIES

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### Abstract

USDA Animal Plant Health Inspection Service (APHIS) Animal Care is the division of USDA that provides leadership for determining standards of humane care and treatment of animals. APHIS achieves compliance through inspection, education, cooperative efforts and enforcement. For more than 40 yr, Congress has entrusted APHIS with the stewardship of animals covered under the Animal Welfare and Horse Protection Acts. APHIS/Animal Care, continues to uphold that trust, giving protection to millions of animals each year, nationwide.

The Animal Welfare Act (AWA) requires that minimum standards of care and treatment are provided to certain mammals bred for commercial sale, used in research, transported commercially, or exhibited to the public. APHIS' Animal Care program enforces the AWA primarily through inspections of regulated facilities. To ensure that compliance with the AWA is continually maintained, all facilities that keep animals regulated under the Act must be licensed or registered with APHIS. APHIS officials—veterinarians or qualified animal care inspectors employed by APHIS and trained to identify potential violations of the AWA and its regulations—conduct unannounced inspections of every licensed or registered facility in the country. APHIS inspectors receive special training in the proper care of marine mammals, exotic animals, and animals used in research. Inspectors also receive extensive training in how to conduct inspections at airport terminals, zoos, and commercial animal breeding facilities, among others.

Animal Care inspectors may either be veterinarians or individuals with considerable experience working with and caring for animals. All inspectors receive extensive training once they are hired into Animal Care. They are responsible for doing unannounced site visits of facilities that are regulated by the Animal Welfare Act (AWA). They also may be involved with the confiscation of animals that are suffering or that are housed in dangerous or unhealthy situations. They occasionally are called on to assist in emergency situations that affect animals such as fires or weather-related disasters.

The APHIS Center for Animal Welfare (CAW) is a newer division of Animal Care, and is physically located in Kansas City, MO. The CAW was established in 2010 to coordinate training and education/outreach, conduct long-term policy analysis and maintain program currency with the advancing science of animal welfare. The Elephant, Big Cat, Primate, Kennel and Training Specialists, along with a biophysicist, all work as a part of the CAW team. The Specialists work to support the inspectors and promote education and training. AC also has an Avian Specialist located at Animal Care Headquarters in Riverdale, MD. There are two regional Emergency Response Specialists, who coordinate Animal Care's assignment from the Department of Homeland Security to assist states in their efforts to include pets as a part of their emergency plans.

The most common job opportunities within Animal Care are inspector positions, followed by Supervisor and Specialist positions. There are a number of student internship and externship positions offered each year. For more information about student externships with the Animal Care program, go to this link:

 $http://www.aphis.usda.gov/animal\_welfare/downloads/meetings/Animal\% 20 Care\% 20 Externship\% 20 Programv 4.pdf$ 

For more information regarding APHIS Student internships, follow this link:

http://www.aphis.usda.gov/audience/students.shtml

# THE USGS NATIONAL WILDLIFE HEALTH CENTER: PAST, PRESENT AND FUTURE

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#### **Abstract**

The National Wildlife Health Center (NWHC) is a science center of the Department of Interior's U.S. Geological Survey, and was established in 1975 in Madison, Wisconsin, USA. The current mission is to provide national leadership to safeguard wildlife and ecosystem health through dynamic partnerships and exceptional science. The NWHC fulfills its mission by conducting an integrated program of research, diagnostics, epidemiologic surveillance, technical assistance, training, and information management and communication on wildlife disease and health issues to wildlife and natural resource managers, decision- and policy-makers, other scientists, and the public. The NWHC is certified by the Centers for Disease Control and Prevention and the U.S. Department of Agriculture to work with disease agents at Biological Safety Level 3. The NWHC also operates the Honolulu Field Station (HFS), located in Honolulu, Hawaii, that carries out the mission of the NWHC and serves the State of Hawaii and U.S. Territories and Freely-Associated States in the Pacific region. Current research focuses on diseases such as white nose syndrome, avian influenza, sylvatic plague, chronic wasting disease, West Nile virus, amphibian diseases, coral and sea turtle diseases, among other wildlife health issues. The NWHC has embarked upon an ambitious new strategic plan with a focus on three goals:

- Serving as a catalyst to establish a collaborative North American Wildlife Health Strategy
  that creates an operational framework to address the most pressing wildlife health issues.
  This Strategy will emphasize the importance of a collaborative approach to mitigate the
  impact of wildlife diseases and other stressors on wildlife, domestic animal, and human
  health.
- 2) Providing nationally comprehensive wildlife health information based on collective knowledge and making this information available to a broad audience of professionals, general public, media and decision makers.
- 3) Conducting exceptional science to anticipate, detect and assess wildlife diseases, and support the management of wildlife and ecosystem health.

# VETERINARIAN'S ROLES IN DISASTER RESPONSE AT THE LOCAL, STATE AND NATIONAL LEVELS

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#### Abstract

Veterinarians have essential roles in disaster preparedness and response and can serve our communities at the local, state, and national levels. Zoo and wildlife veterinarians need to be involved in disaster response planning for their facilities to ensure the safety of the animals and the local community. This lecture will review the various opportunities and important skills needed for veterinarians to participate in disaster preparedness and response. The presenter has over 10 yr of experience in veterinary disaster response as a Veterinary Medical Assistance Team (VMAT) and National Veterinary Response Team (NVRT) team commander, State Animal Response Team (SART) member, and in working with National Alliance of State Animal and Agricultural Emergency Programs (NASAAEP).

#### THE SCIENCE OF MEASURING PERIOPERATIVE PAIN

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#### Abstract

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. If we accept that, like ourselves, our patients experience pain to some degree, then we can move forward with the statement that one goal of veterinarians is to manage pain. However, pain management has consistently been a problem in our profession. In 1993, it was reported that after major surgery in a large referral hospital in the USA less than 7% of cats received any postoperative analgesia and only 19% of dogs received analgesia for more than 8 hr. Unfortunately, the limited use of analgesics for veterinary surgical patients is not limited to the US. In 1999, 30% of British veterinarians agreed with the statement, "A degree of pain is required to stop the animal being too active post surgery." Similarly, veterinarians in Canada indicated that just less than half administered analgesics to surgical patients.

Why not provide analgesics? Is it because our patients cannot verbally communicate, is it because of our attitude towards animals or, as some have suggested, is it because of a lack of understanding and education regarding pain and analgesics? One would hope that the papers published in the 1990s would have stimulated a push towards more education for veterinary students. It may have in some institutions, but in a recent paper two-thirds of health science programs in Canada were unable to identify specific hours designated for the topic of pain in their programs.<sup>5</sup>

A lack of the understanding of pain is a major obstacle. We cannot specifically measure it and therefore there is little positive feedback if we treat it successfully or little negative feedback if we fail to treat it. Perhaps pain cannot be treated scientifically. One veterinarian said, "Pain is an experience that does not lend itself to objective measurements, so the art of medicine should not be overlooked in favor of the science of medicine." I agree, in part. Certainly we need to utilize our intuition and creativity when it comes to the topic of animal pain. However, instead of using art to diagnose and treat pain we should use it to design scientific experiments that allow us to study pain, analgesia and analgesics. Frankly, art is open to interpretation; only reproducible, scientific experiments will provide progress.

Science in the field of animal pain has been prevalent. In a search of "animal pain" on PubMed one finds nearly 45,000 scientific papers. So what is the problem? With that much research it should be perfectly clear how to measure animal pain and how to test various analgesics. The vast majority of these studies in small animal veterinary patients utilize patient behavior as a mechanism to measure pain. In a paper I wrote comparing analgesic protocols after intercostal thoracotomy we used a numerical rating scale (NRS) that evaluated patient behaviors such as patient crying, agitation and movement. This is nice, but as I reflect on that work I wonder, how accurately can I separate mild from moderate agitation in a dog that is recovering from general anesthesia and major surgery? In an effort to provide some objective outcome measures in this

research we combined the NRS with postoperative changes in heart rate and respiratory rate. At first glance this makes sense because we know that these biologic indices increase in the presence of pain. They are also affected by analgesics, anesthetics, stress and fear. So did we measure pain in this study? In a later study we evaluated data that compared the relationship between changes in subjective (behaviors) and objective (HR, RR, pain threshold) measures of pain and found no correlation between the two. Thus, one of our outcome measures may have been an effective tool for estimating patient pain; but, if one was the others were not.

Since this, subjective measures to estimate animal pain have matured. In a series of papers from a research group in Glasgow, the Glasgow Composite Pain Scale (GCPS) was developed. <sup>9</sup> This was shortly followed with a modified version. The GCPS is widely accepted as a validated, gold standard for analgesic studies using subjective outcome measures in small animal patients. 10 The GCPS was validated in a study of 20 clinically normal dogs, 20 dogs with medical conditions, and 117 dogs undergoing surgery. After a scaling model was applied to the descriptors to establish weights for each and create a continuous scale, five observers independently used the scale to score signs of pain in four groups of dogs (control dogs, dogs with medical conditions, and 40 dogs undergoing soft tissue or orthopedic surgery). Scores from each group and from groups of conditions perceived to cause no, mild, moderate, and severe pain were compared. In addition, the scale was applied to 77 dogs undergoing orthopedic or soft tissue surgery and scores were compared with simultaneously derived numeric rating scale (NRS) scores; comparisons were made between surgical groups and with time after surgery. They found that median pain scores differed significantly among the 4 study groups, among pain severity groups, and were typically greater with increasing perceived pain severity. From this they concluded that the measurement scale is a valid measure of acute pain in dogs. This is a step in the right direction. Validation using these methods is made by demonstrating that the scale performed as expected when used to evaluate patient pain after surgeries with various degrees of invasiveness. Validation in this sense is good, and this technique is easily applied in every hospital but, it seems that there is still room for improvement when it comes to making strides towards the scientific study of analgesia and analgesics.

Another approach if we cannot directly measure pain is to measure something the patient does because of pain that can be objectively measured. One common approach is to measure lameness after inflammation is induced. This is done in a research setting by using a urate crystal synovitis model. Pain can be estimated by measuring limb function. Limb function can be measured by visual observation, but this would defeat the purpose by introducing opinion. Limb function can be precisely and objectively measured using gait analysis by measuring ground reaction forces (GRF). This technique has been widely instituted and is the preclinical gold standard to determine if an analgesic or anti-inflammatory drug performs better than a placebo medication or at least as good as a drug that has already demonstrated efficacy. Numerous evaluations of nonsteroidal anti-inflammatories (NSAID) can be found in the literature. Limb function can also be measured in a clinical setting using gait analysis after surgery. If the methods of the study are set that all groups receive identical treatment (premedication, anesthetic, surgery and surgeon), perioperative analgesic techniques can be tested. This technique was demonstrated when use of a perioperative NSAID was found to improve patient use of an operated leg after cranial cruciate ligament surgery as compared to identical treatment but no use of a NSAID in the analgesic

regimen.<sup>11</sup> Another great benefit to this technique is that patient pain can also be estimated in cats. Using client-owned cats limb function was measured in cats after unilateral declaw to effectively study various analgesic techniques (always remember to use a multimodal approach to the treatment of pain) and surgical techniques.<sup>12,13</sup> Although this would not normally be done in a clinical setting, unilateral declaw gave the patient a choice to use the operated leg or to simply walk on three legs. To accept these models we must accept that patients will use the operated leg more if they are less painful. Intuitively, I think this makes sense. Finally, measuring GRF is a well documented way to estimate chronic joint pain in patients and has been used to document the efficacy of both medications and surgical procedures.<sup>14,15</sup>

Beyond gait analysis there are other, intuitive, objective measures of pain. When performing a physical exam we commonly look for and try to semi-quantify patient pain. Examples of this might be checking for back pain by pressing on the spinal column, abdominal pain by pressing on the abdomen or joint pain by pressing on the joint capsule. These clinical techniques that we use every day in practice can be improved and translated into a more objective technique buy using a standardized instrument that measures the amount of pressure applied before the patient's first negative response (pain threshold). Pain threshold algometers are commercially available and have been used in clinical veterinary studies. In a study evaluating the efficacy of various analgesics techniques after a standardized knee surgery an algometer was used to compare groups. This study allowed for documentation that a single, postoperative injection of intra-articular bupivacaine provided better analgesia than intra-articular morphine or intra-articular saline. One limitation to this technique is that it requires the observer interpret the patient's first negative response; having performed this I always wonder, is this threshold when the patient changes their breathing pattern, stops wagging their tail, or when they want to bite you?

Previously I mentioned that the biologic variables HR and RR were ineffective in measuring pain because they were confounded by too many physical and environmental factors. Until recently, I thought this might be the case with all biologic variables. In a recent study of acute pain in dogs induced by urate crystal synovitis changes in patient serum cortisol was studied. All patients were acclimated to the environment, all had cortisol and GRF measured before synovitis and all had cortisol and GRF after synovitis. Before the synovitis, cortisol and GRF remained unchanged over the course of the 24-hr study. After synovitis, GRF decreased and cortisol proportionally increased. In fact, in this study a patient serum cortisol level greater than 1.9 mg/ml indicated lameness with 90% sensitivity. I would suggest that this validates the use of serum cortisol as an objective measure of pain. Use of cortisol has been used in clinical studies evaluating patient pain in the past. In one study, dogs that had pericardectomy performed via thorascopy had lower cortisol levels than dogs that had it performed via open thoracotomy. Is to seems prudent to pay close attention to studies that utilize validated, objective estimates of pain.

Obvious limitations with verbal communication with our patients leave the interpretation of the signs of pain in animals to the opinion of the observer. In a clinical setting my approach is straightforward: surgery causes pain therefore I should provide analgesia. If a patient appears that it may need more analgesic, and it is safe to provide one, I do. When it comes to selection of analgesics I try to cautiously balance the use of my clinical experience with the peer-reviewed scientific literature that was generated using objective outcomes measures or subjective measures that had large patient groups.

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# CREATING GENE FLOW BETWEEN WILD AND CAPTIVE PALLAS' CATS (Otocolobus manul) THROUGH ASSISTED REPRODUCTION WITH FROZEN SEMEN

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#### Abstract

Collection and cryopreservation of semen from free-ranging wildlife offers a novel means to create gene flow into captive populations without removing animals from the wild. In our previous research in Pallas' cats, semen was collected from 11 wild males captured on the Mongolian steppes, frozen in 115 semen straws and imported to the U.S.<sup>3</sup> Our objectives in the present study were to 1) compare post-thaw motility of wild Pallas' cat spermatozoa in two culture media, 2) evaluate post-thaw sperm function using heterologous and homologous in vitro fertilization (IVF), and 3) produce offspring via laparoscopic transfer of IVF-derived embryos (ET) or artificial insemination (AI). Frozen semen straws from eight wild males were thawed and diluted in two media (Ham's F10, FOCM) for motility assays and IVF<sup>2</sup>. Oocytes collected via laparoscopy from gonadotropin-treated domestic cats (167 oocytes, 15 females) and Pallas' cats (73 oocytes, 5 females) were inseminated (5 x 10<sup>5</sup> motile sperm/ml) and cultured in vitro for 2-7 days before either embryo staining or transfer. Comparing culture medium, % sperm motility did not differ over time (P > 0.05); however, heterologous IVF success and blastocyst formation were higher (P < 0.01) in FOCM (64.7% fertilization, 58.5% blastocyst) than in Ham's F10 (29.3% fertilization, 0% blastocyst). For ET, Pallas' cat embryos (n = 37; 51% fertilization in FOCM) were transferred into the oviducts of seven ovulatory Pallas' cats synchronized with gonadotropin (eCG/pLH) treatment, but no pregnancies resulted. To assess the feasibility of laparoscopic oviductal AI as an alternative to IVF/ET, one synchronized Pallas' cat at the Cincinnati Zoo was inseminated with freshly-collected semen (5.3 x 10<sup>6</sup> motile sperm) from the resident male<sup>1</sup>. This female conceived and gave birth to four kittens (three healthy, one stillborn) after a 69 day gestation. Subsequently, oviductal AI was attempted in five Pallas' cats using frozen-thawed Mongolia semen (mean, 2.9 x 10<sup>6</sup> motile sperm/female), but no pregnancies were produced. These findings indicate that Pallas' cat semen collected and frozen in the field from wild males has adequate post-thaw motility and function to obtain high (50-65%) fertilization success using an optimized feline-specific culture medium. Furthermore, our results show that healthy Pallas' cat kittens can be produced following oviductal AI of gonadotropin-treated

168 2012 PROCEEDINGS AAZV CONFERENCE

females with non-frozen semen. However, our goal of producing founder offspring from frozenthawed Mongolian Pallas' cat semen will require further investigation and continued refinement of assisted reproductive methods for this imperiled wild cat species.

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EVALUATING ECHOCARDIOGRAMS AND INDIRECT BLOOD PRESSURES IN WESTERN LOWLAND GORILLAS (Gorilla gorilla gorilla) IN THREE PHASES OF AN ANESTHETIC PROTOCOL

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#### **Abstract**

Until the majority of the great ape population is trained for awake procedures, most will require general anesthesia to perform echocardiograms for cardiac disease assessments. Within the veterinary community there is concern over anesthetic protocols that may exacerbate or artificially induce signs of cardiac disease. Although medetomidine is generally contraindicated in patients with cardiac abnormalities, the combination of ketamine/medetomidine is used frequently by many institutions due to its ease and reversibility. To-date, there have been no published studies to compare physiologic or echocardiographic parameters using different protocols in the same individual. This study collected echocardiographic and blood pressure data on multiple male gorillas with and without cardiac disease. Initially gorillas were given ketamine/medetomidine (historically used in all great apes at Omaha's Henry Doorly Zoo and Aquarium without complication), after adding supplemental sevoflurane, and 15 min after reversing the medetomidine. Measurements were obtained under initial anesthetics and on gas alone. Based on data collected, the anesthetic regimen does affect certain cardiac parameters and indirect blood pressures. Without exception there was a decrease in ejection fraction (range 10-25%) with medetomidine that was not seen after reversal on sevoflurane. There is a potential for increase in chamber size with medetomidine as well as worsening of regurgitant lesions not noted after reversal on inhalant anesthesia. Indirect blood pressures were generally higher on ketamine/medetomidine, lower with the addition of sevoflurane and considerably lower after medetomidine reversal. Results of awake echocardiograms in the same individuals appeared similar to those using inhalant anesthesia with reversal of medetomidine.

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# THE CURRENT STATE OF ORANGUTAN HEALTH IN NORTH AMERICA AND THE ORANGUTAN SSP'S PLANS FOR MOVING FORWARD

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#### **Abstract**

A formal review of the medical conditions affecting captive orangutan species (*Pongo pygmaeus* and *P. abelii*) has not been performed since 1990. The Orangutan Species Survival Plan (SSP) identified the need to update the knowledge on the current state of orangutan health in North America to determine what medical issues are of greatest concern and to help decide where resources should be directed for the future. A survey was completed by 45 of the 55 (81.8%) institutions in North America that house orangutans and are accredited by the Association of Zoos and Aquariums. The survey results indicated that the top three health problems that concern the clinical veterinarians are 1) respiratory infections, 2) heart disease, and 3) obesity. The survey also identified specifics on each institution's preventative health care plans, medical training, use of consultants, and diagnoses of major diseases. To better understand the health conditions affecting orangutans, the SSP conducted an orangutan health workshop from 18-20 May 2012 at the Fort Worth Zoo where professionals from veterinary, human medical, and other specialties collaborated to identify prioritized goals for addressing the diseases of highest concern.

#### **ACKNOWLEDGMENTS**

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# UPDATE: EVALUATION OF CAPTIVE GIBBONS IN NORTH AMERICAN ZOOLOGICAL INSTITUTIONS FOR AN EPIZOONOTIC AGENT: THE GIBBON APE LEUKEMIA VIRUS (GALV)

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#### **Abstract**

The gibbon ape leukemia virus (GALV) is an infectious gammaretrovirus associated with neoplasias in gibbons. Highly related retroviruses have been isolated from other animals (woolly monkey, koalas). <sup>1-4</sup> The virus is shed in urine, feces, and saliva, and can be transmitted *in utero* and via postnatal contact. Since its initial characterization in the 1970's and 80's, the incidence of GALV has not been assessed in gibbons. Investigating the disease status of captive animals as well as factors affecting their health is a critical first step in determining if captive gibbons are infected, and if an etiologic linkage between infection and neoplastic diseases exists. Diagnostic assays developed and validated with National Zoological Park animals (PCR of genomic DNA, co-culture for virus isolation, and ELISA) were used to identify the presence or absence of viral DNA, RNA, and GALV antibodies in 80 captive gibbons representing 29 zoological institutions in North America. Samples were obtained during routine and diagnostic examinations. Studies revealed possible exposure to GALV, but lack of integration or expression of the virus.

#### **ACKNOWLEDGMENTS**

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# COMPREHENSIVE HEALTH ASSESSMENT OF GREAT AND LESSER APES IN TWO MEXICAN ZOOLOGICAL COLLECTIONS

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#### Abstract

In August 2011 fourteen apes were examined at 2 Mexican zoological institutions: seven (3.4) chimpanzees (*Pan troglodytes*), two (1.1) pygmy chimpanzees (*Pan paniscus*), two (0.2) hybrid orangutans (*Pongo* spp) and three (1.2) siamangs (*Hylobates syndactylus*). The health assessments were performed under general anesthesia and included complete physical evaluations, dental and ophthalmic exams, intradermal TB testing, chest and pelvic radiographs, abdominal ultrasound, bronchoscopy (for the two orangutans), echocardiography, and semen collection (for one common chimpanzee and one pygmy chimpanzee). Blood was collected for hematology, serum chemistry and serologic TB testing (Prima TB-Stat Pak, Chembio Diagnostics, Inc; Medford, New York). Tracheal swabs were collected for TB culture and PCR. Fecal samples were collected for parasite testing. The main objectives were to: 1) obtain baseline information on a variety of health parameters for the apes within these collections, 2) guide specific treatment for any health problems identified, 3) learn the current status of these animals regarding important zoonotic risks (i.e. TB) for the staff at each zoo, 4) lead the way for institutions outside the USA to contribute to the Great Ape Heart Project (GAHP). This project was an unprecedented event for Mexican zoological collections.

#### **ACKNOWLEDGMENTS**

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# TREATMENT OF CHRONIC SINUSITIS IN ORANGUTANS (Pongo sp.) BY FUNCTIONAL ENDOSCOPIC SINUS SURGERY

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#### Abstract

Chronic upper respiratory tract diseases (URTD), such as common cold, sinusitis and airsacculitis, are a common health problems in captive orangutans (*Pongo abelii*, *P. pygmaeus*).<sup>2-7</sup> A previous study identified chronic sinusitis as a primary stage of airsacculitis in captive orangutans.<sup>7</sup> Comparable to human medicine, orangutans with diagnosed sinusitis that is unresponsive to longterm intensive medical treatment might be potential patients for minimal invasive functional endoscopic sinus surgery (FESS) to improve their wellbeing and to prevent further disease progression. Orangutans considered for FESS must be examined thoroughly, including a computed tomography scan (CT) to evaluate the upper respiratory tract to display individual anatomic structures. Anesthesia should be regarded as a high-risk immobilization, and anesthetized orangutans should be intubated in a sitting position immediately after induction to prevent pulmonary aspiration of pathologic exudates. For CT scanning, orangutans should be positioned in ventral recumbency for best display of possible fluid levels.<sup>6</sup>

The purpose of the surgery is to re-establish ventilation and mucociliary clearance of the sinuses. Preoperative medical management includes ten days of antibiotics according to drug resistance testing and a three-day course of steroids. The minimal invasive FESS technique also requires extensive training, specialized endoscopic and surgical instruments to ensure best surgical results. Preliminary results of the study in the captive European orangutan population revealed a promising longterm outcome of FESS as a treatment of chronic sinusits and airsacculitis and thus increasing the welfare of orangutans suffering from chronic upper respiratory tract disease.

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# NASAL CARCINOMA IN MEXICAN GRAY WOLVES (Canis lupus baileyi): PREVALENCE DETERMINATION USING COMPUTED TOMOGRAPHY

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#### Abstract

The Mexican gray wolf (Canis lupus baileyi), is the rarest, southernmost, and most genetically distinct subspecies of the North American gray wolves. It is also the smallest subspecies of the gray wolf, and one of the most endangered canids in the world. Since the early 2000's at least 14 clinical cases of nasal carcinoma have been described in the captive population of Mexican wolves in the United States and in Mexico. Although cancer represents only 3.3% of the mortality of the registered Mexican wolf population, the majority of these neoplasms have been categorized as sino-nasal carcinomas (Gaffney, Garner, unpublished data). Preliminary studies suggest that, as in dogs, a genetic component is involved in the carcinogenesis of this neoplasm. Advanced imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) are routinely used for the diagnosis of nasal tumors in dogs. <sup>1-5</sup> Because most nasal tumors involve bony structures, including nasal turbinates and sinuses, CT exams are more commonly used to assess the extent of the nasal disease as well as to aid in differentiating between neoplastic and non-neoplastic processes. In addition CT allows exact disease localization and staging, biopsy guidance and treatment planning. 1,5 Mexican wolves housed at the Brookfield Zoo as well as archived specimens (heads and skulls) from deceased Mexican wolves, were examined using CT to identify changes indicative of nasal disease and determine prevalence of nasal carcinoma on this species.

#### ACKNOWLEDGMENTS

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HOW TO CONDUCT RETROSPECTIVE STUDIES IN THE ABSENCE OF CONFIRMATORY DIAGNOSTICS: AN EXAMPLE FROM A STUDY OF FELINE HERPESVIRUS (FHV) IN CHEETAHS (Acinonyx jubatus)

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### **Abstract**

A common challenge in conducting retrospective epidemiologic studies is incomplete confirmatory diagnostic information to aid in the classification of animal disease status. If cases are limited to those with confirmed diagnostics alone, many true positives would be missed. 1 Similarly, inclusion of all individuals with clinical signs but lacking confirmatory diagnostics may result in significant misclassification error. Results could also be biased if reasons for diagnostic confirmation differ across confirmed and non-confirmed animals. To address the potential for such biases, systematic quantitative methods for identifying clinically compatible (CC) individuals should be used. A population-level study on the epidemiology of Feline herpesvirus (FHV) in 322 cheetahs housed in 6 zoos used a combination of scholarly literature, expert opinion, and exploratory multiple correspondence analysis<sup>2</sup> to determine the distribution of clinical signs among 35 laboratory confirmed (LC) cases of FHV. A final case definition for clinical FHV was then developed, ensuring that the distribution and grouping of signs identified in the LC cheetahs were mirrored in the 61 identified CC cases. The inclusion of both LC and CC cases created a sensitive case definition that is effective for both disease surveillance and developing lists of diagnostic differentials. This study not only highlights the importance of confirmatory diagnostics, which are often lacking in routine case investigations, but also demonstrates methodology that can be used to address diagnostic deficiency in retrospective Although limitations exist, such methods should help improve accuracy when developing case definitions based on non-specific clinical signs or unknown syndromes.

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# AN EVALUATION OF DESLORELIN IMPLANTS FOR CONTRACEPTION IN CAPTIVE SEA OTTERS (*Enhydra lutris*) USING FECAL GONADAL HORMONE CONCENTRATIONS

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## **Abstract**

Captive sea otters are owned by the United States Fish and Wildlife Service (USFWS). The USFWS has requested that sea otters be prevented from breeding in order to save captive space for wild rescued animals that might be deemed non-releasable. Suprelorin<sup>®</sup> or deslorelin, is a contraceptive that has been used in many different species to effectively suppress reproduction but duration of effect may vary between species and individuals. The effects of one to several consecutive deslorelin implants on gonadal reproductive hormones found in fecal samples from six captive sea otters (2 = male, 4 = female) was compared to baseline pre-deslorelin levels for each individual and two control otters (1 = male, 1 = female) housed at three zoological institutions. The longitudinal hormone signatures of different stages of the contraceptive cycle were documented including pre-treatment, initial stimulatory phase, effective contraception and hormone reversal characterized by a return to normal cycling reproductive levels. All sea otters exhibited effective contraception of gonads as evidenced by significantly lower concentrations of fecal reproductive hormones compared to pre-treatment or control animal levels. However, the initial stimulatory phases and duration of contraception were highly variable at 0 to 9 mo, and 6 mo to 4 yr, respectively.

#### **ACKNOWLEDGMENTS**

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# AN OUTBREAK OF VARICELLA-LIKE DISEASE IN GREAT APES AT MELBOURNE ZOO, AUSTRALIA

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#### Abstract

Five gorillas and two orang utans developed signs of Varicella-like disease over a 31 day period. The first case was an adult male gorilla presenting with lethargy and inappetance. Five to eight days later, pruritic vesicular lesions progressively appeared on his face, trunk and hands. He was anesthetized on day 9 and blood collected for serology, returning a strong positive result for Varicella-Zoster (V-Z) IgM and negative for V-Z IgG. A presumptive diagnosis was made of V-Z infection. V-Z serology was subsequently performed on banked serum from ten apes in direct and indirect contact with the affected animal. Three had strong IgG titres and three had borderline titres, indicating prior exposure to V-Z virus. All seronegative and two borderline animals subsequently developed similar clinical signs in two "waves" of onset, commencing 17 and 31 days respectively after presentation of the index case.

Varicella-like illness has been previously reported in young great apes all in very close contact with children, and in two reports, with known exposure to human V-Z infection. Human V-Z virus was isolated from lesions in two of these cases. Although virus was not isolated in the cases reported here, the clinical signs, incubation period and serologic findings were all strongly suggestive of V-Z infection, however the animals only had close contact with zoo staff and there was no known exposure to an infected human. As the virus is capable of travelling long distances, we speculate that the index case resulted from aerosol spread from a zoo visitor.

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# MANAGEMENT OF JOINT LUXATIONS IN BIRDS

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# Abstract

#### Introduction

Few reports of methods for managing luxations in birds have been published.<sup>2</sup> Coxofemoral luxations are frequently the result of trauma during restraint, or the bird struggling when its leg has been ensnared within a cage structure or fence. Elbow luxations primarily occur in raptors secondary to in-flight trauma.

For treatment of luxations, it is crucial to reduce the luxation as early as possible, which minimizes the formation of periarticular fibrosis. The bird's attempts to use the injured extremity often — and very quickly - cause damage to the articular cartilage. In as brief as three days, clinically significant fibrosis occurs and inhibits reduction of the luxation and predisposes the joint to ankylosis. Where articular cartilage is damaged, even with successful luxation reduction and stabilization, it is likely that degenerative changes and osteoarthritis will occur in the future. As many animals with severe degenerative joint disease do not demonstrate overt pain, it is difficult to determine the clinical importance of osteoarthritis in older captive birds, and in free-ranging birds it is even more difficult to assess. Clinically significant arthritis may require years to develop during which time the bird likely will function well.

When managing luxations in birds, it is important to take radiographs after the extremity has been reduced and bandaged. During bandage application, re-luxation is common and must be identified immediately so that appropriate measures can be taken. Although controlled physical therapy under general anesthesia as early as possible will help minimize the effects of bandaging on other joints, it is best to wait 24-48 hr to allow healing to begin and pain to subside. In most cases, primary repair of the damaged tendons and ligaments is not possible so ultimately the joint becomes stable because of the formation of scar tissue. During physical therapy, no effort should be made to move the affected joint as if the scar tissue is disrupted by joint manipulations it may not stabilize. Therapy is generally performed under general anesthesia every other day and involves passive range of motion exercises of all immobilized joints, except the affected joint, for 10-15 min. For example, when managing an elbow luxation, the shoulder and carpus are exercised but not the elbow. Tendons and ligaments heal initially by a disorganized mass of fibrous connective tissue, which is not strong. When stresses are applied to the scar tissue, it reorients along the lines of stress. This process takes a long time (6-8 wks in mammals). The conformation of many joints favors their remaining in a reduced position. While no objective data is documented on how long a joint should be immobilized before applying stress, if the support is removed too early, the joint will be more prone to re-luxation. Generally 7-10 days seems to be a long enough period for most joints to stay reduced after the support is removed.

It is vital to check the bandage daily if at all possible. Serious bandage morbidity can occur very quickly. In birds, damage to the propatagium can occur with figure-of-eight bandages. Part of physical therapy for wing injuries involves massaging the tendon and checking for injury from the bandage. Some birds are predisposed to developing pododermatitis when they must bear weight on only one leg. They also should be checked daily for early signs of bumble foot and appropriate treatment instituted as soon as signs are noted.

# **Luxation of the Shoulder**

Shoulder luxation may involve avulsion of the ventral tubercle of the proximal humerus.<sup>2</sup> The shoulder joint is not a very stable joint and re-luxation is common. If the luxation occurs secondary to ventral tubercle avulsion, surgery to reattach the tubercle results in a stable joint. However, as the bone is fractured, it will take 3-4 wks for healing. If the tubercle is not avulsed, closed reduction is often successful. If the joint does not stay reduced, a trans-articular pin can be placed through the proximal humerus along the deltoid crest and into either the scapula or coracoid. The pin should be placed with the shoulder joint held in a flexed (resting, folded wing) position. Regardless of the technique used, the wing should be bandaged to the body to immobilize the shoulder joint.

# **Elbow Luxation**

Luxation of the elbow is usually the result of severe blunt trauma strong enough to disrupt the ligamentous support. This type of injury occurs infrequently in companion birds but has been reported to occur as frequently as in 12% of raptor patients.<sup>2</sup> Because of the anatomy, luxation usually occurs dorsal, caudal or caudodorsal. Ventral luxations generally occur only in association with fracture of the radius. The wing generally is held with the elbows extended (drooped) and externally rotated. Pain, crepitus and swelling are noted on palpation of the affected wing. The wing should be examined for concomitant soft tissue injury that may affect the prognosis. The presence of open wounds and fractures has been associated with a poor prognosis for return to normal function.<sup>2</sup>

Reduction is accomplished by flexing the elbow to counteract the force of the scapulotriceps muscle that pulls the ulna caudally. Maintaining flexion, the radius and ulna are rotated internally while pressure is applied to the dorsal (lateral) aspect of the radial head to force it into apposition with the dorsal (lateral) humeral condyle. As the cubital joint is extended gently, a pop is often palpable when reduction is complete. In cases with severe ligamentous damage, this pop may not be palpable. If the joint is stable following reduction, it may be supported with a figure-of-eight bandage

If closed reduction is not possible or re-luxation readily occurs, open surgical reduction is recommended. Through a lateral (dorsal) approach to the cubital joint, the common digital extensor is sutured to the scapulotriceps tendon distal to the cubitus. In a study that evaluated 12 cubital luxations in raptors, only three birds could be released. Nine had caudodorsal luxation, none could be reduced closed and reduction was maintained using external skeletal fixation or figure-of-eight wrap. In another study, four of eight raptors with a cubital luxation were released.<sup>2</sup>

If the cubital joint can be reduced either open or closed, but it will not stay in reduction, a transarticular external skeletal fixation device may be applied to maintain reduction. At least two pins are placed in the humerus and two in the ulna. The elbow is flexed into a normal folded position and the pins connected.

# **Luxation of the Carpus**

Usually the carpometacarpus is displaced dorsally relative to the radius and ulna. The bird will hold the wing with the carpus extended and it will be externally rotated at the carpus. Reduction is accomplished by applying traction and (dorsal) abduction of the distal extremity. The carpometacarpus is then toggled into reduction and the carpus is flexed and (ventrally) adducted. With the carpus in flexion a figure-of-eight bandage is applied to maintain reduction. With large birds or chronic luxations, open reduction may be indicated. In cases where laxity is present following reduction and the joint will not stay reduced, a transarticular pin or ESF device may be placed to maintain reduction. The transarticular pin is placed with the carpus in a normal degree of flexion through the main body of the carpometacarpus and into the ulna, which immobilizes the carpus. A fixator can be applied with two pins in the ulna and two in the major carpometacarpus.

# Luxation of the Metacarpophalangeal Joint

Luxation has been reported in two raptors and both were treated by arthrodesis.<sup>8</sup> The bones are small and blood supply tenuous in this location, which makes primary repair impractical. Both birds were treated with a type I external skeletal fixator and both regained full fight and were released.

# **Coxofemoral Luxation**

In most psittacine birds and raptors, the coxofemoral joint is not a tight fitting ball and socket joint. As a diarthrodial joint supported by a round ligament as well as collateral ligaments, it has a substantial amount of cranial to caudal gliding motion with little abduction and adduction. The ventral collateral ligament and the round ligament primarily are involved in maintaining the femoral head within the acetabulum. For luxation to occur, both of these structures must be disrupted.<sup>2</sup> In many species, the dorsal acetabular rim is well developed and extends as the antitrochanter to articulate with the broad, flat femoral neck and trochanter.

Coxofemoral luxations are generally the result of traction and rotational trauma, such as occurs when the leg is caught and the bird struggles to escape. Most luxations are craniodorsal in birds, although cranioventral luxation also has been reported. Closed reduction and stabilization with slings, splints and casts have been recommended. In some cases, the luxation may be reduced and maintained using a transarticular pin. The pin is inserted through the trochanter into the head of the femur, across the joint and seated in the acetabulum. This pin must be inserted carefully by pre-determined measure so to avoid injuring the kidney that lies medially to the acetabulum. The injured limb should be supported using an off weight bearing sling or spica splint to prevent pin migration. In most cases, sufficient production of scar tissue will occur 7-10 days postoperatively

such that the pin safely may be removed. Long-term maintenance of a transarticular pin can predispose to the development of degenerative joint disease and pin migration.

Surgical reduction and stabilization is considered the treatment of choice for acute coxofemoral luxations. A femoral head and neck excision arthroplasty often is indicated for chronic luxations. The approach for both of these surgeries is the craniolateral approach that also allows for the placement of support sutures and access to the joint capsule.<sup>2</sup> In most cases, the joint capsule is torn or even absent as a result of the bird trying to walk on the affected leg. Following reduction of the luxation, stabilization sutures are placed from the trochanter to the dorsolateral iliac crest caudal to the central axis of the femur and from the trochanter to the cranial rim of the acetabulum.<sup>2</sup> The sutures are placed through the bone and with the stifle maintained in a normal standing position, the sutures are tightened. These sutures prevent excessive external rotation of the leg as with dorsal coxofemoral luxation, severe damage usually is present to the muscles that prevent external rotation. The joint capsule (if present) is closed and the iliotrochantericus caudalis and iliofemoralis externus are apposed. An alternative to open reduction is to perform a femoral head and neck excision arthroplasty (FHO). <sup>2,4</sup> For this technique, the rehabilitation is easier and the prognosis is generally good, even for raptors. By craniolateral approach to the hip, the head and neck of the femur are removed with an appropriate sized osteotome or oscillating saw. Rongeurs should be used to ensure that no rough or sharp edges remain at the osteotomy site. Following FHO, a tendency for external rotation of the limb is often present because of the muscle damage caused by the luxation although this problem is not observed with femoral neck fractures.<sup>4</sup> This issue can be countered using the support sutures described for surgical coxofemoral stabilization. In both situations, as polydioxanone suture remains for over 4 mo in birds, but is absorbable, it is an appropriate choice for these anti-rotational sutures.

As with closed reduction, the limb should be supported post-operatively in a spica splint or off weight bearing sling for 7-10 days. It is best to maintain the bird in a cage with smooth walls and a perch near the floor to discourage the bird's attempts to climb. It can be very difficult to achieve postoperative immobilization of the coxofemoral joint in long legged birds. Unlike with open reduction of a hip luxation, early use of the leg is encouraged following excision arthroplasty as a better pseudoarthosis will form. Passive range of motion exercises can be started the day after surgery. In a recent report, a red-tailed hawk (*Buteo jamaicensis*) and a Canada goose (*Branta canadensis*) had virtually no lameness and normal function following FHO with good return to function within 12-48 hr post-operatively.<sup>4</sup>

#### Luxation of the Stifle

In addition to tearing the cruciate ligaments, damage to the collateral ligaments usually is present in birds with stifle luxation and multiple stifle ligament injury is frequent. During the physical examination, a positive drawer sign is elicited and medial and/or lateral collateral instability exists. The tibiotarsus may be located cranial or caudal to the distal femur. Surgical repair of the ligaments may be attempted, especially in large birds; however, in most birds, the size of the ligaments precludes primary surgical apposition.

If the stifle can be reduced closed, a transarticular ESF may be used to maintain the stifle in reduction allowing periarticular fibrosis to stabilize the joint. Two fixation pins minimally should

be placed each in the femur and the tibiotarsus. With the joint reduced and the limb in a normal, standing position, the pins are connected.<sup>7</sup>

If the joint cannot be reduced closed, an open reduction is indicated. A lateral parapatellar approach to the stifle is made and a curved hemostat is used to lever the proximal tibiotarsus into its proper position on the distal femur. Once the joint is reduced, several options to maintain reduction are described. A transarticular pin can be placed from the distal femur into the proximal tibiotarsus to hold the joint in reduction. During closure, the joint capsule is imbricated. The leg is bandaged for 10-14 days to allow fibrous tissue to stabilize the joint before the pin is removed. In one report, cruciate ligament and stifle luxation were managed with open reduction and stabilization<sup>5</sup> with one bird (trumpeter hornbill, *Bycanistes bucinator*) repaired by a fibular head transposition and lateral joint imbrication for cruciate ligament damage and the other bird (African grey parrot, *Psittacus erithacus*) stabilized by lateral imbrication after open reduction. Both birds regained good limb function by 30 days. In these birds, no external coaptation was used, allowing early return to function, which is preferred if the joint is stable after soft tissue repair.

Alternatively, a hole can be created from lateral to medial in the distal femur and proximal tibiotarsus. A suture is passed from lateral to medial in the distal femur and medial to lateral in the proximal tibiotarsus. This approach will create a mattress suture that will mimic the collateral ligaments that are often damaged with stifle luxation. The joint is reduced and the suture tightened. Unfortunately, this suture may not stabilize cranial-caudal movement (drawer), so a transarticular pin, an external fixator, or a bandage can be added to prevent cranial-caudal movement. A third option is to perform an open reduction and place a transarticular fixator with at least two pins each in the femur and the tibiotarsus connected on the lateral aspect of the leg. Once the fixator is applied, soft tissues are imbricated to provide support and scaffolding for scar tissue formation. Finally, a technique described for use in young birds with developmental stifle luxation involves inserting a pin normograde from distal to proximal into the femur and another proximal to distal into the tibiotarsus.<sup>3</sup> These pins are left long and used to align and reduce the luxation. The stifle is placed in a normal standing angle and the pins will cross. Cement is used to bond the two pins cranial to the stifle externally. A disadvantage of this technique is that the pins penetrate the articular cartilage and are exposed externally. In one report, ascending osteomyelitis occurred and ended in amputation.<sup>6</sup>

Where severe damage is present with these articular fractures, arthrodesis may be indicated. Successful arthrodesis and good limb function was reported in a cockatoo (*Cacatua moluccensis*) with a traumatic stifle luxation. When an arthrodesis is performed, the fixator is maintained until evidence of bony union is present radiographically.

Prognosis with avian luxation repair is somewhat dependent on the intended use of the bird. Companion birds and zoo specimens may function without a precise ability to fly; however, with wild birds, hunting birds, and racing pigeons, anything less than perfection cannot be regarded as success. In many birds, some degree of leg dysfunction may be acceptable; however, in raptors, legs are important for obtaining food; in terrestrial birds, they are necessary for survival; and in many species, they are vital for successful reproduction.

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# IRON STORAGE DISEASE SUSCEPTIBILITY PROFILES IN ASIAN HORNBILLS

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# Abstract

From the 54 extant hornbill species, 30 species, represented in 6-8 genera, are native to Asia in the areas of India, Thailand, Indonesia, and adjacent island countries. Many of the Asian hornbill genera have overlying natural territories, notably for this presentation *Aceros*, *Rhyticeros*, and *Buceros*. AZA-accredited facilities (www.aza.org), five hornbill genera and nine species are exhibited most typically and have managed programs; of these taxa, two genera and nine species are Asian hornbills. Hornbills are diverse in their dietary preferences ranging from predominant carnivores and insectivores (African) through non-seasonal omnivores (e.g., *Buceros*) to nearly exclusive frugivores (e.g., *Aceros*). However, all Asian hornbill species have increased protein consumption as animal matter and calcium from figs during the breeding season.

Frequently, Asian hornbills are represented as at risk for iron storage disease (ISD) or secondary hemochromatosis. <sup>2,8</sup> To define ISD for this presentation, it is the histopathologic presence of iron accumulation within the hepatic parenchyma and concurrent presence of hepatopathy. As a problem identified in primarily frugivorous avian taxa, ISD has been described with exceptionally high incidence in species such as toco toucan (*Ramphastos toco*) and Bali mynah (*Leucopsar rothschildi*). In these birds, it is considered highly unusual for adult bird histopathology to not present some degree of iron accumulation and a cause of death attributable to ISD (K. Benson, T. Norton, J. St. Leger, personal communications). <sup>1</sup> However, for hornbills, the program managers and veterinary and nutritional consultants have asserted the presumption of uniform ISD risk for their taxon as inaccurate. This disagreement first was based on lack of sufficient pathology data on which to conclude the issue. Additionally, documented nutritional assessment for the primarily displayed Asian hornbill genera (*Buceros* and *Aceros*) did not demonstrate differences in protein requirements and usage. <sup>2</sup> However, through recent collaboration of a leading private pathology service (Northwest ZooPath) and accumulated Coraciiformes TAG pathology data which now spans 20 yr, new trends have been documented.

A comparison of the two databases is provided for overall data quality and context (Table1). A total of 486 birds were available in the combined databases: individuals of African species (n=237) and Asian species (n=210), and those birds undesignated to species (n=39) which were eliminated from the total count. A further 113 birds were eliminated from analysis as incomplete necropsies were available; many of these were in-nest chick deaths or related to traumatic causes of death so histopathology was not performed. A final count of 185 Asian hornbills was available with complete histopathologies, including four genera of Asian hornbills with greater than 10 individuals per genus: *Aceros* (n=54), *Anthracoceros* (n=19), *Buceros* (n=79), and *Rhyticeros* (n=23).

For each individual bird that entered the final data set, complete gross necropsy and histopathology reports were reviewed to determine primary cause of death that was attributed to one of three categories: hepatic ISD; primary hepatic, but non-ISD, disease; non-ISD and non-hepatic cause. These groupings then were evaluated at both the genus and species level for analysis of ISD risk.

Overall, ISD was categorized as the cause of death in 58 individual hornbills, or 12% of the complete data set including African species. Asian hornbills (n=43) represented 74% of the total number of individuals so affected. Analysis by each Asian hornbill genus with consideration of ISD cases against all deaths (2-5%) and against only Asian species (4-8%) did not distinguish the groups from one another or the same calculations for African hornbills as a group (5% and 10% respectively). Analysis by Asian hornbill genera against the cases of ISD as a denominator were higher [Aceros (26%), Anthracoceros (17%), Buceros (16%), and Rhyticeros (12%)] than African hornbills overall. However, by species evaluation, an increased incidence of ISD was attributed as the primary cause of death in wrinkled (Aceros corrugatus) (24%) and wreathed (Rhyticeros undulatus) (30%) hornbills. In this database, no other individual species with greater than 10 individuals had such high presence of ISD.

The presence of ISD in Asian hornbills is not as prevalent as in the uniformly affected species such as Bali mynah or toco toucans. However, this database assessment has concluded that the more frugivorous Asian hornbill species, specifically in the *Aceros* and *Rhyticeros* genera, indeed should be considered susceptible to ISD. Routinely, it will be encouraged that they be managed with low iron content diet, restrictions on provision of ascorbic acid, and perhaps provision of chelating agents (i.e., tannins) as is routine for other ISD-sensitive species. However, these dietary restrictions should be lifted during breeding season for dam and chick well-being, and are not considered necessary for other Asian hornbill genera.

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**Table 1.** Comparison of two pathologic databases representing 20 yr of accumulated data (1990-2012) for evaluation to determine risk of iron storage disease in Asian hornbill species.

,	$NWZP^a$	TAG <sup>b</sup>
Total birds evaluated	230	256
Total species	20	20
African	9	8
Asian	11	12
Total individuals		
African	84	153
Asian	109	101
Unknown	37	2
Total ISD cases	17	41

<sup>&</sup>lt;sup>a</sup>Database originating with NorthWest ZooPath and inclusive of 1994-2012.

<sup>&</sup>lt;sup>b</sup>Database originating from Coraciiformes TAG Advisor and inclusive of 1990-2007.

# HEMORRHAGIC DIATHESIS IN AVIAN SPECIES FOLLOWING INTRAMUSCULAR ADMINISTRATION OF POLYSULFATED GLYCOSAMINOGLYCAN

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#### Abstract

Polysulfated glycosaminoglycans (PSGAGs) have been used for decades in a variety of species for managing osteoarthritis. Reports on the use of PSGAGs in avian species are scarce. In domestic cats and dogs PSGAG administration has caused prolongation of clotting times to considered an efficacious drug with a wide margin of safety. This publication documents four cases of fatal coagulopathies in different avian species [one Abyssinian hornbill (*Bucorvus abyssinicus*), one barn owl (*Tyto alba*), one Cooper's hawk (*Accipiter cooperi*) and a cockatiel (*Nymphicus hollandicus*)] following the administration of PSGAG (Adequan®, Luitpold Pharmaceuticals, Shirley, New York, 11967, USA). Doses ranged from 0.5-100 mg/kg and were administered at varying frequencies of every other day to once every four weeks. Three of the four birds experienced fatal hemorrhage into the pectoral muscle, while the fourth bled continuously from the injection site. One bird had chronic, severe pre-existing hepatitis and nephritis while the other cases were managed solely for osteoarthritis. This report highlights the occurrence of species sensitivity to PSGAGs and warrants further investigation into the etiopathogenesis of this adverse event.

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UTILIZATION OF Mycobacterium genavense DIRECT PCR ON FECES AS A NON-INVASIVE METHOD TO IDENTIFY INFECTED LADY GOULDIAN FINCHES (Chloebia gouldiae) IN A FREE-FLIGHT AVIARY

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#### **Abstract**

Mycobacterium genavense is a common cause of mycobacteriosis in birds, and an occasional cause of atypical mycobacteriosis in immunosuppressed humans.<sup>3-7</sup>Within zoological institutions housing a variety of birds, diagnosis of avian mycobacteriosis can be challenging due to the lack of reliable ante-mortem tests.8 In 2009, a Lady Gouldian finch (Chloebia gouldiae) was diagnosed on post-mortem examination with Mycobacterium genavense. It had been housed in a free-flight, walk-though, single-species aviary that was contained within a larger free-flight, walk-though, multi-species aviary. In the State of Iowa, M. genavense, as part of the Avian Mycobacteria-Complex (MAC), is reportable to the Iowa State Department of Agriculture.<sup>2</sup> The Blank Park Zoo developed a protocol to identify infected and shedding finches which included necropsy of all deceased birds and annual group fecal M. genavense direct PCR screening using primers MG22 and MG23.1 In 2011, the flock of finches was sub-divided into groups and pooled feces from each group submitted for M. genavense direct PCR which was detected in one pooled sample from a group of 11 finches. These 11 birds were euthanatized and submitted for necropsy. Hepatic granulomas were evident in 7/11 finches and acid-fast organisms were identified in granulomas in 5 of these 7 birds. In 2012, 82 finches were again divided into six groups, and feces pooled for direct PCR testing; these tests were negative. From 2009 to the present, necropsy of deceased finches and birds in the surrounding aviary did not reveal any evidence of mycobacterial disease.

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# AVIAN BORNA VIRUS IN NONPSITTACINE SPECIES

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#### Abstract

The Schubot Exotic Bird Health Laboratories have tested greater than 500 free-living nonpsittaciformes of more than 30 species (Table 1) for avian borna virus (ABV). Birds sampled include hunter-killed ducks; raptors and aquatic birds which died or were euthanatized during rehabilitation; birds which were culled by animal control authorities; passeriformes which were collected for museum study; and rehabilitated pre-release ducks and pelicans from Wildlife Center of Texas. Samples tested include choanal and cloacal swabs, and brain, liver, and splenic tissues. Avian borna virus sequences were amplified using two multiplexed primer sets. One recognized matrix (M) protein genes and the other recognized conserved regions of the nucleoprotein (N) genes. Positive results were verified by repetition, sequencing and culture confirmation of ABV positive animals. Many non-psittaciforme species were identified with avian borna viruses, particularly within the Anseriformes and Charadriformes. However, sample sizes in other avian taxa were low. An improved recovery rate for borna virus from the brain of birds occurred as compared with a very low recovery rate from testing as compared to swabs from choana, cloaca or eliminations. Clinical signs attributable to avian borna virus in psittacine birds such as gastrointestinal tract dysfunction and neurologic signs are seldom noted.<sup>1-3</sup> Sequencing has revealed multiple avian borna virus isolates which are not closely related to the isolate which appears most likely to cause clinical signs in Psittaciformes (ABV4).<sup>3-5</sup>

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**Table 1.** Non-psittaciformes and sample type tested by Schubot Exotic Bird Health Laboratories for Avian Borna Virus.

Tot Tivian Botha viras.					
Order	Brain	Swab <sup>b</sup>	NB Tissue <sup>c</sup>	Pos	Collection Area
Anseriformes	625	60	0	76	TX, NJ, KS
Passeriformes	9	-	73	0	TX/Africa
Charadriformes	47	-	0	6	NY, NJ, NH
Raptors <sup>a</sup>	31	-	-	0	TX
Coraciformes	-	-	3	0	Africa
Piciformes	-	-	7	0	Africa
Pelecaniformes	10	12	-	0	TX
Columbiformes	8	-	-	0	TX

<sup>&</sup>lt;sup>a</sup>Raptors included Falconiformes and Strigiformes.

<sup>&</sup>lt;sup>b</sup>Swab from choana, cloaca, or eliminations.

<sup>&</sup>lt;sup>c</sup>Non-brain tissues of kidney, liver, or spleen.

# THE AVIAN SARCOCYSTIS PREDATOR: NOT THE SPECIES WE THINK

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### Abstract

Sarcocystosis as a cause of avian mortality is well known in captive collections. In one zoological collection, the disease appeared with regularity during the late autumns of 2006-2010 and decimated a parakeet (Melopsittacus undulatus) and cockatiel (Nymphicus hollandicus) Sarcocystosis had been diagnosed previously in this collection in lorikeets aviary. (Trichoglossus haematodus) and generated the first report of the disease in a thick-billed parrot (Rhynchopsitta pachyrhyncha). In the present cases, deceased birds had good condition with ample food in the gastro-intestinal tract. Most deaths were peracute and birds reported as almost dropping in mid-flight. Principal gross necropsy findings were severely congested lungs. Histopathology confirmed pneumonitis with Sarcocystis sp. organisms in the lungs and occasionally in other tissues. A small percentage of birds presented with neurologic signs and survived with treatment of ponazuril (Marquis 15%, Bayer HealthCare, LLC, Shawnee Mission, KS 66201) at 20 mg/kg p.o. s.i.d. x 30 days, although neurologic deficits were not reversible. Deaths occurred sporadically: one a day, then none for 2-3 days then two dead, and none for 2-3 days. Males and females and all age classes were affected. Mortality rate over the season reached 50%. By end November to early December or January, the epidemic had ceased. During the remainder of the year deaths were rarely attributed to sarcocystosis. Other disease conditions detected in this population were liver abscesses, bacterial hepatitis, gastrointestinal nematodes, and aspergillosis but none of these were significant causes of mortality. Other species in the aviary, doves (Streptopelia sp.), pigeons (Columba livia) and golden pheasants (Chrysolophus pictus) were not affected.

Preliminary genetic sequencing of a portion of the ribosomal RNA genes has indicated that the involved sarcoystis species present is not *Sarcocystis falcatula*, but it may be closely related. Evidence of genetic variation between sarcocystis specimens collected from different host species and at different times has been detected at this institution. If *Sarcocystis falcatula* is not the causative agent of this disease, elements of this parasite's life cycle need re-examination. The accepted opossum (*Didelphis viriginiana*) host and cockroach (*Periplaneta americana*) transmission model may not be accurate at this location. Novel *Sarcocystis* species and hosts are being described so the likelihood of other species is real.<sup>2</sup> Use of oral ponazuril for treatment or prophylaxis has not yielded consistent results in this collection supporting a different sensitivity of the target parasite. Considering that treatment of a peracute disease is generally not feasible, disease control must focus on defining and seeking strategies to interrupt the lifecycle of this possibly novel parasite. Current strategy is the development of genetic probes sufficiently robust to identify this *Sarcocystis* sp. through its lifecycle in definitive and dead-end hosts.

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Hexamita (Spironucleus) meleagridis IN COCKATIELS (Nymphicus hollandicus)

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### Abstract

A small, flagellated gastrointestinal protozoan of psittacines has been described sporadically in the literature. It presumptively was identified as *Hexamita* (*Spironucleus*) *meleagridis*, but molecular characterization of this organism had not been performed. This protozoan has been associated with acute and chronic gastrointestinal disease in psittacines including cockatiels (*Nymphicus hollandicus*) with considerable morbidity and mortality. DNA isolated from the droppings of individual birds in a group of cockatiels that persistently shed protozoa was used in a polymerase chain reaction (PCR) to amplify the gene for 16s rRNA of *Hexamita* spp. Sequence analysis of ~1200 bp revealed a 98% identity with *Hexamita* (*Spironucleus*) *meleagridis* isolated from a turkey (*Meleagris gallopavo*) (GenBank accession EF050054). Based on these sequence data, a PCR assay was developed to determine diagnostic predictive value, prevalence of infection, and morbidity and mortality for cockatiels and other psittacines.

# **ACKNOWLEDGMENTS**

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# EVALUATION OF THE THERMAL ANTINOCICEPTIVE EFFECTS OF BUTORPHANOL TARTRATE IN AMERICAN KESTRELS (Falco sparverius)

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# **Abstract**

Partial kappa-opioid agonist and mu-opioid antagonists, like butorphanol and nalbuphine, are currently the recommended opioids for acute pain management in psittacines. <sup>1-4</sup> Pure mu-opioid agonists, like hydromorphone, also have been evaluated previously in birds with conflicting results, <sup>5-9</sup> but they have been shown recently as potential analgesics for American kestrels (*Falco* sparverius). 10 A blinded randomized complete crossover study using foot withdrawal threshold to a noxious thermal stimulus was performed to evaluate the antinociceptive effect and duration of action of butorphanol tartrate. Butorphanol tartrate (1, 3 and 6 mg/kg i.m., Fort Dodge Animal Health, KS 66210, USA) and saline solution (0.9% Saline, Hospira Inc., Lake Forest, IL 60045, USA) were evaluated in 15 kestrels. Baseline thermal withdrawal threshold data were generated prior to drug administration then thermal foot withdrawal threshold measurements were obtained at 0.5, 1.5, 3, and 6 hr following butorphanol administration. Kestrels were assigned an agitationsedation score and monitored throughout the testing period for adverse effects. Butorphanol tartrate caused sex-dependent responses in American kestrels. The increase in mean threshold in females was suggestive of very mild analgesia; however, mild hyperalgesia and agitation, especially at higher dosages, were observed in male kestrels. Butorphanol tartrate might not provide clinically effective analgesia in American kestrels. Further studies with other types of stimulations, formulations, dosages, and routes of administration are needed to fully evaluate the analgesic and adverse effects butorphanol in kestrels and other avian species and its relevance in the clinical setting.

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# SEDATION AS AN ALTERNATIVE TO GENERAL ANESTHESIA IN ZOOLOGICAL COMPANION ANIMAL PATIENTS

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#### Abstract

General anesthesia is used frequently in zoological companion animal medicine for purposes in addition to surgery. Indications include sample collection, diagnostic imaging and minor therapeutics. General anesthesia carries a significantly higher risk than sedation alone. Newer drugs and drug combinations have been found extremely useful and generally safe for sedation in zoological companion animal patients, and provide a viable alternative to general anesthesia. Use of sedation for minor procedures is further amplified when combined with local analgesia.

#### Introduction

Anesthesia is defined by the American Society of Anesthesiologists (ASA) as a pharmacologically induced reversible state of amnesia, analgesia, loss of responsiveness, and loss of skeletal muscle reflexes, or more simply "without sensation". In contrast, sedation is a "drug induced depression of consciousness during which patients cannot be easily aroused, but responds purposefully following repeated or painful stimulation. The advantages of sedation primarily focus on ease of administration, patient safety, and in human medicine, on relative cost. A study comparing death rates in dogs, cats and rabbits indicated a 2.5 times higher death rate in anesthetized verses sedated patients; however numbers of sedated patients were small and significance is uncertain. While not yet scientifically demonstrated, it is hopeful that sedation can provide a safe, effective alternative in exotic species in cases where general anesthesia carries increased risk or is non-essential. Additionally, sedation can be an effective adjunct to physical restraint, a means to reduce stress in hospitalized patients, and potentially a method to reduce patient memory of unpleasant procedures.

Disadvantages of sedation can include incomplete elimination of patient movement, patient semi-awareness, and lack of analgesia. Other disadvantages include risks associated with use of the drugs themselves; however these can be mitigated with careful drug selection and dosing, patient selection, and vigilant monitoring.

The American College of Veterinary Anesthetists (ACVA) has published recommendations for monitoring patients that are sedated without general anesthesia. For these patients, intermittent monitoring using similar parameters as for general anesthesia is recommended. If the patient is sedated to the point where protective airway reflexes are lost, monitoring should proceed as with a fully anesthetized patient (continuous monitoring). Supplemental oxygen, and endotracheal tube and materials to obtain vascular access should be readily available.

# **Drugs Used For Sedation**

Many drugs and drug combinations have been evaluated specifically for sedation in humans, with significant focus on sedation of the critical patient. While anesthetic studies are relatively plentiful, only a few drugs have been investigated specifically for sedation in animal species, most commonly in traditional companion animal species. In general, dosages for sedation are lower than dosages of the same drugs when used for pre-anesthesia, induction or general anesthesia. In zoological companion animals, potential agents include ketamine, xylazine, medetomidine, midazolam, diazepam and opioids (specifically butorphanol in birds), alfaxalone and others. In some zoological companion animal anesthetic studies, complete anesthesia is not achieved; therefore, results are better described as sedation.

### Midazolam

Midazolam is a benzodiazepine sedative with no analgesic effects, and is used with increasing frequency as an alternative to general anesthesia for a variety of procedures in human patients. Midazolam reduces anxiety, and has been shown to produce amnesia in humans and some laboratory species.<sup>3,4</sup>

Reports on the use of midazolam in zoological companion animal patients are scarce, and focus mainly on use in combination with other drugs for anesthesia, but not specifically for sedation. The effects of midazolam can be reversed with flumazenil.

# **Opioids**

Opioids are frequently combined with midazolam for sedation in humans, and this combination has been found useful in birds and mammals. The effects of some opioids can be reversed with naloxone.

# Ketamine

Ketamine is a NMDA receptor antagonist used in combination with other drugs for sedation, and as part of induction and general anesthesia. At low doses, ketamine has a wide safety profile in many species and can be an effective addition to other agents for sedation in zoological companion mammals. Lower dosages are analgesic. Combinations described in humans and animals include ketamine/midazolam, ketamine/opioid and ketamine/dexmedetomidine. Suggested dosages for sedation in zoological companion mammals range from 5-10 mg/kg.

### Dexmedetomidine

Dexmedetomidine is the newest agent for use for light sedation of critical human patients in the ICU, and is believed to have a safety profile superior to benzodiazepines. Dexmedetomidine has also been evaluated and found to be effective alone and with the addition of other agents in cats. The author has used dexmedetomidine in combination with low dose ketamine and an opioid, with or without the use of midazolam for pre-anesthesia and sedation in rabbits. Optimal dosages

of dexmedetomidine for sedation in zoological companion patients are unknown. Effects of dexmedetomidine can be reversed with atipamezole.

# Alfaxalone

Alfaxalone (Alfaxan, Jurox, NSW, Australia) is an injectable anesthetic agent used for induction and maintenance of anesthesia in dogs and cats. Anecdotally it is useful in some zoological companion species as well. The author's experience is with the use of Alfaxalone as a preanesthetic or sedative for reptiles. The drug is available in Australia, and the United Kingdon, but not currently manufactured and distributed in the United States. It can be acquired legally using the Importation of Drugs regulations administered by the US Food and Drug Administration (www.fda.vob/ForIndustry/ImportProgram/ucm173751.htm).

#### **Sedation in Birds**

In the author's experience, response to administration of midazolam and butorphanol for sedation is variable, and ranges from profound to barely perceptible. Onset after intramuscular injection is rapid, within 2-3 min. The profoundly sedated bird does not stand, but rests on the sternum with the head over the back or down. Some rest in a head down and tail up position. Respirations are generally slow and regular. In birds with respiratory distress, respiratory rate and effort is usually improved. In all cases, birds can be roused to a standing position, and react immediately to handling or discomfort. When left undisturbed, the bird returns to a sleeping position. Length of sedation is variable, but ranges from 20 min to several hours, with progressively decreasing level of sedation over time.

The largest factor affecting degree of sedation appears to be overall patient condition and demeanor, with more profound effects seen in ill or calm birds. For this reason, dose modification is based primarily on degree of debilitation. No species, sex or age predilections have been confirmed, but may emerge with expanded usage.

# **Psittacines**

While every effort should be made to practice safe, atraumatic handling techniques, and even more importantly to train young birds to tolerate and accept the medical examination, in some cases, handling and examination produces extreme stress, continuous vocalizations, marked increased respiratory and cardiac rate, and hyperventilation. The author and others have observed cases of anxiety followed by seizures in parrots, in particular African grey parrots. In many of these cases, diagnostic tests & procedures did not indicate an underlying medical etiology for seizure, therefore these are assumed to be stress-induced. Sedation of these patients is extremely useful. The level of sedation varies from bird to bird, with some resting while undisturbed on the sternum, and most dozing while standing. Level of sedation decreases over time, and birds are generally standing and reacting to visual stimulation within 15-30 min. Recovery is improved with administration of flumazenil.

#### **Sedation in Mammals**

Combinations of midazolam and an opioid have variable results in zoological companion mammals, from profound to non-perceptible. In the author's experience, the largest factor influencing effect appears to be clinical condition of the patient, rather than drug selection and/or species. If the initial combination does not provide adequate sedation, the dose may be slightly increased, or additional agents may be added, in particular ketamine and dexmedetomidine in low doses. Use of sedation has been associated with extremely low morbidity and mortality in clinical practice.

# **Sedation in Reptiles**

The author and others have experience with the use of Alfaxan in reptiles in clinical practice. The best uses for Alfaxan in reptiles appear to be the following: a) induction (with or without pre-anesthetics) followed by immediate intubation and maintenance with isoflurane; and b) sedation (with or without other agents) combined with local analgesia for brief, minor procedures. Even when combined with pre-medications, Alfaxan alone does not appear to achieve an acceptable surgical plane of anesthesia at currently explored dosages, and is therefore best described as a pre-anesthetic/sedative agent for this species. Duration of action is variable but in general brief, often no more than 15 min. Full recovery is usually within 1 hr, but can be longer when combined with other agents, especially in debilitated patients. Dosages required appear to be higher in chelonians and green iguanas, and lower in snakes and leopard geckos. The author always begins with the lower end of the dosage range, adding boluses as needed to effect.

# **Indicatons for Sedation in Zoological Companion Animal Patients**

# Handling and Restraint

A number of patients experience stress during handling, and may present danger to the handler. For these patients, efficient, safe restraint (or use of a squeeze cage when applicable) plus administration of sedative agents can be extremely useful.

# **Respiratory Distress**

A number of disease conditions produce variable degrees of respiratory distress in zoological companion animal patients. In some cases, distress is extreme, and handling is risky. Patients in respiratory distress are placed in a gently warmed incubator with oxygen for 10-15 min, then given midazolam by i.m. injection and returned quickly to the incubator. If additional sedation is desired, administer butorphanol by i.m. injection. The author has not noted a single case of worsening respiratory distress in sedated birds or mammals with sedation.

# Diagnostic Sampling and Imaging

While sedation decreases anxiety and struggling during radiography, complete reduction of

patient movement is superior with general anesthesia. However, calm handling and patience results in production of high quality radiographs in patients for which general anesthesia is considered excessively risky. Collection of diagnostic samples, in particular blood is easier in the calm, sedated patient.

# Establishment of Vascular Access

Many patients requiring vascular access are by necessity higher risk patients. Vascular access can be accomplished with the use of sedation, plus local analgesia over the catheterization site. Topical lidocaine gel is followed by injection of lidocaine at the site. Careful movement of the skin away from the vessel of choice is necessary to avoid inadvertent intravenous injection. For intraosseous catheterization, lidocaine is injected subcutaneously over the desired location, and into the periosteum of the bone.

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**Table 1.** Suggested drug dosages for sedation in zoological companion animals. Dosages are based on clinical trial and error only. Note administration is by i.m. injection, which is considerably less stressful than restraint for i.v. injection.

Drug	Dosage	Comments
Midazolam	0.25-0.50 mg/kg	Mammals and birds
	2 mg/kg intranasal (birds)	Doses up to 1-2 mg/kg for rodents have been reported in the literature. In the author's experience, higher dosages are linked with increased cardiopulmonary depression.
Opioid		
Butorphanol	2-4 mg/kg (birds)	
	0.2-0.4 mg/kg (mammals)	Produces marked sedation in ferrets; use lower doses in this species
Buprenorphine	0.01-0.05 mg/kg	Note: analgesic dosages for many rodents are much
Hydromorphone	0.10 mg/kg	higher
Ketamine	5-10 mg/kg	For use in combination with midazolam with or without an opioid
Dexmedetomidine	0.005 mg/kg	For use in combination with midazolam and/or ketamine with or without an opioid
Alfaxalone	5-25 mg/kg (reptiles)	

# SEX DIFFERENCES IN MELOXICAM PHARMACOKINETICS IN FERRETS (Mustela putorius furo) AFTER SINGLE SUBCUTANEOUS ADMINISTRATION

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# **Abstract**

This study investigated the pharmacokinetics of meloxicam, an oxicam class, non-steroidal anti-inflammatory drug (NSAID), in ferrets. The pharmacokinetic properties of a single subcutaneous dose of meloxicam (0.2 mg/kg) in 9 male and 9 female ferrets were determined. Blood samples were collected from ferrets under isoflurane anesthesia by venipuncture of the cranial vena cava into heparinized syringes. Plasma meloxicam concentrations were determined by high pressure liquid chromatography (HPLC). Pharmacokinetic variables were calculated using non-linear mixed effects modeling to take advantage of the population-based sampling scheme and to minimize sample volume collected per animal.<sup>1</sup>

Maximum plasma concentration, volume of distribution per absorption, elimination half-life and systemic clearance per absorption were 0.663  $\mu$ g/mL, 0.22 L/kg, 11.97 hr and 0.018 L/kg/hr, respectively for females and 0.920  $\mu$ g/mL, 0.30L/kg, 17.97 hr and 0.009 L/kg/hr, respectively for males. Significant differences were found in each of the above parameters between male and female ferrets. Analgesic efficacy was not evaluated, however plasma meloxicam concentrations achieved in these animals are considered effective in other species. Sex differences in the pharmacokinetic behavior of meloxicam should be considered when treating ferrets.

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# BLOOD CONCENTRATIONS OF D- AND L-LACTATE IN HEALTHY RABBITS

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#### Abstract

Rabbits kept as companion animals are often presented for gastrointestinal stasis. Clinical evolution of these patients is often difficult to predict with some cases progressing to shock (hypovolemic, endotoxemic, septic) and/or not responding to medical management. Blood L-lactate levels have been shown to provide diagnostic and prognostic value when managing shock in various species.<sup>1</sup> D-lactate concentration increases significantly secondary to damage from bacterial infections.<sup>2</sup> This study determined normal whole blood and serum values of L-lactate and serum values of D-lactate in 25 healthy rabbits and compared three methods of analysis (Point-of-care portable Lactate Pro, Nova Critical Care Blood Gas Analyzer, and High Performance Liquid Chromatography (HPLC)) for L-lactate measurement.<sup>3</sup> D-lactate values were 0.17 ± 0.08 mmol/L. Results of L-lactate were 5.1 ( $\pm$  2.1) mmol/L by HPLC, 6.9 ( $\pm$  2.7) mmol/L with the portable analyzer and 7.1 ( $\pm$  1.6) mmol L with the blood gas analyzer. No significant difference (p>0.05) was found between values obtained with the portable analyzer and the blood gas analyzer. Significant difference was present between the serum L-lactate values obtained by HPLC and the whole blood values obtained with the blood gas analyzer (p<0.01) and portable analyzer (p<0.05). Serum concentrations of D-lactate in healthy rabbits are similar to those of other mammals. L-lactate values in healthy rabbits are higher compared to those of other mammals.

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# CAUSES OF MORTALITY IN CAPTIVE LESSER HEDGEHOG TENRECS (Echinops telefari)

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# **Abstract**

Tenrecs are common as both educational and exhibit animals. The causes of mortality for various species of tenrecs have not been published, aside from a few reports of neoplasia.  $^{1-3}$  A retrospective survey of causes of mortality for lesser hedgehog tenrecs (*Echinops telfairi*) in Association of Zoos and Aquariums (AZA) zoological institutions was conducted. Twenty out of 32 institutions responded with data for 139 living and 92 dead animals. In response to the survey, 26% (60) of the tenrecs were female, 29.4% (68) were male, and 44.6% (103) were unknown gender. Tenrecs in this survey ranged in age from 0 days to 18 yr old, with average ages of 3.7 yr and 6.4 yr for living and deceased tenrecs respectively. Causes of mortality included neoplasia, cardiomyopathy, hepatic lipidosis, renal disease, pneumonia, septicemia, osteomyelitis and trauma. Neoplasia was the most frequent primary cause of death for 18.5% (18) of deceased tenrecs. Additional frequent causes of mortality included cardiomyopathy, hepatic lipidosis and septicemia, all respectively at 7.6% (7). Gender was a notable factor in the overall analysis; male tenrecs were 4.8 times more likely than female tenrecs to have died from cancer ( $P \le 0.05$ ). Results from this retrospective survey will assist in preventive medicine, diagnosis and treatment of lesser hedgehog tenrecs.

### ACKNOWLEDGMENTS

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# A RETROSPECTIVE STUDY OF THE LESIONS ASSOCIATED WITH IRON STORAGE DISEASE IN CAPTIVE EGYPTIAN FRUIT BATS (Rousettus aegyptiacus)

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#### Abstract

Captive Egyptian fruit bats (*Rousettus aegyptiacus*) are one of many species that frequently develop iron storage disease within zoological collections and laboratory colonies. Previous studies have identified a high incidence of iron storage disease in this species. <sup>1,2</sup> The goals of this study were to determine the complete tissue distribution of iron storage in captive adults and the incidence of other pathologic lesions, including neoplasia and infectious diseases, which may be directly or indirectly related to iron overload. In this multi-institutional study, histologic sections from over 100 adult Egyptian fruit bats of both sexes were evaluated with hematoxylin & eosin and Prussian blue staining for iron. Histologic evaluation of iron was based on the grading scheme in Farina et al. (2005) that was proven to significantly correlate with tissue iron concentrations. Additionally, sections of liver and heart tissue were also stained with Masson's trichrome stain to evaluate for the presence and/or severity of fibrosis. Liver and spleen consistently had the largest amount of iron, but iron was also detected in the gastrointestinal tract, renal tubules, pulmonary interstitium, choroid plexus, and reproductive organs. Hepatic and extrahepatic neoplasia was also identified in iron overloaded bats. Hepatocellular carcinomas were the most common neoplasm, followed by cholangiocarcinoma. Metastatic neoplasms with no hepatic involvement were also identified including a carcinosarcoma, heart-based neuroendocrine mass, and a urinary transitional cell carcinoma. Cardiomyopathy was identified in multiple iron overloaded bats. Hepatic abscesses occurred in association with increased iron storage in multiple cases, although a common etiologic agent was not identified.

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# MYCOBACTERIOSIS IN THE BLACK AND RUFOUS ELEPHANT SHREW (Rhynchocyon petersi)

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# **Abstract**

The black and rufous elephant shrew (Order Macroscelidea) is classified as vulnerable (IUCN); breeding programs were established in AZA facilities in 2000. Mycobacteriois is the most common cause of death in captive adults of this species. This presentation describes mycobacterial infection in 13 black and rufous elephant shrews from five institutions.

Common clinical findings are lameness and joint swelling +/- associated bony lysis. Weight loss, lethargy, respiratory signs, internal granulomas, and non-regenerative anemia may develop as infection progresses. Diagnosis is confirmed by acid fast staining, culture, and PCR of granuloma aspirates or biopsies. *M. intracellulare* is identified most frequently.

Multi-drug antibiotic protocols including azithromycin, rifabutin, and ethambutol have been administered based on recommendations in humans and appear relatively well tolerated.<sup>3</sup> Medical and surgical management have had limited success; infection often results in euthanasia. Mycobacteriosis is often widely disseminated at necropsy. Histologic findings include pyogranulomatous and necrotizing periarticulitis, synovitis, osteomyelitis, lymphadenitis, pneumonia, vasculitis, pericarditis, myocarditis, and hepatitis. Suppurative inflammation is observed more frequently than is typically reported with mycobacterial infection in other species.<sup>4,8</sup>

Common sources of *M. intracellulare* infection include soil and municipal water where it can remain viable for extended periods.<sup>2,7</sup> Management practices to decrease environmental contamination within enclosures are recommended. Compromised immune function, possibly secondary to nutritional, genetic, or viral factors, may contribute to this species' susceptibility to infection.<sup>5,6,9</sup> Further investigation of the nutritional requirements, genetic diversity, and immune status of this species as well as of antibiotic sensitivity patterns and pharmacokinetics is warranted to improve prevention and treatment.

### ACKNOWLEDGMENTS

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# DIAGNOSIS AND TREATMENT OF ATYPICAL MYCOBACTERIAL INFECTIONS IN PALLID BATS (Antrozous pallidus)

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#### Abstract

A group of 16 male pallid bats (*Antrozous pallidus*) was collected from the wild in central Texas in May of 2010 for the purpose of display. Case #1 was an adult male, presented January 2011, with a swollen discolored area one centimeter in diameter on the chest. The bat was examined under general anesthesia. Four swellings identified over the chest and ventrum were biopsied, cultured, opened and flushed. No physical exam findings of external injuries to explain the abscesses were identified. Treatment with systemic antibiotics was initiated. Routine aerobic, anaerobic and fungal cultures were negative. Five acid fact bacilli were identified from the biopsy of the tissue overlying the largest abscess. Samples were submitted for mycobacterial culture and sensitivities. The organism was grown and identified as belonging to the *Mycobacterium abscessus* group. The bat was treated with marbofloxacin at 25 mg/kg q24h, and azithromycin 20 mg/kgq24h.

Cases #2 and #3 were identified in May 2011. They were also treated with marbofloxacin and azithromycin. Investigation to identify the source of the mycobacterium included mycobacterial cultures from all insect cultures and water sources. The same *Mycobacteria abscessus* group was cultured from a water hose interior. The water hose was used to provide drinking and cleaning water for the group. The water hoses were removed from the husbandry routine. Bats were treated 4-6 mo past any occurrence of disease.

Case #1 died February 2012. The bat had been treated for 13 mo. He was the most severely affected and had continued reoccurrence of abscesses with multiple subcutaneous calcified nodules. This bat also had multicentric lymphoma. Cases #2 and #3 completed courses of treatment and appear to be recovered, without recurrence of lesions. A fourth case was identified in February 2012. This bat had one isolated abscess. The abscess was drained and the bat started on a similar treatment protocol.

# A NEW SUPRAGLOTTIC DEVICE AS ALTERNATIVE FOR RABBIT ENDOTRACHEAL INTUBATION

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### Abstract

In rabbits, anesthetic risks are significantly higher than in dogs and cats.<sup>2</sup> During prolonged anesthesia, assuring a patent upper airway is vital to increase the chances of survival.<sup>2</sup> Currently, the most common method to achieve this is endotracheal intubation.<sup>5,8</sup> This method of intubation is complicated by the rabbit's oropharyngeal anatomy and tendency to develop laryngospasm during intubation.<sup>5,8</sup> In addition, post-intubational complications may occur, such as respiratory arrest, laryngeal/tracheal injury or edema, or development of tracheal strictures.<sup>4,5,9</sup>

Because of the difficulties of intubating rabbits, alternative approaches to manage the airway, such as the use of supraglottic airway devices, have been investigated. However, the use of such devices to date have primarily involved experimental studies with human pediatric devices or prototypes for use in laboratory animals. 1,6,7,10,11 In 2009, a novel supra-glottic airway device (v-gel®, DocsInnovent Ltd, London, UK) was developed with the use of rabbit cadavers. After refinement of the prototype, which was designed specifically to fit the rabbit's oropharyngeal anatomy, clinical trials were performed to validate its use in clinical practice. To date the v-gel® has been used in >200 rabbits. In >90% patients, a patent airway was established quickly and easily on the first attempt, and successfully maintained during both spontaneous and mechanically controlled ventilation with minimal leakage of isoflurane. Minor complications (e.g., linguocyanosis, gastric inflation, insertion difficulties due to improper anesthetic depth or dental issues) were encountered in <5% of patients. In addition, recovery was usually quick and uneventful. Results demonstrate that the v-gel® provides an attractive and practical alternative to endotracheal intubation in rabbits.

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# THE CONTRACEPTIVE HEALTH SURVEILLANCE PROGRAM: THE VETERINARIAN'S IMPORTANT ROLE

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# **Abstract**

Contraception is commonly used to manage captive breeding programs. Yet, like any other pharmaceutical, contraceptives are not without risk. In order to define and characterize such risks, contraception is evaluated by comparing the occurrence of adverse reactions in contracepted animals to those occurring in control animals. Because zoo species are usually not included among those for which the label is approved, similar to most of the drugs used by zoo veterinarians, contraceptives are used off-label and efficacy and safety information are gathered while they are being used. Veterinarians rely on this type of information to oversee the health of each individual, but in the case of reproductive health, the species will also benefit.

Since its inception by Dr Linda Munson, the Contraceptive Health Surveillance Program has played an important role in documenting adverse effects of contraceptives such as melengestrol acetate (MGA) through gross pathology and histopathology. Examples of this are the increased risk of endometrial hyperplasia and mineralization in felids and canids with MGA exposure, the risk of mammary adenocarcinomas in MGA-treated animals, <sup>5,8,9</sup> and the inflammatory response in felids to pZP vaccination. The program has also played a key role in documenting that certain conditions are not associated with contraceptive use, such as leiomyomas in felids, ovarian lesions in felids and canids, <sup>7,8</sup> and cystic endometrial hyperplasia in elephants. Currently the effects of deslorelin in canids are being evaluated in collaboration with the Wildlife Contraception Center and Species Survival Plans. <sup>4,10</sup>

Historically, contracepted animals have been compared to non-contracepted as controls; however, more recently it has become clear that there is a difference between non-contracepted parous and non-contracepted nulliparous animals.<sup>2</sup> Non-contracepted females that are housed alone may be exposed to repeated infertile cycles and concomitant endogenous hormones. The time since the last parturition (number of barren cycles) may be a risk factor for certain lesions.

Presently, the Contraceptive Health Surveillance Project tissue archive contains more than 2000 reproductive tracts. Most of them are female, but as newer methods are used such as deslorelin in males, it becomes even more important to continue to contribute to this archive. In spite of the large number of cases, there are some species which are poorly represented, for example hyenas, rodents (porcupines and beavers), small carnivores, and bears. These findings and the ability of the Reproductive Health Surveillance Program to provide information on adverse effects and normal aging pathology of the reproductive tract are possible, thanks to the veterinarians who have submitted the reproductive tracts of contraceptive-treated and non-treated animals along with their complete reproductive histories.

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# IMMUNE MEDIATED HEMOLYTIC ANEMIA SECONDARY TO DISSEMINATED B-CELL LYMPHOMA IN A CALIFORNIA SEA LION (Zalophus californianus)

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# Abstract

A 10-yr-old male California sea lion (*Zalophus californianus*) presented with a 2-mo history of waxing and waning clinical signs associated with central nervous system disease (bouts of unresponsiveness, dull mentation/altered attitude, tremors, mild head tilt, mydriasis, difficulty swallowing). Serial bloodwork, radiographs, and ultrasound were unremarkable. Broad serologic testing revealed no infectious causes. Mentation improved dramatically and clinical signs resolved with high dose prednisone (2.5 mg/kg, p.o., b.i.d.). Efforts to decrease the dose resulted in a return of clinical signs. Over the following 20 days a thrombocytopenia and severe immune mediated hemolytic anemia (IMHA) developed. Hematocrit decreased from 46% to 12% with slide auto-agglutination and poor regenerative response. Treatment with prednisone, azathioprine (2 mg/kg, q24hr, p.o.), leflunomide (4.25 mg/kg, q24hr, p.o.), erythropoietin, iron, and other medications was unsuccessful in stopping progression and the animal died.

At gross examination, abdominal and thoracic lymph nodes were markedly enlarged and effaced by a pale tan, soft, and friable mass. Histologic and immunohistochemical findings confirmed advanced, disseminated multicentric B-cell lymphoma. Altered mentation was attributed to neoplastic dissemination throughout the meninges and superficial cerebral cortex. Blood cell breakdown likely occurred in the tumors, bone marrow, and vasculature secondary to neoplastic invasion. IMHA and thrombocytopenia was compounded by myelophthisis and direct blood loss from a large necrotic mesenteric tumor. Gastric ulceration (despite prophylactic famotidine and sucralfate) was present, presumably related to corticosteroid administration. Leflunomide drug levels were tested and found to be in therapeutic range, suggesting it could be of benefit for adjunctive therapy of primary IMHA in this species, despite the poor success in this case of secondary IMHA.

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# PREVALENCE AND MANAGEMENT OF OSTEOARTHRITIS IN ASIATIC BLACK BEARS (Ursus thibetanus) RESCUED FROM BILE FARMS IN CHINA

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# **Abstract**

Medical conditions including degenerative joint disease have been documented in captive bears.<sup>1</sup> Since 2000, Animals Asia has rescued 277 Asiatic black bears (Ursus thibetanus) and Eurasian brown bears (Ursus arctos arctos) from bile farms in China where they are housed in cages in strict confinement for up to 30 yr and develop chronic infections and inflammation from bile extraction sites, cut teeth and untreated wounds.<sup>2</sup> Of 129 bears that have died since 2000, 18 bears (14%) were humanely euthanatized due to progressive hindlimb paresis/paralysis. 144 rescued Asiatic black bears have been radiographed since 2009 at the China Bear Rescue Centre (CBRC) at the time of abstract submission. Of these, 96 (67%) exhibited joint pathology, 102 (71%) exhibited spinal pathology, and 83 (58%) exhibited a combination of joint and spinal pathology. Of 143 surviving resident bears, 34 (23%) receive medications to manage clinical gait abnormalities. Radiographic pathology and clinical gait abnormalities are not consistently predictive of one another. Over 60 bears (41%) are therefore routinely monitored for clinical gait abnormalities due to radiographic evidence of spinal and/or joint pathology. Medical management includes nutraceutical joint protectants with the addition of NSAIDs such as meloxicam at a standard loading dose of 0.2mg/kg SID followed by a maintenance dose of 0.1mg/kg SID. As lameness progresses, tramadol is trialed at 2-4mg/kg BID followed by gabapentin at 3.5mg/kg SID initially, increased up to 6mg/kg BID. In addition to weight management, specially designed dens and enclosures are incorporated to minimize stress on joints and spines.

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# THE UNITED STATES DEPARTMENT OF AGRICULTURE AND THE ZOO ANIMAL HEALTH NETWORK: A MODEL FOR GOVERNMENT COLLABORATION WITH ZOOS AND AQUARIUMS

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### **Abstract**

Since 2007, the United States Department of Agriculture (USDA) and the Association of Zoos and Aquariums (AZA) have collaborated under the Zoo Animal Health Network (ZAHN) umbrella on multiple projects supporting preparedness for all-hazards emergencies at zoological institutions. Included in these projects is a pilot surveillance program for highly pathogenic avian influenza in three zoos, animated online training materials for zoological and governmental personnel on surveillance for influenzas, and extensive, multi-annexed best practice guidance for emergency planning for the zoological community. Information and materials from these projects are all accessible online through http://www.zooanimalhealthnetwork.org. In addition to these projects, the USDA and AZA facilitated two tabletop exercises this past year linking zoos with their local first responders, state and federal agricultural and public health officials, and livestock industry representatives. The first exercise, called the "Zoo Foreign Animal Disease Coordination Exercise" and orchestrated by the Kansas Department of Agriculture, simulated a national outbreak of Foot and Mouth Disease and involved 10 Kansas zoos. The second exercise, called "Flu at the Zoo" and run by the University of Illinois, drew participants from sixteen zoos in and around Illinois and officials from 10 states and the District of Columbia and simulated an outbreak of Highly Pathogenic Avian Influenza that began in wildlife and spread to zoo animals and then zoo staff. As funding streams diminish for the federal government and zoological institutions, collaborative projects such as these become the model for all parties to accomplish mutually beneficial goals for zoological all-hazards emergency preparedness.

# A FLUKE OF A DIAGNOSIS!: PARASITIC CONJUNCTIVITIS IN AN OSTRICH (Struthio camelus)

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# Abstract

Parasitic conjunctivitis is an uncommon finding in avian species. An adult 15-yr-old female ostrich (Struthio camelus) presented with a two week history of blephorospasm and epiphora. On examination a follicular conjunctivitis was diagnosed, characterized by a mild heterophilic inflammation which responded well to 0.3% ciprofloxacin and 0.1% diclofenac sodium ophthalmic drops along with parenteral enrofloxacin (Baytril 100, 8.5mg/kg q24hr). Three days after the cessation of therapy the conjunctivitis returned. Subconjunctival triamcinolone OU (Kenalog-10, 10mg/ml, 2.5mg OU) proved ineffective. On subsequent examination a carpet of 1.4 - 5 mm long pink organisms, tentatively identified as *Philophthalmus megalurus* (based on geographic distribution and morphologic characters of adults and eggs)<sup>1, 2</sup> were seen attached to the conjunctiva. The closely related fluke, *P. gralli*, has been diagnosed in ostrich in Zimbabwe and Brazil.<sup>3-5</sup> Philophthalmus megalurus transmission involves a freshwater snail and ingestion of metacercariae encysted on solid surfaces (e.g., vegetation, crustaceans). Initial treatment consisted of hypertonic saline ophthalmic drops and manual removal. Despite repeated treatments heavy infestation persisted. Treatment was altered to levamisole injectable (137.5mg/ml solution, 80mg), topically OU along with manual removal q1wk.<sup>3</sup> After three weeks of treatment clinical signs had resolved completely and flukes were only occasionally seen on the conjunctiva but during treatments large numbers were found to be coming out from under the nictitating membrane. After seven weeks of treatment no flukes could be seen during examination and only a rare, non-motile, discolored fluke was removed after topical treatment with levamisole. Two months after treatment there has been no recurrence of clinical signs.

# ACKNOWLEDGMENTS

The authors thank all of the keepers, veterinary and laboratory technicians who made diagnosis and treatment of this case successful.

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# EVALUATION OF METOMIDATE HYDROCHLORIDE AS AN ANESTHETIC IN LEOPARD FROGS (Rana pipiens)

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# **Abstract**

Metomidate hydrochloride is an imidazole-based, non-barbiturate hypnotic drug primarily used as an immersion sedation and anesthetic agent in freshwater and marine finfish. 1-3 To the author's knowledge, there is no documentation in the literature of its use in amphibians. In this study, seven male and four female leopard frogs (Rana pipiens) were induced with metomidate (Metomidate hydrochloride powder, Western Chemical, Inc., Ferndale, WA 98248 USA) via immersion bath at a concentration of 30 mg/L for 60 min. The pH of the induction solution ranged from 7.63 to 7.75. Each frog was then removed from the induction solution, rinsed, and recovered in 80°F (26.6°C) amphibian ringer's solution. <sup>4</sup> After 210 min in the ringer solution, the frogs were transferred to moist paper towels for recovery. Heart rate, gular and abdominal respiration rates, righting reflex, superficial and deep pain withdrawal reflexes, corneal and palpebral reflexes, and escape response were monitored and recorded at defined intervals during both induction and recovery. The average time to loss of righting reflex and escape response was 17.36 min and 17.82 min, respectively. Metomidate produced clinical sedation in all frogs (n=11). Surgical anesthesia was achieved in only 27% (3/11), with an anesthetic duration ranging from 9 min to 20 min. Recovery times were extremely prolonged and varied, with a range from 313 min to greater than 600 min. Our findings suggest that metomidate hydrochloride is unsuitable as a sole anesthetic agent in leopard frogs, and further research is needed to evaluate its suitability in other amphibians.

# **ACKNOWLEDGMENTS**

The authors thank Dr. John Hawke and Judy Wiles with the Department of Pathobiological Sciences, Louisiana State University School of Veterinary Medicine, for their knowledge and expert technical advice

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# MANAGEMENT OF AN OUTBREAK OF COWPOX IN A GROUP OF CAPTIVE CHEETAHS (Acinonyx jubatus soemmeringii)

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### Abstract

Cowpox is a virus in the family Poxviridae. In the United Kingdom wild rodents are the reservoir host and in these species it does not cause overt disease. However, when cowpox crosses out from its reservoir hosts it can cause disease in other species.<sup>2,3</sup> Domestic cats are most often affected, although many species, including man, can be. 4 Here we report the management of a group of nine captive cheetahs (Acinonyx jubatus soemmeringii) during a cowpox outbreak. A family group of five cheetahs developed clinical signs and were immediately isolated. The index case, a 4-mo-old female, was anesthetized and sampled due to the development of a focal raised ulcerative nodular lesion on the rostral lower lip. Cowpox was diagnosed by clinical signs, PCR of tissue and blood and histopathology. Treatment, including supportive care with non-steroidals, covering antibiotics, feline interferon omega and monolaurin was given to all cheetahs with clinical signs. Two of the five cheetahs that developed clinical signs died or were euthanatized. Case follow up includes opportunistic serum antibody titres against cowpox for all cheetahs at the facility and investigation of vaccination. In addition, future potential treatments such as novel anti-viral drugs, immune stimulants or immune modulators and nutritional supplements are being investigated. Finally, ongoing survey work is being done to further characterize and quantify this emerging infectious disease in captive wild species across Europe.

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EXHIBIT FORAGE LARVAL SURVEY FOR GASTROINTESTINAL NEMATODES FROM EXOTIC ARTIODACTYLIDS AT DISNEY'S ANIMAL KINGDOM® AND DISNEY'S ANIMAL KINGDOM LODGE®

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# **Abstract**

Internal nematode parasites are a significant health concern in domestic and non-domestic ruminants resulting in morbidity and mortality. In the southeastern US as well as in other warm, humid climates, this is primarily due to the abomasal worm, *Haemonchus* spp. <sup>1,2</sup> Disney's Animal Kingdom<sup>®</sup> and Disney's Animal Kingdom Lodge<sup>®</sup> utilize a multifaceted, holistic parasite control program that keeps drug resistance prevention in mind integrating diagnostic tools with strategic parasite control focusing on both animal and environment. One component of this program includes forage larval counts (FLC). Exhibit populations, forage populations, seasonal changes and rain accumulation can all influence what worm populations are present in exhibits.<sup>1,2</sup> FLC (expressed in larvae/kg forage dry matter [DM]) is a diagnostic test that identifies "hot zones" for strategic environmental control.<sup>2</sup> A 2-yr investigation of worm populations on the savannah exhibits at Walt Disney World® using FLC showed variability by exhibit region and season. This information has proven helpful for developing animal collection and exhibit management strategies, fecal removal schedules and savannah forage maintenance, including irrigation strategies. FLC is not an in-house test and requires a partnership with a university parasite laboratory. Fecal sampling and monitoring frequency is program-dependent and may not be critical to an institution's strategic parasite control program. If testing is indicated, performing monthly or alternate month sampling is recommended for the first year to identify areas of concern. Follow-up annual or biennial testing may be indicated to monitor for any significant change in population trends.

# **ACKNOWLEDGMENTS**

The authors thank the technicians and husbandry teams at Disney's Animal Kingdom<sup>®</sup> and Disney's Animal Kingdom Lodge<sup>®</sup> for their tireless hours of fecal and forage processing to make this project possible as well as the technical support team at Louisiana State University parasitology laboratory for sample processing and testing.

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# OCCURRENCE OF Toxoplasma gondii, PAPILLOMAVIRUS AND POXVIRUS INFECTIONS IN BRAZILIAN DOLPHINS

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### **Abstract**

Infectious diseases are considered biologic threats in different dolphin populations.<sup>1,2</sup> Among them, *Toxoplasma gondii*, Papillomavirus and poxvirus are well documented worldwide.<sup>2</sup> This abstract describes the presence of these diseases in Brazil. *Toxoplasma gondii* was seen in a Guiana dolphin (*Sotalia guianensis*) from the Brazilian Southwest. Tissue cysts and groups of tachyzoites were observed in lung, liver, kidney, adrenal gland, eye and intestinal samples, mostly surrounded by mononuclear cells and necrosis. Immunohistochemistry was performed using a polyclonal antibody to *T. gondii*. All tissues were positive to this protozoan agent. By ultrastructural assessment, tachyzoites were observed being engulfed by Kupffer cells and within glomerular tufts in the liver and kidney samples, respectively.

Papillomas were observed in the oral mucosa and surrounded the genital slit of a rough-toothed dolphin (*Steno bredanensis*) from the Brazilian Southeast. Histologically, these masses were composed of epithelial hyperplasia, elongation of the dermal papillae, koilocytosis, some bizarre mitoses in the basal epithelium and mild dermatitis. Inclusion bodies were not observed. Ultrastructurally, round to hexagonal intra-nuclear viral particles approximately 40 nm in diameter, compatible with Papillomavirus, were observed in the epithelial cells.

Poxvirus-like lesions ("tattoo lesions") were diagnosed in a Guiana dolphin from the Brazilian Northeast. Grossly, it was observed as an irregular, dark skin lesion near the dorsal fin. Histologically, acidophilic cytoplasmic inclusion bodies were observed within epithelial cells.

These findings show that Brazilian dolphins are exposed to infectious agents that cause morbidity and mortality. Furthermore, the presence of these infectious diseases may represent an important tool to assess the marine environmental conditions along the Brazilian coast as related to incidence of infectious disease.

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# ECHOCARDIOGRAPHIC, ELECTROCARDIOGRAPHIC, AND RADIOGRAPHIC ANALYSIS IN THE GREEN IGUANA (Iguana iguana)

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# **Abstract**

Cardiac disease can cause significant morbidity and mortality in captive reptiles, but antemortem diagnosis and monitoring are hindered by a lack of standardization in diagnostic procedures. The authors have developed methods to standardize echocardiography, electrocardiography and cardiac radiography in apparently healthy adult iguanas. Echocardiographic anatomy was published descriptions. 1,2 with reference to necropsy specimens and Echocardiographic examination allowed reliable visualization of the great vessels, atria, and ventricle, as well as the associated valves. Intracardiac chamber diameters tended to increase with body size, while great vessel diameters were less reliably correlated. The indistinct endocardial surface of the ventricular myocardium prevented measurement of internal diameter, but the measured change in outer diameter between systole and diastole may provide an index of systolic function. Systolic function was also assessed by pulse-wave Doppler measurement of ventricular outflow velocities. Color Doppler imaging showed that insufficiency of the atrioventricular and left aortic valves was common, with atrioventricular regurgitation present in over 60% and aortic regurgitation in over 75% of the population. A six-lead electrocardiogram allowed reliable identification of P waves, QRS complexes, and T waves, with complexes and timing similar to those previously reported in reptiles.<sup>3,4</sup> Radiographic visualization in the right lateral view allowed repeatable measurement of the width of the heart perpendicular to the sternum, which may prove a useful indication of generalized cardiomegaly. This study describes a method for standardizing cardiac diagnostic testing which may facilitate diagnosis and monitoring of heart disease in iguanas and other lizards.

# **ACKNOWLEDGMENTS**

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# CAPTURE OF SANDHILL CRANES (Grus canadensis tabida) USING ALPHA-CHLORALOSE

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# Abstract

The International Crane Foundation has captured greater sandhill cranes (Grus canadensis tabida) in Wisconsin for long-term ecologic research using oral delivery of alpha-chloralose (AC). The goals of this study were to assess the efficacy of modest changes implemented in 2002 in drug deployment (regimented baiting limited to early fall) and post-capture treatments (fluid administration) intended to reduce capture-associated morbidity and mortality, especially exertional myopathy (EM). 317 captures made between 1990 and 2011 were reviewed. Capture efficacy (the proportion of capture attempts where all cranes in a targeted social group were successfully immobilized) improved from 65% to 72% following the aforementioned changes in 2002; however there was no statistically significant difference in sedation scores. The proportion of cranes that were diagnosed with EM decreased from 7/188 (3.7%) to 3/129 (2.3%), and the overall mortality observed among the captured cranes decreased from 9/188 (4.8%) to 4/129 (3.1%). Time in confinement (elapsed time between capture and release, including processing and recovery in a portable pen) was reduced by 3 to 4 hr in birds that received subcutaneous fluids compared to those that did not ( $F_{2,213} = 6.6$ , p = 0.002), but no preventive association was found between fluid administration and the development of EM. The findings of this follow-up study suggest that these management changes in bait deployment resulted in modest improvement in the efficacy of the field capture technique and were associated with decreased morbidity and mortality rates with little change in sedative effect. This method is associated with very low morbidity compared to alternative practices used to capture groups of cranes.

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# INFECTIOUS PATHOGENS AND RESISTANCE TO DISEASES RELATED TO URSIDS: ARE MICROPARASITES A FACTOR IN THE URSID THREATENED SPECIES MANAGEMENT PLANS?

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### Abstract

The Carnivora comprise 15 families<sup>1</sup> and they are identified as one of the mammal groups most threatened by infectious agents.<sup>2-4</sup> However, in the case of Ursids, several authors have suggested that members of this family have a high resistance to infectious diseases<sup>5-11</sup> and therefore infectious disease wouldn't be relevant for their management. In order to document the relationship between pathogens (viruses, protozoa, and bacteria), susceptibility to infection and clinical disease in ursids, a literature review was conducted.

Reports (which included pathology, parasitology, molecular diagnostics, isolation and serum titers) document susceptibility to infection by 43 different pathogens. Additionally, at least 65 clinical reports documented disease caused by 20 pathogens, with viruses being the most common pathogen type associated with clinical disease. Although these reports mostly document individuals being affected rather than entire wild populations, it is very important to take infectious diseases into account for ex-situ and translocation management programs. Thus, biosecurity and preventive medicine protocols may be established for selected pathogens as an important issue for captive bear populations and translocation programs. In conclusion, further studies about the relationship of infectious pathogens and *Ursid* family may be conducted.

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# CIBZ: ONE HEALTH IN SOUTH AMERICA FROM THEORY TO PRACTICE

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### Abstract

South America has huge wildlife diversity, but there is scarce data available about diseases and a limited number of wildlife veterinarians and financial resources for health management. Buin Zoo Conservation and Research Department (CIBZ) was created in 2010 with "One Health" as a philosophy with the mission to address wildlife health management based on scientific criteria. The goal of CIBZ is to serve as a tool to answer questions such as "What", "Who", "Where" and "When" in wildlife disease research and to develop management proposals using interdisciplinary and inter-institutional working networks.

Three programs have been created: education and training, disease surveillance, and the management of health issues in the region. After 2 yr through the first program more than 400 students and health professionals in the region have been exposed to different issues regarding wildlife and zoo animal health. Through the second program a serum and tissue bank has been established to allow health screening in more than 500 captive wild mammals for different infectious pathogens (*Brucella abortus*, *Salmonella* sp., *E. coli*, *Mycobacterium avium paratuberculosis*, *Leptospira interrogans*, MRSA, Canine Distemper Virus, Bovine Viral Diarrhea, *Neospora caninum*, *Toxoplasma gondii*, *Cryptosporidium* sp., *Giardia* sp.). Currently CIBZ is working to provide information on the health status of four endangered mammal species in the region (Andean bear, Chilean Huemul, Darwin's Fox and Chilean Pudu). The third program involves the implementation of zoological medicine into local wildlife conservation programs. Through these efforts the standards of wildlife health management are being raised in natural and artificial environments in South America.

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# TRANS-COELOMIC ULTRASOUND FOR REPRODUCTIVE MONITORING IN A FEMALE FIJIAN BANDED IGUANA (Brachylophus bulabula)

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### Abstract

The Fijian banded iguana (*Brachylophus bulabula*) is a highly arboreal, endangered iguana that is rarely encountered in the wild. Yolk coelomitis is an important cause of death in captive female iguanas, though much about this disease physiology remains poorly understood. Affected iguanas often present with non-specific or no clinical signs of illness and plasma biochemistry results are difficult to distinguish from those of normally gravid females.

The Houston Zoo houses 2.1 iguanas, with one pair and one single male housed separately. The female iguana has been monitored closely via monthly trans-coelomic ultrasounds from 3 to 7 yr of age (June 2008 to May 2012). Ultrasounds are performed under manual restraint in a warm water bath, using a Sonosite 180 Vet Plus ultrasound machine and 10-5 MHz linear transducer. The iguana laid one clutch before ultrasounds began in 2007, and laid 5 more clutches between June 2010 and May 2012. Despite being in a breeding situation, none of the clutches have produced fertile eggs. Monthly average follicular diameters ranged from 0 cm (no structures seen) to over 4 cm when ova are mature, just before oviposition. Ten blood samples taken over the 4-yr time period show intermittent marked elevations in total WBC and in plasma levels of calcium, phosphorus and protein, with the lowest values occurring shortly after eggs are laid. Routine, non-invasive monitoring of the Fiji iguana has helped to establish expected cycling patterns and has the potential to predict when ovostasis and possibly yolk coelomitis is likely to develop.

# DYNAMIC Salmonella SHEDDING IN A COLLECTION OF ZOO REPTILES

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#### Abstract

A *Salmonella* prevalence study was conducted on 200 reptiles at Copenhagen Zoo and followed up by a longitudinal study (n=83) within three weeks of the first sampling. The overall prevalence was found to be 35% (69/200) with significant differences noted between snakes (62%), chelonians (36%) and lizards (15%). The longitudinal study revealed that *Salmonella* fecal shedding status (positive or negative) changed in 25% (21/83) of the reptiles. A total of 30 serotypes were detected and two different serotypes were isolated from 28% (10/36) of the reptiles testing positive in both sampling times. Sixteen serotypes were isolated more than once, and five of these were isolated from more than one species. *Salmonella* ser. Eastbourne was the predominant serotype in both the cross-sectional (22/69) and the longitudinal study (15/44). The data support the theory of dynamic *Salmonella* carriage and shedding.<sup>1</sup>

# **ACKNOWLEDGMENTS**

The authors thank the reptile staff at Copenhagen Zoo's for assistance during the sampling.

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# EOSINOPHILIC PLAQUE IN FOUR RELATED SUMATRAN TIGERS (Panthera tigris sumatrae): REVIEW OF DIAGNOSTIC AND MANAGEMENT CHALLENGES

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# **Abstract**

From January to March 2011, four related Sumatran tigers (sire and three juvenile littermates) simultaneously developed single to multiple well-demarcated, erythematous and ulcerated plaques on the skin of the back or at the tail base. Skin lesions were diagnosed histologically, as eosinophilic plaques, characterized by chronic eosinophilic diffuse to perivascular dermatitis with acanthosis and ulceration. Due tomarked ulceration, advanced cases of eosinophilic plaque, such as these, can resemble herpetic dermatitis and insect bite hypersensitivities. Eosinophilic plaque is distinguished, from the other feline eosinophilic skin diseases, by prominent acanthosis, spongiosis and mucinosis and the absence of eosinophilic degranulation ("flame figures"), granulomatous inflammation or intranuclear inclusions. Two of the four tigers also developed lip lesions consistent with indolent ulcers and one tiger developed an oral plaque suggestive of an eosinophilic granuloma. While eosinophilic plaques are often related to an underlying allergic hypersensitivity, indolent ulcer and eosinophilic granuloma often lack a direct association with clinically evident hypersensitivity. At the same time as the lesions developed, the keepers observed a significant increase in the biting stable fly (Stomoxys calcitrans) population. Therefore, treatment included oral prednisone, intralesional Depomedrol, topical hydrocortisone and fly control (repellant and exhibit foliage removal). Near complete resolution was observed within 3 mo, by late April to early May 2011. Previously published cases of oral eosinophilic granulomas in tigers have been treated with corticosteroids, surgical removal, cryotherapy and antibiotics with variable resolution.<sup>2</sup> The juvenile littermates were exported or are currently being prepared for exportation to other institutes without reports of further recurrence.

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The authors thank Dr. Verena Affolter for her expertise.

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MANAGEMENT OF UTERINE FIBROIDS AND OVARIAN CYSTS WITH LEUPROLIDE ACETATE IN AN ALLEN'S SWAMP MONKEY (Allenopithecus nigroviridus)

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# **Abstract**

A 13-yr-old female Allen's swamp monkey (*Allenopitchecus nigroviridis*) presented with intermittent excessive vaginal bleeding and a history of irregular menstrual cycles. This animal had a melengesterol acetate implant left in for 6.5 yr before it was removed 4 yr prior to clinical presentation.

On examination, abdominal ultrasonography revealed a subjectively thickened endometrium with an irregular, mottled appearance. Additionally, three uterine fibroids (leiomyomas) were identified, ranging from 7 to 9 millimeters in diameter. The right ovary was found to have both a simple cyst and follicular cyst, and the left ovary also contained a follicular cyst.

After consultation with an OB/GYN, a treatment protocol, consisting of intramuscular injections of leuprolide acetate (Lupron Depot® 3.75 mg suspension, Abbott Laboratories, North Chicago, IL 60064 USA) monthly for 6 mo was elected. Leuprolide acetate has been used in human patients for treatment of endometriosis and uterine fibroids. Recheck ultrasound at 3 mo showed a decrease in fibroid diameter and resolution of all ovarian cysts. At 7 mo, there was a normal contour to the uterine body with only one uterine fibroid remaining. Complete blood counts and serum biochemical profiles were also assessed at times of examination and found to be within normal limits during treatment.

The animal clinically had no further vaginal bleeding. Gastrointestinal side effects have been reported in humans with leuprolide acetate (product insert), and this animal had a two-day period of abdominal distention suspected to be intestinal gas that spontaneously resolved. No other adverse reactions were observed.

# **ACKNOWLEDGMENTS**

The authors thank the mammal and veterinary staff at the Toledo Zoo for their assistance with this case.

# **UROLITHIASIS IN CHELONIANS: 38 CASES (1987 - 2012)**

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# **Abstract**

Urolithiasis is commonly reported in chelonians.<sup>2-5</sup> The aims of this retrospective study were to evaluate the presentations, clinicopathologic, imaging and surgical procedures performed in chelonians with urolithiasis. The medical records of client owned chelonians presented to the University of California, Davis Veterinary Medical Teaching Hospital (VMTH) between 1987 and 2012 were reviewed and 38 cases with confirmed urolithiasis were identified. The inclusion criteria was confirmation of urinary calculi through computed tomography (13/38), ultrasound (5/38), post mortem examination (21/38) or surgery (20/38). Cases that had radiographs with only a suspicion of the presence of urinary calculi were not included. The most common species represented was the desert tortoise (Gopherus agasizzii) (29/38). Of the 34 patients that the sex was reported, 18 were male and 16 were female. Sixteen patients presented either as a referral for suspected urolithiasis or for signs directly associated with the clinical manifestation of urolithiasis including constipation, egg binding, and/or cloacal prolapse. Thirty-one patients had blood work available for analysis. For the desert tortoises, the mean hematology and plasma biochemistry values that were outside of reference intervals included packed cell volume, heterophil count, concentrations of aspartate aminotransferase, total protein, and globulin. Of the 20 animals that had surgical intervention for their calculi, 60% received a plastronotomy (12/20) and 3 cases received lithotripsy intervention. Twelve of the 38 presented cases had calculi analyzed and were all composed of 100% urate. Urate urolith prevention strategies, including diet and environmental changes should be evaluated further in chelonians.

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# HEMATOLOGIC AND PLASMA BIOCHEMISTRY VALUES IN FREE-RANGING AND CAPTIVE WESTERN POND TURTLES (Emys marmorata)

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# **Abstract**

The western pond turtle is listed as a Species of Special Concern by the California Department of Fish and Game (CDFG) and the species is limited to the west coast of the United States and Mexico, ranging from Washington state to northern Baja California.<sup>4</sup> It is a common wildlife patient in veterinary hospitals and wildlife rehabilitation centers within its geographic range.<sup>2,4</sup> Two populations in northern California, a free-ranging population from a university campus habitat (n=20) and a captive population from a zoological collection (n=10), were sampled in September 2011. Complete blood cell counts, plasma biochemistries, and Salmonella spp. cultures from cloacal swabs were performed. Individual parameter values that were noted to be significantly different between the two populations included heterophil, azurophil, eosinophil and monocyte counts, albumin, aspartate aminotransferase, calcium, glutamate dehydrogenase, globulin, sodium, total protein and uric acid concentrations. Parameter values that were noted to be significantly different between male and female individuals within the free-ranging population included creatine kinase and phosphorus concentrations. Salmonella cloacal cultures from all turtles were negative and many of the values obtained in this study are similar to those published for other Emydid turtles. The hematologic and plasma biochemistry values reported for this free-ranging population may be used as reference interval for this species; however, differences between the two populations investigated highlights how factors including nutrition and environmental quality may induce changes in commonly evaluated hematologic and plasma biochemical parameters.

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# PREPUTIAL APLASIA, ECTOPIC TESTES, AND SUSPECTED INTERSEX IN A CHINESE MUNTJAC DEER (Muntiacus reevesi)

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### **Abstract**

A 6-vr-old intact male Chinese muntiac deer (Muntiacus reevesi) was examined because of blood in the exhibit. The source of bleeding was the distal penis. Examination revealed a reduced penis, preputial aplasia and ectopic testes. Paraphimosis and paralysis of the penis resulted secondary to an absent inner lamina of the prepuce. The testicles were subcutaneous, caudal to the umbilicus, and cranial to the penis in a cranial-caudal orientation. No body wall defect was identified. A reproductive examination was performed under general anesthesia, which revealed normal male internal accessory sex glands and normal ultrasonographic structure of the testicles. Given the external abnormalities and risk of continued trauma to the penis, partial penile amputation and castration were elected. A Williams' phallectomy and castration were performed without complication. Serum estradiol, progesterone, and testosterone before castration were 18.43 pg/ml, 4.37 ng/ml, and < 0.01 ng/ml, respectively. Two weeks and 2 mo after castration, estradiol and progesterone values were 31.99 pg/ml, 34.7 pg/ml; and 1.23 ng/ml, 5.28 ng/ml, respectively with persistently low (<0.01 ng/ml) testosterone. High estrogen and low testosterone can be seasonally normal in white-tailed deer, however elevated progesterone is likely explained by the presence of ovarian tissue or atypical Cushing's syndrome.<sup>2,3</sup> Histopathology of the testicles did not demonstrate ectopic ovarian tissue. Abdominal ultrasound failed to identify reproductive tissue. Persistent progesterone suggests this muntjac deer is possibly intersex with internal active ovarian tissue. This is believed to be the first reported case of preputial aplasia, ectopic testicles, and possible intersex in any cervid.

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# BODY CONDITION SCORES FOR DESERT TORTOISES

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# **Abstract**

Body condition scoring is a visual appraisal system that estimates average body energy reserves without using scales, calipers, or calculators. Since individuals can vary in size and shape, weight alone is not a good indicator of body condition. The body condition score (BCS) is based on an evaluation of muscle mass and fat deposits in relation to skeletal features and has been adapted to the desert tortoise. This score is dynamic and should improve if the animal is eating and body energy reserves increase. Conversely, the score will decrease if inanition persists or body energy reserves are depleted. A tortoise's body condition will change with life stage, stage of reproduction, season of the year, drought, food availability, and disease. Therefore, this management tool can be used to monitor and compare populations over time.

BCS ranges from one to nine, with one being emaciated and nine being extremely obese.

Assigning a BCS is a two-step process. The numbers are divided into 3 groups.

STEP 1: Choose the grouping that best describes the tortoise at the current point in time.

- a) Under-condition (1-3): best assessed by degree of temporalis muscle atrophy and prominence of the sagittal crest;
- b) Good condition (4-6): best assessed by degree of temporalis muscle development
- c) Over-condition (7-9): best assessed by degree of subcutaneous fat deposition.

STEP 2: More accurately define the score by selecting one of the three numbers within the respective group. Choose the best fit for that individual at the current point in time.

# **ACKNOWLEDGMENTS**

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INTRAOCULAR PRESSURE MEASUREMENT BY APPLANATION TONOMETRY: BASELINE ASSESSMENT IN EXOTIC CARNIVORES AND NON-HUMAN PRIMATES

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# Abstract

Intraocular pressure (IOP) is measurement of fluid pressure within the anterior chamber of the eye. Glaucoma or uveitis may present with abnormal IOP. Applanation tonometry measures IOP by the force required to flatten the cornea. 4,6,7,10

In domestic animals, IOP can be measured under manual restraint with topical anesthesia.<sup>1,2</sup> Although anesthesia can affect IOP,<sup>2</sup> general anesthesia seldom can be avoided in exotic species.<sup>3,5,8,9</sup> Normal IOP reference ranges have been established for domestic species, but little information is available for exotics.

In this retrospective study of one zoological collection, IOP measurements (n=100) were collected over a 5-yr period at a single institution in 22 mammalian species opportunistically during annual examinations. In 73% of the individuals, measurements were obtained more than twice at repeated physical examinations, and in 25% of the individuals, measurements were made from juvenile to adulthood. Anesthetic protocols were maintained consistently within each species. Typically during measurement, animals were positioned laterally. Measurements (in mm of Hg) were obtained in triplicate using a Tonopen®XL (Medtronic, Jacksonville, FL 32216, USA) and dependent eye was recorded.

IOP of the right and left eye was compared by paired t-test by species. No difference was identified at p<0.05 significance. Normal IOP (18mm of Hg) within mammalian species studied was generally consistent. Therefore, differences greater than two standard deviations from this baseline may indicate underlying ocular pathology. Caution should be exercised in interpretation of IOP between eyes of laterally recumbent animals as IOP may be elevated artifactually in the dependent eye. <sup>1</sup>

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INVESTIGATION OF EPIDEMIOLOGIC AND NUTRITIONAL FACTORS ASSOCIATED WITH A GLOBAL EPIZOOTIC OF TRANSITIONAL CELL CARCINOMA IN FISHING CATS (*Prionailurus viverrinus*)

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### **Abstract**

Transitional cell carcinoma (TCC) of the urinary bladder has been previously reported in fishing cats (*Prionailurus viverrinus*) maintained in North American zoos, <sup>1,3</sup> but the pathogenesis and prevalence of TCC are unknown. In this study, our objectives were to: 1) investigate the prevalence of TCC in captive fishing cats in North America and internationally, 2) evaluate risk factors possibly associated with TCC occurrence in North American zoos, and 3) begin assessing nutritional parameters in fishing cats to explore a possible link between diet and TCC. A combination of email survey of zoo veterinarians and pathologic surveillance identified 29 confirmed cases of TCC in fishing cats housed in North American zoos since 1995, representing ~35% of all fishing cats (>5 yrs of age) that died during this time period. Notably, TCC was diagnosed in three imported founders originating from three different fishing cat range countries (Thailand, Cambodia, Sri Lanka). Additional TCC cases (n = 13) were observed in fishing cats housed in European and Australian zoos. Epidemiologic analysis of data from the Fishing Cat International Studbook determined that genetic relatedness, geographic region, number of transfers between zoos, and gender were not (P > 0.05) correlative factors for TCC. Nutritional analysis of serum samples (n=58) from 42 fishing cats (including 19 TCC cases) in 17 North American zoos found increased (P = 0.032) saturated fatty acid and increased (P = 0.048) palmitic acid and decreased (P = 0.022) gamma-linolenic acid (GLA) concentrations in cats affected with TCC versus cats without TCC. Vitamins A and E, and antioxidant levels did not differ (P > 0.05). These findings indicate that TCC is a global disease concern, occurring at an epizootic level in captive fishing cats with no identifiable demographic risk factors. Because fishing cat diets in North American zoos are comprised primarily of beef with very little fish (~20%, on average), we suspect that TCC occurrence may be influenced by dietary factors. Beef-based diets are substantially higher than fish in saturated fatty acids, a dietary component correlated with TCC in humans<sup>2</sup> and found in the present study to be higher in fishing cats with TCC. Similarly, levels of GLA, a tumoricidal fatty acid, were lower in TCC-affected cats. These observations suggest that increasing fish composition of zoo diets to more closely mimic diets of wild fishing cats may be warranted as a preventative measure to reduce TCC-related morbidity and mortality.

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# WHAT A DISASTER! CONTINGENCY PLANNING TOOLS FOR THE ZOOLOGICAL COMMUNITY

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### Abstract

Hurricane Katrina proved to be a seminal moment in the management of animals in disasters. As a result of that devastation and confusion, new legislation was proposed and enacted to assist with evacuation and sheltering of household pets and service animals. This call to prepare for disasters extended to the managed wildlife community as well, but would require a different approach. A proposed rule change to the Animal Welfare Act, in final stages of the approval process, will require contingency planning and training of personnel in United States Department of Agriculture (USDA) licensed facilities.

Contingency planning is currently mandated by several States and accrediting bodies (Association of Zoos and Aquariums, Global Federation of Animal Sanctuaries, etc.) but there are licensed exhibitors and other wildlife owners who have done little contingency planning. A flexible plan can guide the decision making process in times of crisis; however, designing a plan is often challenging and time consuming. A working group assembled by Lincoln Park Zoo's Zoo Animal Health Network, in cooperation with the United States Department of Agriculture Animal Care, created tools to assist facilities in drafting useful contingency plans. A multitude of references are provided, along with best practices and lessons learned.

# COMPARISON OF TWO ANESTHESIA PROTOCOLS FOR CELIOSCOPIC SEXING IN JUVENILE BLANDING'S TURTLES (Emydoidea blandingii)

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# Abstract

The isolated population of Blanding's turtles (*Emydoidea blandingii*) in Nova Scotia have been designated as threatened since 1993. Living at the northern periphery of the species' range, this population is considered particularly vulnerable due to cold temperatures prolonging incubation time, potentially skewing sex ratio towards males and decreasing breeding success. I

Two anesthesia protocols were compared in a group of 2-yr-old Nova Scotia Blanding's turtles undergoing celioscopic sexing as a part of a conservation project. Ninety-four turtles were randomly attributed to one of the two following protocols: morphine (0.5 mg/kg)-dexmedetomidine (0.05 mg/kg)-ketamine (10 mg/kg) i.m. or butorphanol (0.5 mg/kg)-dexmedetomidine (0.05 mg/kg)-ketamine (10 mg/kg) i.m. Only the dexmedetomidine was reversed after the procedure. Body weight, carapace length as well as duration and ease of coelioscopy did not differ statistically between the two groups. Induction and recovery times were also not statistically different between protocols. Level of anesthesia was significantly deeper in the turtles who received the morphine protocol. However, three turtles from the morphine group died postoperatively. The first case of mortality was due to an anaphylactic reaction. The two additional mortalities were suspected to be caused by impaired ability to thermoregulate.

While the morphine protocol provided a deeper level of anesthesia, morphine increased the risk of postanesthetic mortality in Blanding's turtles. Turtles should be monitored closely if morphine is used as they appear to be a heat sensitive species.

### ACKNOWLEDGMENTS

The authors thank Dr. Henrik Stryhn for statistical assistance as well as the students and staff for assistance with the turtles.

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# LESSONS LEARNED: EMERGENCY EVACUATION OF ROOSEVELT PARK ZOO

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### Abstract

On May 30, 2011, the Roosevelt Park Zoo in Minot, North Dakota began to evacuate the majority of its collection. The Souris River that divides the zoo grounds in half was flooding due to increased runoff and rainfall. An evacuation plan was in place but was revised that day due to a higher than expected crest. A small staff of six full-time employees, the director, and the contract veterinarian worked with volunteers to evacuate nearly one hundred and twenty animals in 38 hr. With the exception of three animals, all were evacuated without tranquilization. Some animals were sent to a safe location near Minot while others went to local farms, ranches, or zoological institutions in North Dakota. Animals requiring more permanent housing went to zoological institutions in Kansas, Minnesota, and South Dakota. On May 31<sup>st</sup> the river stopped rising and the zoo was not flooded. On June 19 the river rose again due to record rainfall upstream. The zoo as well as one-third of Minot's population evacuated again with the knowledge that flooding was inevitable. The remaining animals were evacuated to safe locations and supplies and offices were relocated to park district property. By June 23<sup>rd</sup>, the zoo grounds were flooded with 9 to 12 feet of water.

Federal Emergency Management Agency (FEMA) officials began working with the park district to plan the recovery effort. The waters receded in mid-July and work began in August to clean and disinfect. Large amounts of garbage and mold growth were removed. Buildings sustained heavy damage and the perimeter fence was gone. The majority of animals remaining in the Minot area were relocated to other institutions across the country. Currently, repairs to the entrance building are nearly complete, those to the clinic are in progress, but the majority of barns and exhibits are not repaired. No animals have returned due to the absence of the perimeter fence. Lessons learned during the flood event have included preparing and training staff for a disaster, moving animals safely during an emergency, caring for stressed animals after the event, documenting important information during and after the evacuation, and recovering the zoo grounds after the waters receded.

### **ACKNOWLEDGMENTS**

The author thanks the staff and volunteers who assisted in the evacuation and the zoological institutions who have accepted and cared for the animals of Roosevelt Park Zoo since the flood.

# NASAL ADENOCARCINOMAS IN TWO AFRICAN WILD DOGS (Lycaon pictus)

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# Abstract

Nasal adenocarcinomas in domestic dogs are known for their insidious onset, lack of visible nasal deformity, and local aggression. Indeed, nasal discharge is often the only clinical sign seen until very late in the neoplastic disease process. Limited reports of adenocarcinomas are present in wild canids. This case report describes a nasal adenocarcinoma in two 11-yr-old male captive African Wild Dogs (*Lycaon pictus*). Clinical signs included chronic, intermittent, non-antibiotic-responsive epistaxis for 7 mo. Other signs included slowly progressive anorexia, and palpable bone disruption late in the disease. Diagnosis was made using bloodwork, physical exam, skull radiography, cytology and histopathology of a nasal biopsy, as well as nasal computed tomography (CT). Due to the poor prognosis and declining condition, euthanasia was elected. The masses were excised at necropsy and confirmed as a nasal adenocarcinoma without evidence for metastasis. These cases can be compared to nasal adenocarcinomas in domestic dogs, with implications for screening and diagnosis in wild canids.

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# USE OF COMPUTED TOMOGRAPHY AS AN IMAGING GUIDE FOR CASTRATION IN THE CRESTED PORCUPINE (Hystrix africaeaustralis)

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### Abstract

There is little published information describing the male reproductive anatomy of the African crested porcupine (*Hystrix africaeaustralis*). Traditional radiographic and ultrasonographic imaging modalities generally fail to provide adequate anatomic information due to interference from the quills. A 3-yr-old, 16.6-kg, male porcupine was presented for castration. After inconclusive palpation on pre-surgical examination, computed tomography (CT) was used to determine relevant reproductive anatomy and develop a surgical approach for castration. CT imaging confirmed that the testes were located immediately lateral to the prepuce beneath subcutaneous adipose tissue. Testes measured 5cm in length with a diameter of 1.6 cm. Based on CT imaging, a precise pre-scrotal surgical approach was used for castration. Without CT imaging, a wider and more invasive surgical approach would have been necessary, resulting in unnecessary tissue damage and longer healing time. Technologic advancements and decreases in cost are making CT technology more widely available for use with non-domestic species. By providing greater visualization and knowledge of anatomic structures, CT imaging and 3D reconstruction can be of great benefit for surgical procedures, particularly in species where extensive anatomic data does not currently exist.

# **ACKNOWLEDGMENTS**

The authors thank the staff at VIZUA<sup>TM</sup> for their assistance in image rendering for this and other cases.

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USE OF DEXMEDETOMIDINE, MIDAZOLAM, KETAMINE AND REVERSAL WITH ATIPAMEZOLE FOR CHEMICAL IMMOBILIZATION OF GIANT ANTEATERS (Myrmecophaga tridactyla), LESSER ANTEATERS (Tamandua tetradactyla) AND SILKY ANTEATERS (Cyclopes didactylus) KEPT IN CAPTIVITY

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#### **Abstract**

There are very few reports regarding anesthesia of anteaters and there is almost nothing about chemical immobilization of silky anteaters. Over the last 3 yr, the author has tested a new combination for chemical immobilization of three species of anteaters during routine veterinary procedures at "Parque Zoologico Huachipa." The anesthetic combination consisted of ketamine (4±0.25 mg.kg<sup>-1</sup>), dexmedetomidine (20±5μg.kg<sup>-1</sup>) and midazolam (0.1 mg.kg<sup>-1</sup>), administered in one syringe and applied via intramuscular (i.m.) injection. Ten minutes after initial injection, cardiac frequency, oxygen saturation, respiratory frequency, and rectal temperature were monitored every 10 min. The following parameters related to anesthetic quality were also assessed: induction time, effective period of the anesthesia, recovery time, muscle relaxation score, presence or absence of salivation, and protective reflexes. After 50 min of anesthesia, dexmedetomidine was reversed with atipamezole (0.20±0.05 mg.kg<sup>-1</sup>), administered i.m. A rapid time of induction was observed in three species (3.63  $\pm$  3 min.). Recovery was quick and without excitement. Recovery times were different for the three species: 4±2, 8±2 and 4±1 min, in silky, lesser and giant anteaters respectively after administration of atipamezole. Total recovery was achieved at 12±4, 24±3 and 25±6 min in silky, lesser and giant anteaters respectively. Good muscle relaxation and no salivation were observed. No alterations of vital functions were observed during anesthesia. Based on the results, this protocol could be considered as an excellent choice for pharmacologic contention of captive anteaters.

# **ACKNOWLEDGMENTS**

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# A CASE OF TUSK FRACTURE IN A 40-YEAR-OLD FEMALE AFRICAN ELEPHANT (Loxodonta africana)

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### Abstract

An estimated 40-yr-old female wild-born African elephant (Loxodonta africana) housed at an AZA-accredited institution was evaluated for a 48-hr history of fracturing her right tusk within the enclosure. The fracture occurred at the labial margin and a portion of the fracture site extended obliquely below the labial margin; moderate bleeding (later determined to be of gingival origin) and discomfort were present. A literature review of different techniques for estimating coronal pulp length within the tusk revealed both the traditional method of measuring the distance of the labio-dental fold to the eye and assuming a 1:1 ratio of the pulp cavity, as well as the more recent formula that has been published describing findings that suggest the coronal pulp length varies based on age and sex of the animal, but will not extend 300 mm past the lip.<sup>2</sup> According to both measurement techniques, a fracture site at the labial margin should have exposed the pulp canal of the tusk requiring further therapeutics such as vital pulpotomy, endodontics or extraction; however, on clinical examination, there did not appear to be any pulp exposure. The fractured portion of the tusk was removed without sedation. Due to the individual's age and other health concerns, conservative medical treatment with flunixin meglumine (Banamine: Intervet Inc./Merck Animal Health; Summit, NJ 07901) 1500 mg p.o. q24 hr for 3 days, then q 48 hr for 6 days, omeprazole (Gastrogard: Merial; Duluth, GA 30096) 3.42 g p.o. q 24 hr for 2 days, and doxycycline 9000 mg p.o. q 24 hr for 7 days was elected over sedation or anesthesia for more definitive therapy. Eighteen months after initial presentation, no abscessation had been noted and the tusk had begun to regrow. Despite the severity of the fracture, no pulp had been exposed and the tusk was not devitalized.

### **ACKNOWLEDGMENTS**

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# EVALUATION OF THREE TEST KITS FOR THE MANUAL COUNTING OF LEUKOCYTES IN WHOLE BLOOD IN WILD AND CAPTIVE RING-TAILED LEMURS (Lemur catta)

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### **Abstract**

The Becton Dickinson Unopette<sup>®</sup> leukocyte count test has been used as part of health evaluations of wild ring-tailed lemurs at the Beza Mahafaly Special Reserve since 2003. Production of the Unopette<sup>®</sup> was discontinued in 2009. The goal of this project was to select a Unopette<sup>®</sup> replacement test that is accurate and yields results comparable to historical Unopette<sup>®</sup> results. This project compared the Whi-pette test (Exotic Animal Solutions, Inc., Hueytown, AL 35223 USA), the LeukoChek<sup>TM</sup> test (Biomedical Polymers, Inc., Gardner, MA 01440 USA), and a 2% glacial acetic acid test with 1) an automated leukocyte count performed at a United States reference laboratory and 2) the Unopette<sup>®</sup> manual leukocyte count performed in the field.

Leukocyte count tests are considered acceptable if the results are within 15% of the standard test result. For the first part of this study, the standard test was the automated leukocyte count. The Whi-pette and LeukoChek<sup>TM</sup> test kits performed similarly, both with 66% of results within the acceptable range, whereas 22% of the acetic acid test results were within the acceptable range. For the second part of the study, the standard test was the Unopette<sup>®</sup> test. Compared with the Unopette<sup>®</sup> test kit, the Whi-pette and LeukoChek<sup>TM</sup> tests had 73% and 77% of results within the acceptable range respectively, whereas the acetic acid test had 69% of results within the acceptable range. The Whi-pette and LeukoCheck<sup>TM</sup> tests appear to be equally acceptable replacements for the Unopette<sup>®</sup>, whereas the acetic acid test is not an acceptable replacement.

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# GROSS AND COMPUTED TOMOGRAPHIC ANATOMY OF THE LACRIMAL DRAINAGE SYSTEM OF SNAKES

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#### Abstract

Objective: Unique anatomic characteristics of the lacrimal drainage system in snakes may predispose them to obstruction with subsequent clinical complications including distension of the subspectacular space (pseudobuphthalmos) or infection (subspectacular abscessation). This study was designed to define lacrimal duct anatomy in snakes.

Animals studied: Twenty snakes of 10 different species.

Procedures: Direct observation following injection of fluorescein into the subspectacular space, microtomographic imaging following injection of one of three contrast agents into the subspectacular space, gross dissections following injection of latex into the subspectacular space, and histopathologic observations.

Results: Microtomographic imaging following post-mortem injection of barium provided the clearest images. Fluorescein and iodinated contrast agents were not useful. Microtomographic images and gross dissections revealed a single mucosal opening into the lacrimal duct through the ventronasal palpebral-conjunctival space (in the region of the ventral orbital rim). The lacrimal duct then passed in a rostral and ventral direction through a prefrontal foramen. It completed two 90° turns as it passed between the vomer and hypochoanal cartilage before entering the medial aspect of the Jacobsen's organ duct mouth at the rostral aspect of the palate. Of the nine additional snake species dissected, specimens fell into one of three groups based on similar anatomic characteristics of the lacrimal duct; *Python regius, Boa Constrictor, Lampropeltis calligaster*.

Conclusion: This study is the first to utilize 3D reconstruction of micro CT images to provide an accurate anatomic representation of the lacrimal duct of boid snakes with barium contrast while identifying the challenges faced with current imaging modalities.

# OUTBREAK OF Pterygodermatites nycticebi IN CALLITRICHIDS OF THE ROYAL ZOOLOGICAL SOCIETY OF ANTWERP

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# **Abstract**

In September 2010, an outbreak of *Pterygodermatites nycticebi* occurred in a group of 24 callitrichids: 4 Goeldi's monkeys (Callimico goeldii), 1 white-fronted marmoset (Callithrix geoffroyi), 1 common marmoset (Callithrix jacchus), 4 pygmy marmosets (Callithrix pygmaea), 11 golden-headed lion tamarins (Leontopithecus chrysomela, GHLT), and 3 emperor tamarins (Saguinus imperator subgrisescens). A 3-mo-old Goeldi's monkey was treated with injectable ivermectin (Ivomec® – Merial, Brussels, Belgium) at 0.17 mg/kg b.w. and survived, but an 8-moold GHLT died suddenly with hemorrhagic enteritis caused by multiple worms. In the same week, two more 3-mo-old GHLT died of trauma and pneumonia, both with few worms. Since then, feces from all callitrichids were examined monthly. Following a positive fecal exam, flubendazole (Flubenol<sup>®</sup> 5% - Janssen Pharmaceutica, Beerse, Belgium) at 5 mg/kg b.w. for 3 days was administered orally. Egg sizes of P. nycticebi (32 - 45 µm x 22 - 36 µm) and Physaloptera sp (39 - 50 μm x 23 - 34 μm) overlap, which complicates differentiation. <sup>1-7</sup> Since Physaloptera sp was reported before in Antwerp Zoo, we assumed these eggs to be Physaloptera.<sup>3</sup> Adult nematodes however, showed the characteristic features of P. nycticebi: two subventral rows of combs, three buccal teeth, vulva near level of oesophago-intestinal junction.<sup>2</sup> Eradication of P. nycticebi in enclosures with natural substrates is impossible because of its indirect life cycle with cockroaches as intermediate hosts. <sup>4,6,7</sup> As yet, proper monitoring and treatment prevented disease and death.

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# THE DEVELOPMENT AND TESTING OF A DUAL FUNCTION UNDERWATER DART RIFLE

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#### Abstract

A dual function or amphibious underwater dart rifle<sup>3</sup> has been designed by the authors of this abstract. It is a double barrel projector, with one barrel for launching tranquilizer darts and another barrel for launching net darts. In sub-aquatic environments, the rifle launches or projects tranquilizer darts, which are designed to mimic the anatomy and fluid dynamics of the sword fish (*Xhipiasgladius*).<sup>10</sup> The net dart has an outer shell made of pieces ofglass tubing, which form a shell enclosing a net. Each segment of glass tubing is joined to the outer edge of the net. The barrels are 0.59 m in length and the length of the rifle is 1.54 m. The approximate weight is 9 kg. The darts are propelled by the energy released from the combustion of a stoichiometric mixture of hydrogen and oxygen in the firing chamber.<sup>8</sup>Gas pressure in the firing chamber is amplified due to the presence of a blow pipe mouth piece at the exit aperture of the firing chamber.<sup>2-4</sup>

#### Introduction

Special operation units of the naval forces of the US and Russia have been solving a military armament problem, which is shared with aquatic veterinarians around the world. The problem is the development of an amphibious rifle, which can be used under water and above the water surface or on land in the medium of air. In the case of aquatic veterinary medicine it is the development of an amphibious dart rifle, which can be used in sub-aquatic environments and on land. This research team has adapted a multi-step experimental approach to solve the problem of developing an amphibious tranquilizing dart rifle. The rifle we are developing utilizes aerodynamics principles derived from the blow pipe used by the indigenous people of the Amazon. Amazon.

### **Materials and Methods**

Aerodynamic and aerostatic studies conducted by the authors, have demonstrated that the Amazon blow pipes are governed by the major gas laws, Bernoulli's Principle and Poisuelle's Principle.<sup>1-2</sup> The blow pipe barrel was examined with it found to be a rigid tube in which there is a pressure differential due to the forced expiratory volume at one end, where the operator is

introducing a large volume of air from her/his lungs into an extremely small space, the mouth piece of the blow pipe. Ethnotoxicology studies were then conducted on artifacts of the blow pipe, including the darts and blow pipe barrel. Kinematic, bio-mimetic and electrochemical thermodynamic studies were conducted on micro-rockets launched by the energy released from the combustion of hydrogen and oxygen. Utilizing the data from the hydrogen rocket studies, the aerodynamics studies and the ethnotoxicology studies, an amphibious dart rifle has been developed by the authors, with double barrels and magazines for net darts and tranquilizing darts (Figure 1). The tranquilizing darts are dart syringes, encased in a plastic body, which mimic the morphology of the sword fish. In development is an optical system consisting of a regular underwater telescopic camera and a camera with night vision capabilities. The images from the scopes are relayed to 12 cm x 10 cm LCD screen attached to an optical loop and worn as a head gear (Sea Viewer Products, www.seaviewer.com). The image of the target animal can be viewed on the LCD screen, which can be used for aiming the rifle, when in use under water. A laser spotter on the rifle is used for precise location of targets.

### **Results**

An amphibious dart rifle was developed. Figure 1 illustrates the components of the tranquilizing dart. Figure 2 is a diagram of the tranquilizing dart rifle without attachable gas propulsion units.

### **Conclusions**

A stoichiometric ratio of 2 parts of hydrogen to 1 part of oxygen in the firing chamber of an amphibious tranquilizing dart gun provides energy for the expulsion of a dart at muzzle velocities equivalent to darts fired by conventional charges or air pressure.

# Discussion

There is a need for detailed hydrodynamic and hydrostatic studies of the behavior of the underwater darts. Studies will be done to fully understand the motion of the darts under water and improve the function of the net darts. There is also a need to improve the optics of the rifle under water. An operator of the rifle should, with ergonomic ease, view targets through the lens of the diving goggles and remotely adjust the telescopes. The operator should also be able to switch from full spectrum or white light view to IR viewing.

#### **ACKNOWLEDGMENTS**

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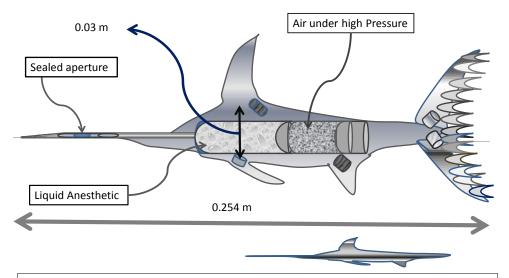


Figure 1. Sub-aquatic dart gun syringe, exploiting the fluid dynamical advantages of the sword fish

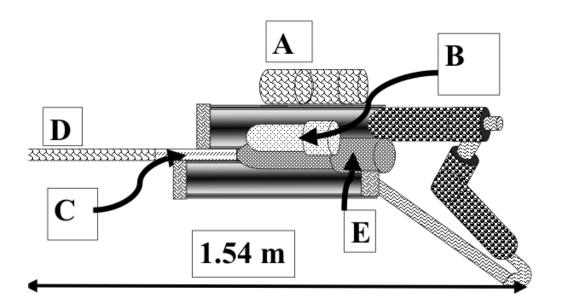


Figure 2. Amphibious Dart Rifle without the magazines: A. Housing for scope and periscope; B. Adapter for the net dart barrel; C. Compartment for insertion of tranquilizer dart magazine; D. Barrel for tranquilizer dart and; E. Firing Chamber

# SEDATION OF WHITE SEABASS (Atractoscion nobilis) WITH KETAMINE-DEXMEDETOMIDINE TO FACILITATE CAPTURE AND TRANSPORTATION

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#### Abstract

The California Science Center has maintained white seabass (*Atractoscion nobilis*) in both the exhibit kelp tank and in holding tanks. Moving white seabass was previously done with nets and physical restraint, frequently resulting in torn nets and injuries to both fish and staff. Based on limited information on using ketamine-medetomidine sedation in fish, we decided to give this a try. Eight white seabass transfers have been performed using this technique. Drugs were delivered by rapid hand injection. With some fish, trained behaviors were used for drug delivery, and in other situations the injections were given opportunistically. In one case the injections were delivered with a modified spear gun setup. The spear gun functioned well, but we had some problems with compressed air powering the darts under water.

Fish received ketamine, 10-12 mg/kg, and dexmedetomidine, 0.05-0.06 mg/kg, by intramuscular injection. Fish ranged in size from 8 to 22 kg. Maximum sedation occurred in 20-30 min. Fish were still swimming, but much less responsive to stimuli and easily maneuvered into a stretcher for transport and procedures. Six fish were reversed with atipamizole, 0.5-0.6 mg/kg i.m. Two fish were sedated for capture and transport of 6-8 hr. In these two fish the sedation was not reversed. They handled the transport well and there was no additional sedation used for transfer on arrival. No adverse effects were seen and all sedations were considered successful.

LAUNCHING OF THE UNITED STATES DEPARTMENT OF AGRICULTURE (USDA) AND THE ASSOCIATION OF ZOOS AND AQUARIUMS (AZA) PILOT SURVEILLANCE PROGRAM FOR AVIAN INFLUENZA: PRELIMINARY RESULTS

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#### **Abstract**

Since 2007, the USDA/APHIS Animal Care Program and the AZA have worked through a cooperative agreement on a pilot voluntary surveillance program for avian influenza in three zoos, using three regional state laboratories in the National Animal Health Laboratory Network. Samples from avian species positive for highly pathogenic avian influenza (HPAI) subtypes (H5 and H7) will be forwarded to the National Veterinary Services Laboratory (NVSL) for final processing. The purpose of this program is three-fold: test this avenue for early detection of avian influenza, particularly in rare and protected zoo populations of birds; establish a baseline prevalence rate in three large zoos; and evaluate the utility of zoos as sentinels for disease. Results from the launching of this program presented here will be posted on a password-protected on-line network and used for epidemiologic analysis, adding another facet to our understanding of potential areas of emergence for HPAI.

# THIRTY YEARS OF MORTALITY ASSESSMENT IN WHOOPING CRANE (Grus americana) REINTRODUCTIONS: PATTERNS AND IMPLICATIONS

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#### **Abstract**

We reviewed postmortem data to identify primary causes of mortality in reintroduced whooping cranes (Grus americana) and assess their potential for mitigation in future reintroduction efforts. In total, 240 cases from three populations were reviewed for causes of death, including the Rocky Mountain migratory population (n = 24, release dates 1975-1989), the Florida resident population (n = 186, 1993-2005), and the Wisconsin migratory population (n = 30, 2001-Traumatic injury was the leading cause of mortality among the reintroduced whooping cranes, most commonly from predation (n = 120 or 50%, range 8-58% per project) or collision with fixed structures such as electrical power lines or fences (n = 22 or 9%, range 3-46%). Disease of infectious etiology (including confirmed cases of bacterial, viral, fungal and parasitic infection) was the second leading cause of mortality (n = 19 or 8%, range 3–17%). The data were limited by the large number of undetermined causes of death due to scavenging and decomposition of carcasses (n = 64 or 27%, 8-40%). Molting and poor roosting behavior or habitat quality may have increased the risk of predation in these populations. Preventive measures for power line collisions (marking devices) are impractical except at significant roost or migration stopover sites. Health evaluations of release candidates should continue in order to minimize losses from endemic or emerging diseases and prevent the introduction of novel pathogens into native ecosystems.

**264** 

### PREPARING TO ANESTHETIZE A GIRAFFE IN A CONFINED AREA

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#### Abstract

Planning and preparation are key components to a successful giraffe anesthesia. Since no two facilities will be exactly the same, it is important to understand the basic requirements of the work area as well as things to avoid. The substrate should create suitable traction to ensure adequate footing. It should also be thick enough to cushion the animal when it falls. The area should be large enough to accommodate the animal in lateral recumbency and to allow people and equipment to move safely around the animal. Two exits are desirable to prevent personnel from becoming trapped in the enclosure with the animal. Hazards such as hayracks and concrete drinkers should be removed or padded. Eight to ten people are necessary to position, roll, or move the giraffe as needed. Personnel should have duties assigned in advance to prevent chaos and to facilitate working simultaneously to keep anesthesia time to a minimum. Animals should be fasted when possible. During anesthetic induction, the animal may fall over backwards, hit its head against the wall, splay, or even wedge itself into a corner and thus is unable to fall. Staff should be prepared to push, pull, or trip the animal (using ropes) to facilitate recumbency. Reducing stall size with hay bales and lining the walls with a few rows of hay bales may reduce trauma. A neck board is used to support the head and neck at a 45-60 degree angle. The neck must be kept flat and any kinks in the cervical vertebrae should be quickly remedied. The head should be maintained above the level of the rumen and should be supported immediately once the animal goes down. Problems during recovery are similar to induction. Giraffe usually stand with the rear limbs first and they do need enough room to rock and roll sternal. Ropes and straps may be useful to prevent the animal from going over backwards until it regains its balance.

# **Planning**

Planning and preparation are key components to a successful giraffe anesthesia. If this is the first time a giraffe anesthesia is performed at a given facility, planning should commence well in advance and the procedure reviewed multiple times. Essential personnel include but are not limited to animal managers, animal care staff, veterinarians, veterinary technicians, and facility and maintenance personnel.

- •Review the facilities.
- •Review personnel safety (escape routes, working around the animal, accidental narcotic exposure).
  - •Review goals of the procedure and time line.

- •Discuss the logistics of the procedure and anticipate problems. It is helpful to review scenarios of what can go wrong and how the staff should respond. This may help to prevent problems but also prepares the staff and generates an emergency equipment list.
- •Identify roles and establish the chain of command. Personnel should have duties assigned in advance to prevent chaos and to facilitate working simultaneously to minimize anesthesia time.
  - Solicit input and address questions and concerns.

# **Enclosure Size and Shape**

Carefully evaluate the options for where the procedure could take place. Indoors or outdoors? Corral or stall? What's the weather going to be like? Is there adequate lighting? Is there vehicle access? Is there electricity? What are the walls of the area made of? How much access is there to the animal? Since no two facilities will be exactly the same, it is important to understand the basic requirements of the work area as well as things to avoid. The area should be large enough (at least 20' in one direction) to accommodate the animal in lateral recumbency and to allow people and equipment to move safely around the animal. If an area seems too large, the size and shape can be altered using stacked hay bales, rubber pads or mats, or even mattresses. Giraffe can flip over backwards and this can be fatal. Consideration should be given as to how to break the fall and prevent the head from striking a hard surface. Reducing stall size with hay bales and lining the walls with a few rows of hay bales is one option as noted above. Ideally, two exits should be available to prevent a person from becoming trapped in the stall with the animal. It is preferable to have access to the head via catwalks. Alternatively, scaffolding or ladders can be used to access the head of the giraffe if necessary. Solid or chain link walls are acceptable. Horizontal bars alone can be problematic. Walls should extend to the ceiling or at least be shoulder height. Areas with moats should not be used unless a temporary wall can be created to prevent the animal from falling into the moat. The temporary wall needs to be strong enough to stay in place against the weight of the giraffe.

# **Substrate**

The substrate should create suitable traction to ensure adequate footing that prevents the animal from slipping. It should also be thick enough to cushion the animal when it falls and while laying in lateral recumbency. Playground sand works well. The chosen substrate should produce a minimum amount of dust. If this cannot be avoided, the surface can be lightly sprayed with water to reduce dust. Additional substrate can be used to even out the stall surface and correct and slopes.

# **Enclosure hazards**

Hazards such as hayracks and concrete drinkers should be removed or padded. Cover any sharp objects along the walls and carefully scan and cover exposed chain link if there are areas that may cause injury.

# **Equipment**

Organize medical equipment so it can be moved easily in and out of the stall. Working out of crates or grips will limit litter in stall and facilitate the rapid clearing of the stall. Vehicles should be loaded such that emergency equipment and supplies are within easy reach. Nonmedical equipment needs include

- •4 nylon straps at least 3 inches wide, 20 ft long
- •4 Lariats or 1" cotton ropes to restrain legs
- •4-2 inch straps to hold the head and neck to neck board (optional)
- •4" thick pads
- •Large rubber inner tubes (optional if additional padding needed)
- •30' long 2" cotton rope
- Padded board or ladder to support the neck (8' long for an adult)
- •8' step ladder
- Generator
- Extension cords
- Portable lights
- Hay bales to support neck board (3-5)
- •Feed bags or pads for support of limbs during hoof trimming
- •10' length of PVC pipe or bamboo for giraffe manipulation
- Winch
- Access to skip loader or Bobcat
- •Eye drape or cover
- •Ear plugs
- •Blankets/towels
- Oxygen tank and accessories
- •If using pulley system thru the roof:
  - oHalter
  - oCollar with clips
  - o2-1" cotton guide ropes, 40' long
  - oPulley in ceiling with snatch block

# **Herd management**

Where will the other animals be housed immediately prior to and during the procedure? When will the target animal be separated from the herd? It is best to keep the daily routine as normal as possible to reduce stress.

# **Fasting Instructions**

Animals should be fasted when possible to reduce the risk of regurgitation. It is most important to restrict food that is easily fermentable such as grain and pellets for 48 hr. Hay is okay to feed

as it might help to create a mat of fiber on the rumen surface. Water should be withheld for 12-24 hr (weather dependent).

### **Anesthetic Induction**

Much like flying a plane, anesthetic induction and recovery are the two most critical times during anesthesia. Possible scenarios during induction include:

- •The animal sinks into a dog sitting position then slides into sternal recumbency. At this point, the animal can be safely approached and the eyes covered. After the drugs have taken effect, the animal can be pulled into lateral recumbency, esp. if prevented from doing so on its own due to leaning up against a wall.
- •The animal hugs the wall/corner and head presses or otherwise wedges itself into a corner preventing it from falling. Ropes, PVC or bamboo pole can be used to repel or pull the animal from corner/wall.
- •It continues pacing or circling despite ataxia. Staff should be prepared to trip or cast the animal with ropes (using ropes). Supplemental anesthesia should also be considered.
- •The animal may become rigid, extend its head and neck in a stargazing posture, and fall over backwards. Limiting head and spinal trauma can be done by adequately preparing the stall. Distracting the animal with a pole may keep the center of gravity forward over the front limbs or a rope can be tossed over the back of the neck and withers to try to keep the animal from falling backwards.
- •Rear legs may splay especially if there is not much traction. This is corrected by repositioning the animal.
- •The animal may hit its head against wall so consideration should be given to how to best reduce or prevent this impact.
  - •The animal may fall suddenly from a standing position.

# **Positioning**

The head and neck are supported by 2-3 people as soon as possible during the induction period. It is best to cover the eyes and place ear plugs at this point. Noise should be kept to a minimum. 8-10 people are usually necessary to pull, rotate, spin, or otherwise relocate and reposition the animal in the stall. It takes at least 4 people per side of the animal to roll the animal over, and another 3 to support the head and neck. The legs up tucked up against the body and the giraffe is gently pushed over. It works best to loop 3" straps around the animal to assist in pulling it over. The animal should be pulled away from walls as much as possible such that there is safe access around the animal. Giraffe are usually positioned in lateral recumbency, legs extended with the down limbs pulled forward, and with the head and neck supported at a level above the rumen (usually 45-60 degrees).

#### Maintenance

Once the animal is properly positioned, the neck board is placed under the down shoulder to support the head and neck. The cervical vertebrae should be maintained in a straight, flat, and natural position with no kinks. Any kinks that develop should be quickly corrected. Massaging

the neck muscles may be beneficial. The nose is usually pointed downwards and the tongue pulled out of the mouth to clear the airway and facilitate drainage of saliva. Padding the down hip, shoulder, and down limbs may be necessary if the procedure will be prolonged or if the substrate is hard. Caution should be used when working around the legs, as the animal may kick without warning. Caution should also be used when supporting the head as an animal can lift or throw its head backwards and injure personnel. Ropes or straps can be preplaced on the limbs to reduce spontaneous movement.

# **Recovery**

Problems encountered during recovery are similar to induction. Most problems are associated with the animal being unable to find its center of gravity. Giraffe usually stand with the rear limbs first. They do need enough room to rock and roll sternal so should not be positioned with the feet against a wall. Pre-placing 2 straps or ropes under the base of neck may be useful to prevent the animal from going over backwards until it regains its balance. Once the procedure is completed, all equipment and supplies except for the neck board should be removed from the stall. All 4 limbs are pushed up against the body (alternating hind and front limbs) as best as possible. The animal can be supported in this position so it cannot roll back on to its side. All unnecessary personnel should leave the stall. Exits must remain clear. Four people are usually needed to stay with giraffe and assist with neck and head control during the initial recovery process. Once the animal starts to move or react to stimuli, the neck board can be removed, but it will still be necessary to control the head above the rumen. The animal may suddenly swing its head. At this point, all personnel should move away to prevent injury. The eyes can remain covered as this will keep the animal calm. The eye covered should be tied or taped at this point as it should be able to fall off as the animal moves. Four staff members (one or two on the end of each strap or rope) can be used to facilitate manual control of the neck and head once the animal is standing. These can be removed once the giraffe has control of its head.

# Post-anesthesia

The giraffe should be monitored for 12-24 hr following anesthesia. Drug recycling can occur 6-72 hr post recovery. Clincial signs include lethargy, dullness, decrease response to external stimuli, dull eyes, inappetance, salivation, drooping tongue, ataxia, leaning against a wall, or incoordination. Administering additional antagonist 6-8 hr after the procedure is strongly recommended.

# EVALUATION OF A BOVINE COMMERCIAL COLOSTRUM REPLACER AND PASSIVE TRANSFER IN SPRINGBOK CALVES (Antidorcas marsupialis)

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### Abstract

Failure of passive transfer (FPT) is the inadequate absorption of immunoglobulins from colostrum that occurs in ruminant neonates. FPT has been shown to increase the risk of diarrhea, enteritis, septicemia, arthritis, omphalitis, pneumonia, and mortality in crias, calves, kids, and lambs.<sup>1-4</sup> In zoologic establishments FPT can be a common occurrence in hand-raised ruminant neonates fed insufficient amounts of colostrum replacer and or poor quality colostrum replacer. The efficacy of specific colostrum replacers at achieving serum IgG concentration consistent with adequate passive transfer and tests to assess FPT have been intensely studied in domestic ruminants but few studies are available in non-domestic ruminants. This research assessed a commercially available bovine colostrum replacer's (Land O Lakes) ability to achieve serum immunoglobulin concentrations consistent with adequate passive transfer in Springbok calves, (Antidorcas marsupialis). The hypothesis of the study was that feeding Land O Lakes commercial bovine colostrum replacer to Springbok calves at a dose of  $\geq 4.65$ g of IgG per kg of animal's body weight will result in a proportion of neonates with adequate passive transfer similar to those that nursed maternal colostrum. The study determined the sensitivity and specificity of various tests (serum total protein, glutaraldehyde, gamma-glutamyl-transferase, globulin, and sodium sulfite) in determining passive transfer status in Springbok calves. The morbidity and mortality until weaning was compared between Springbok calves fed colostrum replacer and those that nursed maternal colostrum.

#### **ACKNOWLEDGMENTS**

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# CHARACTERIZATION AND EPIDEMIOLOGY OF HELICOBACTER INFECTION IN ZOO ANIMALS

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### **Abstract**

Helicobacter species have exceptional genetic and phenotypic adaptability which has rendered them widely successful and allowed for rapid changes in host-bacterium dynamics. It is now recognized that helicobacters are a significant cause of morbidity and mortality in humans and numerous animal taxa, producing local lesions (gastrointestinal inflammation, ulceration, and cancer) and systemic disease in some animals and having either no discernible effects or beneficial influences in others.<sup>3</sup> Yet, little is known about their ecology on a broad scale, including levels of host switching and factors related to disease expression. In this study, we conducted a cross-sectional fecal survey of 261 individuals and groups of primates and carnivores to determine helicobacter status and identify phylogenetic strains. PCR and DNA sequencing analyses were performed and univariate odds ratios were calculated to correlate broad health characteristics with helicobacter status, presence of multi-infection, and sharedgenotypes. Eighty-one percent (64/79) of species and 63% (138/220) of all surveyed individuals (70% of primates; 55% of carnivores) were positive for helicobacter infection with 79 distinct genotypes identified. Presences of multi-infection or infections with shared genotypes were corroborative with host-switching and were associated with mild clinical signs and management characteristics. Epidemiologic analyses provided insight into the dynamics of helicobacter infections in a zoological setting and were valuable for advancing awareness of anthropogenic effects on infection in animals.<sup>2</sup>

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